XII INTERNATIONAL CITRUS CONGRESS
VALENCIA / SPAIN

BOOK OF ABSTRACTS

NOVEMBER
18th - 23rd
2012

Valencia
Conference
Centre

THE XII INTERNATIONAL CITRUS CONGRESS

"CITRUS AND HEALTH"

2012 INTERNATIONAL CITRUS CONGRESS
VALENCIA / SPAIN

18th-23rd November 2012

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WELCOME MESSAGE

On behalf of the International Society of Citriculture (ISC) and the Organizing Committee I wish to warmly welcome you to the 12th International Citrus Congress (ICC 2012) and welcome to the city of Valencia.

The ICC 2012 is taking place from 18 to 23 November, at the Valencia Conference Center. More than 720 scientific contributions will be presented in this congress to highlight the advances in citrus research. The Organizing Committee with the collaboration of the selected conveners have built a scientific program which will provide a balanced the overview among basic and applied citrus research, to be presented in parallel sessions. Of special consideration are the seven plenary sessions and the eleven workshops focused to look at recent advances and future prospects on specific citrus issues, related to the most important scientific and technological problems. The main objective of the whole program is to bring together members from the citrus community for a thorough and lively discussion on each theme. The program also places a special emphasis on the main theme 'Citrus and Health' focused from different perspectives. Thus I would like to meet you all in these sessions!

In addition to this exciting scientific program, you will benefit of being in the third largest city of Spain. The legacy left by more than 2000 years of different cultures and civilizations shape the the city's monuments and streets, and also their habitants. Valencia is a city that never sleeps, with an extensive cultural a lineup start-studded at any time of year with festivals, concerts, shows and exhibitions. The excellent climate and the attractive cityscape allow outdoors activities as café terraces, seaside terraces, parks and garden activities throught all the year. I'm sure that all you will enjoy the experience of living Valencia.

Last but not least, I wish to thank to the Organizing Committee, collaborators, conveners and Sponsors for their extraordinary efforts to make the 12th International Citrus Congress possible.

I expect that the ICC 2012 will be a great scientific success and look forwards to meeting all you during these days.

Dr. Luis Navarro
President of the ISC
Chairman of the Organizing Committee of the ICC 2012

THE 12th ICC COMMITTEES

Honor Committee

ICC Honor Committee President
His Majesty the King, JUAN CARLOS I

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Councelor for Agriculture and Water of the Murcia Region.

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Dr. LUIS NAVARRO
Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Valencia, Spain.

ICC Vice-chairman
Dr. EDUARDO PRIMO
Centro de Citricultura y Producción Vegetal, IVIA.

ICC Secretariat
Ms. TANIA NAVARRO
Fundación AGROALIMED, Valencia, Spain.

Scientific Programme coordinator
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Centro de Protección Vegetal y Biotecnología, IVIA.

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Centro de Protección Vegetal y Biotecnología, IVIA.

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D. FRANCISCO LLATSER
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D. FRANCISCO JOSÉ MARTÍNEZ
Comité de Gestión de Cítricos.

Collaborators

Dr. ANTONIO VICENT
Centro de Protección Vegetal y Biotecnología, IVIA.

Dr. GEMA ANCILLO
Centro de Protección Vegetal y Biotecnología, IVIA.

D. JOSÉ JUÁREZ
Centro de Protección Vegetal y Biotecnología, IVIA.

Dr. PABLO ALEZA
Centro de Protección Vegetal y Biotecnología, IVIA.

Dr. MARÍA ANGELES FERNÁNDEZ
Centro de Citricultura y Producción Vegetal, IVIA.

Dr. MARÍA ANGELES FORNER
Centro de Citricultura y Producción Vegetal, IVIA.

D. PABLO LEMOS
Sección Informática, IVIA.

D. FRANCISCO ARENAS
Consejería de Agricultura de la Junta de Andalucía.

Dr. JOSÉ IGNACIO PORRAS
Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA).

Dr. M.ª CARMEN VIVES
Centro de Protección Vegetal y Biotecnología, IVIA.

GENERAL INFORMATION

The city of Valencia

Located in the eastern coast of Spain, Valencia has a population of 800.000 inhabitants, being the third largest city of Spain. This location is the responsible of the mild temperate Mediterranean climate observed in the city, with an average annual temperature of 17ºC with warm summers and very mild winters. The rainfall period is at the beginning of spring and in autumn.

There are two official languages in Valencia, the Spanish (Spain's official language) and Valenciano. But as the first touristic destination, English is spoken in almost all cafeterias, bars, etc. Feel free to ask to the service.

Travelling info

Standard electrical voltage is 220-240V AC, 50 Hz, with electrical plug type C (standard in all Europe, except UK). Check for a transformer and voltage adapter if necessary with regard to your country. Adapters are available in most hardware stores (like El Corte Inglés).

Banks are open from Monday to Friday between 8:30 to 14:30, some are open on Saturday morning but exceptionally from October to April. Automatic tellers are 24-h open and mainly housed at the bank entry. Most hotels, restaurants and shops accept the main credit cards (American Express, VISA, Mastercard, 4B, Access and Diners Club). But if you want to exchange your currencies, you will find exchange offices at the airports, in some hotels and in all banks.

Shops are mainly open from 09:00 to 21:00, some are open till 22h, and in some periods till midnight.

Bar and Restaurants are mainly open during all day, but in some cases a continental timetable applies. Generally they serve breakfast between 07:30 to 10:00, lunch between 13:00 to 15:00 and dinner from 20:30 to 22:30. Hotels have usually wider services, so ask in your hotel.

Interest phone numbers

- Emergency services, 112
- Local Police, 092

Registration and Technical Secretariat

The ISC resitration counters will be located in the Valencia Conference Center lobby. Check operational hours in the corresponding panel.

The technical secretariat corresponds to:

Viajes El Corte Inglés SA.
División de Congresos, Convenciones e Incentivos
Gran Vía Fernado el Católico, 3, bajo.
46008 Valencia
Phone: +34 963 107 189
Fax: +34 963 411 046
Contact: **Erika Hvidkjaer Sanjuan**
Email: citruscongress2012@viajeseci.es

Exhibits – Trade Show

The exhibition area is located in the main lobby of the Valencia Conference Center (see map).

Delegates are encouraged to visit them. Opening hours are the same as for the ICC 2012.

Poster presentations

All posters are required to be mounted on Sunday, November 18 from 16:00 to 19:00, and will last till November 23. Poster boards are numbered consecutively and accordling to session number. The poster identification number can be found on the abstract book and in the list provided in the web site, and should be included in the right upper corner of your poster to allow match with poster board. The poster presenter should mount their posters on the corresponding poster board unless authors' decision on use the Poster printing service.

The poster presenter (underlined) is requested to be stationed by their poster during the first hour of the assigned session to facilitate discussion.

All postes are to be removed between 18:00-19:00 on Friday, November 23. Remaining posters after this time will be removed by the organization without possibility of recovery.

Speakers, audio visual and Sci-Technical desk

Authors are kindly requested to bring their PowerPoint presentations on a USB to upload the presentation to the established PC-network at least 24h in advance. No personal computers will be allowed in the auditoriums for the presentation to keep the session schedule.

Slide preview computers will be available to check the presentation with the software to be used in the Valencia Conference Center.

Session conveners will be able to check out a laser pointer for the speakers, and to make an advertisement two minutes before the end of allocated presentation time. Presentation would be interrupted if time is surpassed to maintain the programme schedule.

Congress badges

Congress participants are kindly requested to wear their Congress Badges at all times for identification and for security reasons. Congress badge will be required to access to lunch hall.

Congress badge and corresponding tickets will be requested for participation in the Mid-congress tour and for the congress official dinner.

SUPPORTERS

- The 12th ICC has been organized by the **International Society of Citriculture (ISC), Instituto Valenciano de Investigaciones Agrarias (IVIA)** and **Fundación AGROALIMED**.

- The Organizing Committee would like to give thanks to the following Spanish institutions which had provided funds to develop this event.

- **Generalitat Valenciana (GVA), Consejería de Agricultura, Pesca, Alimentación y Agua**.

- **Generalitat Valenciana (GVA), Consejería de Educación, Formación y Ocupación**, project number AORG/2012/011.

- **Instituto Nacional de Investigaciones y Tecnología Agraria y Alimentaria (INIA)** project AC2012-00028-00-00.

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TECNOLOGÍA

Las actividades de investigación que GCM realiza son:

- Programa de obtención de cítricos.
- Programa de irradiación.
- Programa de evaluación de patrones.
- Nuevas metodologías agronómicas de cultivo.
- Importación y evaluación de variedades, acuerdos internacionales.
- Adquisición de derechos de variedades.
- Evaluación variedades experimentales.
- Proyecto Citrusgenn: desarrollo de herra mientas biotecnológicas y genómicas para facilitar la generación y la selección de nuevas líneas y variedades de cítricos.
- Vivero de cítricos certificado.

TECHNOLOGY

The investigation activities within GCM are:

- Investigation program to develop citrus.
- Irradiation program.
- Rootstocks evaluation program.
- Development of new agronomic methodology for the plant.
- Import & evaluation of varieties from around the world, including International agreements.
- Purchase of varietal rights.
- Experimental evaluation of varieties.
- Project Citrusgenn: using the biotechnology & genomics tools to facilitate the generation & se lection of new lines & varieties of citrus fruit.
- Certificate citrus nursery.

GCM Variedades Vegetales, AIE
Contact: Charo Marín
direccion@gcmcitrus.com

GCM
Variedades Vegetales

Investigación y desarrollo
de nuevas variedades de cítricos

Research and development
of new citrus varieties

INVESTIGACIÓN

GCM Variedades vegetales A.I.E. es una empresa especializada en la investigación y el desarrollo de nuevas variedades de cítricos. Cuyo objetivo es aportar valor añadido al proceso desde el obtentor al consumidor, ofreciendo nuevas y excitantes variedades.

Innovación a través de nuevas
variedades de cítricos: VALOR AÑADIDO

INVESTIGATION

GCM Variedades Vegetales A.I.E. is a company specializing in the investigation & development of new citrus varieties. Our objective is to enhance the added value within the process from the creator to the consumer, offering new & exciting varieties.

Product innovation through
new citrus varieties: ADDED VALUE

DESARROLLO

Tiene un extenso proyecto para la recuperación y evaluación de nuevas variedades. Entre las actividades del proyecto, está el uso de las herramientas de la biotecnología y genómica para la obtención de nuevas variedades de mandarina triploides y diploides sin semillas en colaboración con diferentes organismos de investigación.

DEVELOPMENT

We have an extensive project for the evaluation of new varieties. Incorporated within our project, we use the tools of biotechnology & genomics for obtaining new triploides and seedless diploid mandarins, whilst collaborating with different investigation bodies.

m7

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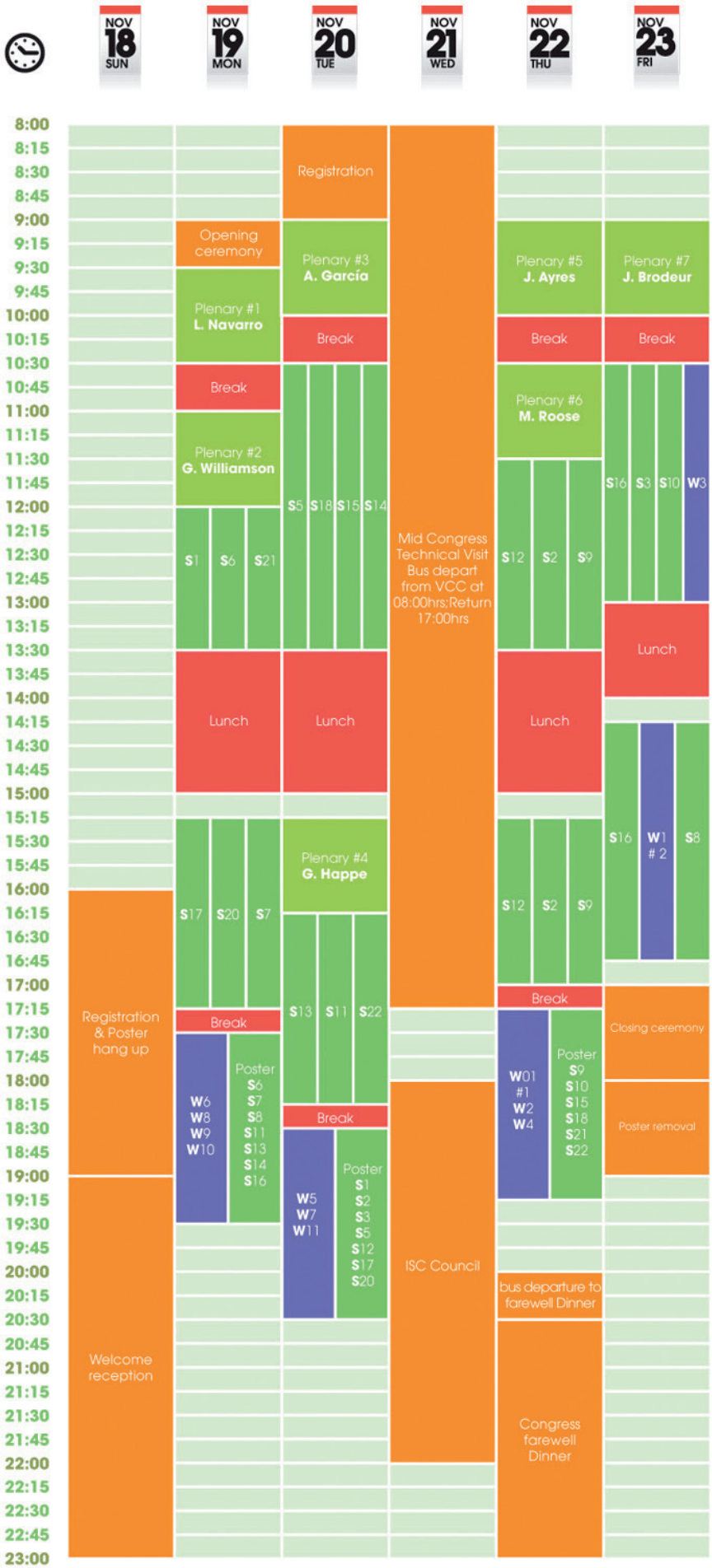
2012 INTERNATIONAL CITRUS CONGRESS
VALENCIA / SPAIN

Scientific Programme

ICC 2012 PROGRAMME AT GLANCE



SCIENTIFIC PROGRAMME

Note: Sessions S04 Citrus biochemistry and S19 Nursery and plant propagation have been removed from Programme.

PLENARY CONFERENCES

Note: All plenary conferences will take place at Auditorium 1

- **PC01 The Spanish citrus industry**
  Luis Navarro
  Instituto Valenciano de Investigaciones Agrarias (IVIA), Spain
  Monday 19th, 9:30-10:30h
- **PC02 Citrus and health**
  Gary Williamson
  School of Food Science and Nutrition, University of Leeds, UK
  Monday 19th, 11:00-12:00h
- **PC03 The importance of citrus for the juice and beverage industry**
  Ademerval Garcia
  Grove 2 Glass Trading GmbH, a 'The Coca-Cola Company' subsidiary, Switzerland
  Tuesday 20th, 9:00-10:00h
- **PC04 Food safety, social compliance and sustainability, in relation to commercial fruit and vegs strategies with special reference to citrus**
  Gé Happe
  European Sourcing Director Ahold, The Netherlands
  Tuesday 20th, 15:15-16:15h
- **PC05 The experience of Huanglongbing control in Brazil**
  Antonio J. Ayres
  Fundo de Defesa da Citricultura (FUNDECITRUS), Brazil
  Thursday 22nd, 9:00-10:00h
- **PC06 New genetic and genomic tools for citrus breeding**
  Mikeal L. Roose
  University of California, Riverside, USA
  Thursday 22nd, 10:30-11:30h
- **PC07 Biological control and citrus: a long time fruitful story**
  Jacques Brodeur
  Plant Biology Research Institute, Montreal University, Canada
  Friday 23rd, 9:00-10:00h

WORKSHOPS

The purpose of workshops will be to look at recent advances and future prospects on specific citrus issues, related to hot topics. The main objective is to bring together members from the Citrus community for a thorough and lively discussion on each theme. To promote discussions and active interactions, the convener of each workshop will make an introduction on the topic and then open and manage the debate. Conveners will make decisions on the best way to organize the debate according to the characteristics of each topic. We encourage delegates willing to participate in these workshops to get in touch with the conveners, as you will see their e-mails are available. Workshops are programmed every day after regular sessions with a format of 2 hours (except W01 and W03), thus providing enough time for discussion.

- **W01 'HLB Control'**
  Convener: J. Bové
  Université de Bordeaux Ségalen - INRA Center, France; joseph.bove@wanadoo.fr
  Thursday 22nd (17:15-19:15h, at Auditorium 1) and Friday 23rd (14:15-16:15 h, at Auditorium 2)

- **W02 'New mandarin varieties'**
  Convener: P. Aleza
  Instituto Valenciano Investigaciones Agrarias (IVIA), Spain; aleza@ivia.es
  Thursday 22nd (17:15-19:15h, at Auditorium 2)

- **W03 'Procedures for the exploitation of protected and/or patented varieties'**
  Convener: M. Iborra
  Norma Agrícola, Spain; miborra@normagricola.com
  Friday 23rd (10:30-12:30h, at Room 4)

- **W04 'New perspectives in pest control'**
  Convener: A. Urbaneja
  Instituto Valenciano Investigaciones Agrarias (IVIA), Spain; aurbaneja@ivia.es
  Thursday 22nd (17:15-19:15h, at Auditorium 3)

- **W05 'Dwarf citrus trees in high-density plantings'**
  Convener: K.D. Bowman
  U.S. Horticultural Research Laboratory, USA; Kim.Bowman@ars.usda.gov
  Tuesday 20th (18:30-20:30h, at Auditorium 2)

- **W06 'Molecular identification of varieties'**
  Convener: T. Shimizu
  National Institute of Fruit Tree Science, Japan; shimizu@affrc.go.jp
  Monday 19th (17:30-19:30h, at Auditorium 3)

- **W07 'Mechanization of citrus harvest'**
  Convener: E. Molto
  Instituto Valenciano Investigaciones Agrarias (IVIA), Spain; molto_enr@gva.es
  Tuesday 20th (18:30-20:30h, at Room 4)

- **W08 'Global citrus industry collaboration on MRL regulatory issues'**
  Convener J.R. Cranney Jr.
  California Citrus Quality Council, USA; jcranney@calcitrusquality.org
  Monday 19th (17:30-19:30h, at Room 5)

- **W09 'Global conservation strategy for citrus genetic resources'**
  Convener: K. E. Hummer
  U. S. Department of Agriculture - Agricultural Research Service National Clonal Germplasm Repository, USA; Kim.Hummer@ars.usda.gov
  Monday 19th (17:30-19:30h, at Room 4)

- **W10 'Research activities to support the potential biological effects of citrus fruits in human health'**
  Convener: Francisco A. Tomás-Barberán
  Centro de Edafología y Biología Aplicada del Segura del CSIC (CEBAS-CSIC), Spain; fatomas@cebas.csic.es
  Monday 19th (17:30-19:30h, at Auditorium 2)

- **W11 'Quarantine security for tephritid fruit fly pests in citrus'**
  Convener: N.J. Liquido
  USDA-APHIS-PPQ, Center for Plant Health Science and Technology, Plant Epidemiology and Risk Analysis Laboratory, USA; Nicanor.J.Liquido@aphis.usda.gov
  Tuesday 20th (18:30-20:30h, at Room 4)

SESSION 01: Citrus germplasm and phylogenetics

Oral presentations at Auditorium 1, on Monday 19th from 12:00 to 13:30.
Poster session on Tuesday 20th

Conveners: Dr. Gema ANCILLO (Instituto Valenciano Investigaciones Agrarias, Spain) and Dr. Robert KRUEGER (U. S. Department of Agriculture - Agricultural Research Service, USA).

Oral ID	Title
S01O01	Citron germplasm in Yunnan, China Karp D., Krueger R.R., Kahn T., and Hu X.
S01O02	Cryopreservation of citrus for long-term conservation Volk G.M., Bonnart R., Shepherd A., Krueger R.R., and Lee R.F.
S01O03	Genetic diversity and population structure of the mandarin germplasm revealed by nuclear and mitochondrial markers analysis. Garcia-Lor A., Luro F., Ancillo G., Ollitrault P., and Navarro L.
S01O04	Study on genetic diversity of 39 citron germplasm resources with SCoT and ISSR markers Zhang S.W., Huang G.X., He X.H., Pan J.C., and Ding F.
S01O05	Origin of dwarf, thornless-type trifoliate orange varieties deduced from genome-wide genotyping analysis Shimizu T., Yoshioka T., Kita M., and Ohta S.
S01O06	Multilocus haplotyping by parallel sequencing to decipher the interspecific mosaic genome structure of cultivated citrus. Curk F., Ancillo G., Garcia-Lor A., Luro F., Navarro L., and Ollitrault P.

Poster ID	Title
S01P01	*Citrus ichangensis* in Sichuan and Chongqing Zhang Y., and Zhou X.
S01P02	Diverse genetic resources of citrus in North-East region of India Huidrom S.D., Singh S. R., and Handique P.J.
S01P03	Observations of graft compatibility between *Citrus* spp and related Aurantioideae taxa Siebert T., Kahn T., and Krueger R.R.
S01P04	Fertility relationships among *Citrus* and its relatives in the subfamily Aurantioideae Siebert T., Ellstrand N., and Kahn T.
S01P05	Characterization of seed and embryo abortion during fruit development in several citrus cultivars pollinated by Nishiuchi Konatsu (*Citrus tamurana*) and preliminary trial of embryo rescue of aborting embryos Honsho C., Tsuruta K., Ryuto K., Sakata A., Kuroki S., Nishiwaki A., and Tetsumura T.
S01P06	Diversity analysis of citrus fruit pulp acidity and sweetness: toward to understand the genetic control of the fruit quality parameters Luro F., Gatto J., Constantino G., and Pailly O.
S01P07	Screening a core collection of citrus genetic resources for resistance to *Fusarium solani* Krueger R.R., and Bender G.S.
S01P08	Nuclear phylogeny of *Citrus* and four related genera Garcia-Lor A., Curk F., Snoussi H., Morillon R., Ancillo G., Luro F., Navarro L., and Ollitrault P.
S01P09	New insights on limes and lemons origin from targeted nuclear gene sequencing and cytoplasmic markers genotyping Curk F., Garcia-Lor A., Snoussi H., Froelicher Y., Ancillo G., Navarro L., and Ollitrault P.
S01P10	Diversity of citron (*Citrus medica*) and phylogenetic analysis of related citron hybrids using molecular markers and essential oil compositions Luro F., Venturini N., Costantino G., Tur I., Paolini J., Ollitrault P., and Costa J.

Poster ID	Title
S01P11	Analysis of genetic diversity in Tunisian citrus rootstocks Snoussi H., Duval M.F., Garcia-Lor A., Perrier X., Jacquemoud-Collet J.C., Navarro L., and Ollitrault P.
S01P12	Characteristics of Pompia a natural citrus hybrid cultivated in Sardinia Mignani I., Mulas M., Mantegazza R., Lovigu N., Spada A., Nicolosi E., and Bassi D.

SESSION 02: Citrus genetics and breeding

Oral presentations at Auditorium n. 2, on Thursday 22nd from 11:30 to 17:00.
Poster session on Tuesday 20th

Conveners: Dr. Patrick OLLITRAULT (Centre de Cooperation International en Recherche Agronomique pour le Développement - Instituto Valenciano Investigaciones Agrarias, CIRAD-IVIA, Spain) and Dr. Jude GROSSER (Citrus Research and Education Center, USA).

Oral ID	Title
S02O01	The triploid mandarin breeding program in Spain. Navarro L., Aleza P., Cuenca J., Juárez J., Pina J.A., Ortega C., Navarro A., and Ortega V.
S02O02	Triploid seedless mandarin breeding in France Froelicher Y., Bouffin J., Dambier D., and Ollitrault P.
S02O03	Mechanism of 2n gametes formation and centromere mapping in citrus Aleza P., Cuenca J., Juárez J., Navarro L., and Ollitrault P.
S02O04	Experiences in the development, release and commercialization of new irradiated citrus varieties from the citrus breeding program at the University of California Riverside Williams T.E.
S02O05	Citrus breeding program in Chile Martiz J., and Montañola M.J.
S02O06	Citrus breeding in South Africa: the latest developments in the programme run by the ARC-institute for tropical and subtropical crops Sippel A.D., Bijzet Z., Froneman I.J., Combrink N.K., Maritz J.G.J., Hannweg K.F., Severn-Ellis A.A., and Manicom B.Q.
S02O07	Highlights of the University of Florida, Citrus Research and Education Center's comprehensive citrus breeding and genetics program. Grosser J.W., Gmitter Jr. F.G., Ling P., and Castle W.S.
S02O08	Advance in *S* allele determination, *S* allele frequencies and *S*-genotyping in citrus. Wakana A., Handayani E., Kim J.H., Miyazaki R., Mori T., Sato M., and Sakai K.
S02O09	Integrated profiling of furanocoumarins (FCs) in grapefruit and derived hybrids toward selection of low FCs varieties. Chen C., Gmitter Jr. F.G., Cancalon P., and Greenblatt D.J.
S02O10	Breeding new grapefruit-like varieties with low furanocoumarin contents. Weissberg M., Yaniv Y., Sobolev I., Fidle L., and Carmi N.
S02O11	Genotypic variation of rind colour in citrus tangor 'Kiyomi' progenies. Combrink N.K., Bijzet Z., Sippel A.D., Booyse M., and Labuschagne M.T.
S02O12	Rootstock effects on mandarin fruit traits under control and salinity conditions Raga V., Bernet G.P., Carbonell E.A., and Asíns M.J.
S02O13	Extension of a citrus genetic linkage map for QTL mapping of freeze tolerance Hong Q.-B., Ma X.J., Gong G.-Z., and Peng Z.C.
S02O14	Location of a chromosome region linked to Alternaria Brown Spot resistance from the evaluation of triploid mandarin populations Cuenca J., Aleza P., Iborra E., Vicent A., Ollitrault P., and Navarro L.

Poster ID	Title
S02P01	Investigating the parentage of 'Orri' and 'Fortune' mandarin hybrids Barry G.H., Gmitter Jr. F.G., Chunxian C., Roose M.L., and McCollum G.
S02P02	Simple PCR-based tolls provide molecular identification of the main rootstock used in Sao Paulo State, Brazil Coletta-Filho H.D., Pompeu-Jr J., Francisco C.S., and Machado M.A.
S02P03	Assignment of SNP allelic configuration in polyploids using Competitive Allele-Specific PCR: application to triploid citrus progenies Cuenca J., Aleza P., Navarro L., and Ollitrault P.
S02P04	The parentage analysis contributes to the validation of high throughput SNP genotype calls of citrus Fujii H., Nonaka K., Kita M., Shimada T., Endo T., Kuniga T., Ikoma Y., and Omura M.
S02P05	Comparative values of SSRs, SNPs and InDels for citrus genetic diversity analysis Ollitrault P., Garcia-Lor A., Terol J., Curk F., Ollitrault F., Talon M., and Navarro L.
S02P06	Preliminary research on genes and proteins related to a spontaneous mutant of *Citrus reticulata* Zeng J.W., Yi G.J., Jiang B., Zhong Y., and Zhong G.Y.
S02P07	Allelic diversity of *Ferritin II* and *PMT IV* genes related to iron chlorosis in some citrus rootstocks Aka Kacar Y., Simsek O., Donmez D., Boncuk M., Yesiloglu T., and Ollitrault P.
S02P08	Genetic mapping of QTLs associated with drought tolerance in citrus Dutra-Souza J., Cristofani-Yaly M., Machado M.A., and Oliveira A.C.
S02P09	Evaluation of resistance of 'Pera' (*Citrus sinensis*) genotypes to Citrus Canker in field and green-house conditions Goncalves-Zuliani A., Nunes W.M.C., Zanutto C., Croce Filho J., and Nocchi P.T.M.
S02P10	Susceptibility of mandarins and tangors to Citrus Leprosis under greenhouse conditions Nunes M.A., Pereira J.A., Freitas-Astua J., Novelli V.M., and Bastianel M.
S02P11	Development of CTV resistant citrus rootstocks using hybridization Pinar H., Seday U., Unlu M., and Uzun A.
S02P12	Lime Bush (*Citrus glauca*) hybrids resistant to CTV Smith M.W., Gultzow D.L., Newman T.K., and Parfitt S.C.
S02P13	Determination of self-incompatibility status of some clementine (*Citrus clementina*) genotypes by histological analysis Aka Kacar Y., Aslan F., Eti S., Boncuk M., Simsek O., Yildirim B., Yalcin Mendi Y., Yesiloglu T., Distefano G., and La Malfa S.
S02P14	Genotyping for male sterility (MS) and MS gene mapping with RAPD markers in citrus, especially with precocious flowering seedlings from a cross of HY16 x grapefruit Dewi P.S., Wakana A., Tanimoto Y., Fujiwara Y., and Sakai K.
S02P15	Embryological studies on 'URS Campestre', a new seedless orange cultivar Guerra D., Santos R. P. dos, Montero C. R. S, Schwarz S. F., and Bender R. J.
S02P16	Mechanism of seedlessness in a new lemon cultivar Xiangshui (*Citrus limon*) Zhang S.-W., Huang G.H., Ding F., He X.-H., and Pan J.C.
S02P17	Differences in the genetic structure of citrus triploid hybrids recovered from 2x X 2x and 4x X 2x sexual hybridisations Aleza P., Cuenca J., Juárez J., Ollitrault P., and Navarro L.
S02P18	Efficient haploid production on 'Wilking' mandarin by induced gynogenesis Jedidi E., Kamiri M., Poullet T., Ollitrault P., and Froelicher Y.

Poster ID	Title
S02P19	Differences in ploidy levels of interploidal crosses progenies between diploids and tetraploid somatic hybrids in citrus Kamiri M., Srairi I., Poullet T., Ollitrault P., and Froelicher Y.
S02P20	Gametic configuration and inheritance of SSR markers in tetraploid interspecific and intergeneric citrus somatic hybrids Kamiri M., Stift M., Srairi I., Chahidi B., Poullet T., Costantino G., Dambier D., Ollitrault P., and Froelicher Y.
S02P21	Haploid and polyploid hybrids obtained from cross of diploid citrus Liu J.J., Chen K.L., He J., and Guan B.
S02P22	Chromosome redundancy and phenotypic variation in autotetraploid trifoliate orange seedlings Oh E.U., Chae C.W., Kim S.B., Park J.H., Yun S.H., Koh S.W., and Song K.W.
S02P23	New cybrids resulting from asexual pathway: a promise of the cybridization for creating new rootstocks and varieties. Dambier D., Petit F., Barantin P., and Ollitrault P.
S02P24	Several seedless citrus hybrids selected from tangor x 'Ponkan' cross Liu J.J., Chen K.L., He J., and Guan B.
S02P25	New citrus hybrids: selection and genetic studies Novelli V.M., Bastianel M., Fernandes K.A., Misságlia J., Latado R.R., Cristofani-Yaly M., and Machado M.A.
S02P26	Characterization of fruits of hybrids between 'Sunki' mandarin (*Citrus sunki*) and sour orange (*Citrus aurantium*) Schinor E.H., Michielin T.H.V., Simonetti L.M., Cristofani-Yaly M., Pompeu Jr. J., and Bastianel M.
S02P27	Evaluation of clementine x 'Kara' mandarin hybrids Seday U., Kafa G., Uysal O., Polatoz S., Uzun A., and Gulsen O.
S02P28	Distribution of leaf features in citrus hybrids obtained from clementine Uysal O., Kafa G., Seday U., and Polatöz S.
S02P29	Optimal dose of gamma irradiation for mutation induction in mandarins Handaji N., Benyahia H., Arsalane N., Mouhib M., Ibriz M., Srairi I., Chahidi B., and Bourachde Y.
S02P30	Effect of budwood irradiation on seed number of clementine, 'Nova' and 'Robinson' mandarins Kafa G., Seday U., Uysal O., and Polatöz S.
S02P31	Three variegated clementines Kafa G., Seday U., Uysal O., and Polatöz S.
S02P32	New low seeded mandarin (*Citrus reticulata*) and lemon (*Citrus lemon*) selections obtained by gamma irradiation in Chile Montañola M.J., and Martiz J.
S02P33	Radiosensitivity of seeds and nodal segments of citrus rootstocks irradiated *in vitro* with γ-rays from ^{60}Co Tallón C.I., Porras I., Perez-Tornero O.
S02P34	Studies on mutation breeding for seedless and low-seeded cultivars of citrus Tang X.L., and Ma P.Q.
S02P35	Agronomic performance of twenty six clones of 'Pera' sweet orange in São Paulo state, Brazil Carvalho S.A., Latado R.R., Silva L.F.C., and Müller G.W.
S02P36	Distribution of fruit characteristics in the group of citrus Setoka nucellar seedlings Park J.H., Yun S.H., Koh S.W., and Chae C.W.

Poster ID	Title
S02P37	'Qing-ougan': The green-peel mandarin mutation of 'Ougan' in Zhejiang province, China Xu J.G., Ke F.Z., and Huang J.Z.
S02P38	Hybridization-based citrus breeding program in Turkey Uzun A., Gulsen O., Seday S., and Kafa G.
S02P39	'BATEM Fatihi': A new orange selection from cv. 'Washington navel' Eryılmaz Z., Dal B., Tuncay M., Balkılıç R., and Gübbük H.
S02P40	A new orange cultivar 'BATEM Baharı' selected from cv. 'Valencia Late' Eryılmaz Z., Ercişli S., Gübbük H., and Tuncay M.

SESSION 03: Citrus genomics

Oral presentations at Auditorium 2, on Friday 23rd from 10:30 to 13:00.
Poster session on Tuesday 20th

Conveners: Dr. Manuel TALÓN (Instituto Valenciano Investigaciones Agrarias, Spain) and Dr. Fred GMITTER Jr. (Citrus Research and Education Center, USA).

Oral ID	Title
S03O01	Genome sequence analysis and comparisons reveal ancestral hybridization and admixture events in the origins of some citrus cultivars Gmitter Jr. F.G., Ollitrault P., Machado M.A., Reforgiato-Recupero G., Talón M., Roose M.L., Navarro L., Wu G., Jaillon O., Morgante M., and Rokhsar D.S.
S03O02	Sequencing of 150 citrus varieties: linking genotypes to phenotypes Terol J., Carbonell J., Alonso R., Tadeo F.R., Herrero A., Ibáñez V., Muñoz J.V., López-García A., Hueso L., Colmenero-Flores J.M., Conesa A., Dopazo J., and Talón M.
S03O03	Whole genome sequencing and mapping analysis for identifying polymorphism among 11 citrus varieties Shimizu T., Yoshioka T., Nagasaki H., Kaminuma E., Toyoda A., Fujiyama A., and Nakamura Y.
S03O04	Sweet orange genome: sequencing, annotation and beyond Xu Q., Ruan X., Chen L.L., Chen D.J., Zhu A.D., Chen C.L., Ruan Y., and Deng X.X.
S03O05	A reference genetic map of *Citrus clementina*; citrus evolution inferences from comparative mapping Ollitrault P., Terol J., Chen C., Federici C.T., Lotfy S., Hippolyte I., Ollitrault F., Bérard A., Chauveau A., Cuenca J., Costantino G., Kacar Y., Mu L., García-Lor A., Froelicher Y., Aleza P., Boland A., Billot C., Navarro L., Luro F., Roose M.L., Gmitter Jr. F.G., Talón M., and Brunel D.
S03O06	The chromosomes of citrus: from a unifying nomenclature to the evolution of karyotypes Silva S.C., Mendes S., Moraes A.P., Marques A., Mirkov T.E., Iglesias D.J., Ibáñez V., Tálon M., Soares Filho W.S., Guerra M., and Pedrosa-Harand A.
S03O07	Analysis of the clementine floral transcriptome uncovers candidate genes involved in self-incompatibility Caruso M., Lo Cicero L., Distefano G., Merelo P., La Malfa S., Tadeo F.R., Talón M., Lo Piero A.R., and Gentile A.
S03O08	Citrus gene function analysis using a viral vector based on the *Citrus leaf blotch virus* genome Agüero J., Velázquez K., Vives M.C., Pina J.A., Navarro L., Moreno P., and Guerri J.

Poster ID	Title
S03P01	Expression analysis of genes differentially expressed in stem tissue of two lemon cultivars Koutsioumari E.M., and Voloudakis A.E.
S03P02	Transcriptome profile analysis of Citrus Canker resistance of Chinese citron (*Citrus medica*) via massively parallel mRNA sequencing Li D.Z., Dai S.M., Li N., and Deng Z.N.
S03P03	Characterization of genes associated with two agronomically important traits in citrus: drought tolerance and fruit color Costa M.G.C., Cidade L.C., de Oliveira T.M., Martins C.P.S., Mendes A.F.S., Pereira S.L.S., Pedrosa A.M., Gesteira A.S., Girardi E.A., Coelho-Filho M.A., Soares Filho W.S., and Machado M.A.
S03P04	Expression of *Ptcor8* gene induced by low temperature as related to cold resistance in citrus Long G.Y., Song J.Y., Luo K., Deng Z.N., Li N., and Gentile A.
S03P05	Cloning and characterization of a prolin-rich protein gene *CsPRP4* from citrus Ma Y.Y., Zhang L.Y., Zhu S.P., and Zhong G.Y.
S03P06	Ploidy and gene expression in clementine Niñoles R., Aleza P., Castillo M.C., Navarro L., and Ancillo G.
S03P07	Expression of flowering genes in different shoot types in citrus Muñoz-Fambuena N., Mesejo C., González-Mas M.C., Iglesias D.J., Primo-Millo E., and Agustí M.
S03P08	Shortening the juvenile phase in *Arabidopsis* plants by ectopic expression of citrus transcription factors Castillo M. C., Navarro L., and Ancillo G.
S03P09	Differential expression of proteins related to primary metabolism in 'Moncada' mandarin leaves with contrasting fruit load Muñoz-Fambuena N., Mesejo C., Agustí M., Tárraga S., Iglesias D.J., Primo-Millo E., and González-Mas M.C.
S03P10	Proteins related to stress and redox state of 'Moncada' mandarin leaves with contrasting fruit load Muñoz-Fambuena N., Mesejo C., Agustí M., Tárraga S., Iglesias D.J., Primo-Millo E., and González-Mas M.C.
S03P11	Genomic compositions and origins of *Citrus sinensis, Citrus clementina* and *Citrus poonensis* as revealed by genome-wide analysis of citrus ESTs Zhong G.Y., Wu B., Zeng J.W., Jiang B., and Yang R.T.
S03P12	Genomic sequencing and comparative analysis of *Citrus platymamma* (Byungkyool) by using next-generation sequencing technology Chung S.J., Han S.I., Jin S.B., Kim Y.W., Yun S.H., Riu K.Z., and Kim J.H.
S03P13	Fast and cost-effective DNA marker typing method with accuracy for citrus by direct PCR and multiplexed post-labeling Ohta S., Yano K., Kurita Y., Kita M., Yoshioka T., Nesumi H., and Shimizu T.
S03P14	Data mining and systems biology for identifying key genes involved in citrus quality Silva E.M.A, Bernardes L.A.S., Ollitrault P., Bonatto D., and Micheli F.
S03P15	Genotyping and mutation scanning by high resolution melting (HRM) analysis of citrus EST-SNPs and SSRs Distefano G., La Malfa S., Caruso M., Lo Piero A.R., Nicolosi E., and Shu-Biao W.

SESSION 05: BIOTECHNOLOGY

Oral presentations at Auditorium 1, on Tuesday 20th from 10:30 to 13:00.
Poster session on Monday 19th

Conveners: Dr. Leandro PEÑA (Instituto Valenciano Investigaciones Agrarias, Spain) and Dr. Xiuxin DENG (Huazhong Agricultural University, China).

Oral ID	Title
S05O01	Unlocking the molecular secret of blood orange Butelli E., Licciardello C., Reforgiato-Recupero G., and Martin C.
S05O02	A novel carotenoid cleavage activity responsible for the biosynthesis of citrus fruit-specific apocarotenoids Rodrigo M.J., Alquézar B., Carmona L., Alós E., Al-Babili S., and Zacarías L.
S05O03	Biosynthesis and regulation of carotenoids in citrus callus Xu J., Gao H.J., Deng X.X., and Zhang H.Y.
S05O04	Terpene downregulation triggers innate immunity and resistance to fungal pathogens in orange fruits Rodríguez A., Shimada T., Cervera M., Alquézar B., Gadea J., Gómez-Cadenas A., De Ollas C., Rodrigo M.J., Zacarías L., and Peña L.
S05O05	Improving *in vivo* antioxidant properties of orange fruits through increased β-carotene content Pons E., Alquézar B., Rodríguez A., Martorell P., Genovés S., Llopis S., González N., Ramón D., Rodrigo M.J., Zacarías L., and Peña L.
S05O06	RNA interference against the three *Citrus tristeza virus* genes encoding silencing suppressors confers complete resistance to the virus in transgenic 'Mexican' lime plants Soler N., Plomer M., Fagoaga C., Moreno P., Navarro L., Flores R., and Peña L.
S05O07	Further verification of the resistance of 'C-05' citron to Citrus Canker disease Liu L.P., Li D.Z., Li J.H., Li N., and Deng Z.N.
S05O08	Genetic transformation of citrus species aiming to increase tolerance to drought stress Carlos E.F., Ariyoshi C., Felício M.S., Shimizu P., Souza G.V., Kudo S.A., Silva L.M., Silva A.J.M., Cação S.M.B., and Vieira L.G.E.
S05O09	Targeted cybridization in citrus and transcript profiling of a male sterile cybrid pummelo containing cytoplasm from satsuma mandarin Guo W.W., Zheng B.B., Xiao S.X., Grosser J.W., and Deng X.X.
S05O10	*In vitro* anther culture of several cultivars of *Citrus sinensis* and *Citrus clementina*. Cardoso J.C., Abdelgallel A., Chiancone B., Rocha Latado R., Lain O., Testolin R., and Germanà M.A.
S05O11	Bioethanol production from mandarin (*Citrus unshiu*) peel waste Choi I.S., Wi S.G., and Bae H.J.

Poster ID	Title
S05P01	Screening and analyzing the root-specific genes of *Poncirus trifoliata* Yao L.X., Chen S.C., He Y.R., Lei T.G., Xu L.Z., Liu X.F., and Peng A.H.
S05P02	Comprehending crystalline β-carotene accumulation by comparing engineered cell models and natural carotenoid-rich system of citrus Cao H.B. , Zhang J.C., Xu J.D., Ye J.L., Yun Z., Xu Q., Xu J., and Deng X.X.
S05P03	Analyses of expressed sequence tags (ESTs) of 'Barnfield' navel orange via constructing of a subtractive cDNA library between oleocellosis and healthy rind Wei Z.X., Hong L., Cheng C.F., Wu C.Q., and Qi J.R.

Poster ID	Title
S05P04	Control of flowering time in citrus through ectopic overexpression of *flowering locus T* (*FT*) and *apetala1* (*AP1*) from sweet orange Rodríguez A., Cervera M., and Peña L.
S05P05	Overexpression of a chromoplast-specific *lycopene* β-*cyclase* (β-*LCY2*) gene in orange fruit Alquézar B., Pons E., Rodríguez A., Rodrigo M.J., Zacarías L., and Peña L.
S05P06	Genetic transformation of *Citrus sinensis* with *attacin A* gene driven by phloem tissue specific promoters for resistance to *Candidatus* Liberibacter spp. Tavano E.C.R., Harakava R., Vieira M.L.C., Mourão Filho F.A.A., and Mendes B.M.J.
S05P07	*D-limonene* downregulation in transgenic sweet orange provides protection against Citrus Black Spot symptoms induced in detached fruits Kava-Cordeiro V., Rodríguez A., Vicent A., Glienke C., and Peña L.
S05P08	Resistance to *Citrus tristeza virus* in *Citrus sinensis* transgenic plants Muniz F.R., Harakava R., Rezende J.A.M., Moore G.A., Febres V.J., Mourao Filho F.A.A., and Mendes B.M.J.
S05P09	Production of a recombinant miraculin protein in transgenic citrus cell suspension cultures Jin S.B., Sun H.J., Kim Y.W., Bachchu M.A.A., Yun S.H., Riu K.Z., and Kim J.H.
S05P10	Transgenic sweet orange rootstocks overexpressing a osmotin like PR-5 protein show resistance against Phytophthora Foot Rot in the field Peris J.E., Vicent A., and Peña L.
S05P11	Evaluation of transgenic oranges expressing the *stx ia* gene for resistance to Citrus Canker Marques V.V., Miller A.M., Bagio T.Z., Souza G.V., Meneguin L., Vieira L.G.E., Pereira L.F.P., and Leite J.R.R.P.
S05P12	Transgenic 'Mexican' lime plants expressing the gene *p23* from *Citrus tristeza virus* (CTV) under the control of a phloem-specific promoter display symptoms closely similar to those accompanying virus infection Soler N., Fagoaga C., López C., Moreno P., Navarro L., Flores R., and Peña L.
S05P13	Efficient deletion of the selectable marker gene from transgenic citrus via the Cre/loxp system Zou X.P., Peng A.H., Liu X.F., He Y.R., Xu L.Z., and Chen S.C.
S05P14	Assessment of pollen-mediated transgene flow in citrus under experimental field conditions Pons E., Navarro A., Ollitrault P., and Peña L.
S05P15	Functional analysis of a citrus transcription factor with mature fruit-specific expression using transgenic tomato Endo T., Shimada T., Fujii H., Sugiyama A., Nakano M., Ikoma Y., and Omura M.
S05P16	Effect of the citrus *lycopene* β-*cyclase* transgene on carotenoid metabolism in transgenic tomato fruits Guo F., Zhou W.J., Zhang J.C., Xu Q., and Deng X.X.
S05P17	Role of the sweet orange *tau* glutathione transferases (*csgstu*) in transgenic tobacco plant detoxification from xenobiotics Lo Cicero L., Madesis P., Tsaftaris A., and Lo Piero A.R.
S05P18	An alternative transformation method in citrus using cell-penetrating peptides (CPPs) Jensen S.P., Febres V.J., and Moore G.A.

Poster ID	Title
S05P19	Establishment of a transient expression system in citrus via agro-infiltration Dai S.M., Li F., Yan J.M., Li D.Z., Li R.H., and Deng Z.N.
S05P20	Improvement of transformation efficiency in citrus Yang L., Hu W., Xie Y.M. , Li Y., and Deng Z.N.
S05P21	Improvement of genetic transformation with mature explants of sweet orange (*Citrus sinensis*) Xie Y.M., Yang L., Hu W., Li Y., and Deng Z.N.
S05P22	Efficient propagation and rooting of citrus rootstocks adult explants using different basal medium and plant growth regulators Tallón C.I., Porras I., and Pérez-Tornero O.
S05P23	Optimization of *in vitro* organogenesis and plantlet regeneration of sweet orange from mature tissue Castro L.M., Mendes B.M.J., and Mourão Filho F.A.A.
S05P24	Applications of citrus shoot-tip grafting *in vitro* Juárez J., Aleza P., and Navarro L.
S05P25	Microshoot tip grafting *in vitro*-a technique for establishment of disease free scion bank of *Citrus reticulata* var. Khasi mandarin Sanabam R.S., Huidrom S.D., and Handique P.J.
S05P26	Elimination of *Spiroplasma citri* by somatic embryogenesis from citrus stigma and style culture: preliminary results Frasheri D., Moujahed R., Djelouah K., Carra A., Carimi F., Valentini F., and D'Onghia A.M.
S05P27	The study on callus induction of citrus anther culture *in vitro* Luo J.Q., Xu J.G., Wang P., Ke F.Z., and Nie Z.P.
S05P28	Induction of tangerine embryogenic calli from unfertilized or immature ovules Machado M.P., de Bona C.M., and Biasi L.A.
S05P29	Chromosome instability in 'Carrizo' citrange x *Citrus macrophylla* somatic hybrids Pensabene G., Ruíz M., Aleza P., Olivares-Fuster O., Ollitrault P., and Navarro L.
S05P30	Preliminary results on biotization of encapsulated *in vitro*-derived propagules of 'Carrizo' citrange (*Citrus sinensis* × *Poncirus trifoliata*) Germanà M.A., Micheli M., Chiancone B., Bianco C., Casales F., and Defez R.
S05P31	Biodegradable films made from PLA-limonene blends for food active packaging applications Arrieta M.P., López J., Ferrándiz S., and Peltzer M.
S05P32	PLA and PLA-PHB incorporated with limonene for biodegradable food packaging Arrieta M.P., López J., Ferrándiz S., and Peltzer M.
S05P33	Shoot tip grafting (STG) in commercial production of disease free, quality-bud grafts of citrus in Central India: the present status Vijayakumari N.

SESSION 06: Fruit physiology

Oral presentations at Auditorium 2, Monday 19th from 12:00 to 13:30.
Poster session on Monday 19th

Conveners: Dr. Manuel AGUSTÍ (Instituto Agroforestal del Mediterráneo – Universidad Politécnica de Valencia, Spain) and Dr. Alfredo P. GRAVINA (Universidad de la Republica, Uruguay)

Oral ID	Title
S06O01	Effect of male-female interaction and temperature variation in citrus pollen performance Distefano G., Hedhly A., Las Casas G., La Malfa S., Cicala A., La Rosa G., Herrero M., and Gentile A.
S06O02	Abscission of reproductive structures in citrus and its control with brassinosteroids and girdling Pérez M.C., Almenares G., Torres W., Aranguren M., Betancourt M., García M.E., Núñez M., Llauger R.E., Mahouachi J., Iglesias D.J., and Talón M.
S06O03	"To fall or not to fall, that's the question!" Molecular mechanisms underlying organ abscission in citrus Tadeo F.R., Agustí J., Merelo P., Estornell L.H., Cercós M., Terol J., Domingo C., and Talón, M.
S06O04	Carbohydrate control over carotenoid build-up in citrus is conditional on fruit ontogeny Fanciullino A.L., Poiroux-Gonord F., Poggi I., and Urban L.
S06O05	Largely altered terpenoids production in red-fleshed fruits of 'Cara Cara' navel orange (*Citrus sinensis*) in comparison with pale-fleshed Seike navel orange Xu J., He M., Liu X., Gao H.J., Wang Z., Deng X.X., Cheng Y.J., and Zhang H.Y.
S06O06	Endogenous factors affecting fruit color development in navel sweet oranges Gambetta G., Gravina A., Fasiolo C., Martínez-Fuentes A., Reig C., Mesejo C., Bentancur O., and Agustí M.

Poster ID	Title
S06P01	Effect of continuously high temperature throughout a growth period on flowering, physiological fruit drop and fruit quality in satsuma mandarin Sato K.S., Fukamachi H., and Ikoma Y.
S06P02	Evaluations of potential fruit abscission and characteristics of seeded and seedless fruit abscission in several citrus species during physiological fruit drop Kitajima A., Li X., Habu T., Kataoka K., Nakazaki T., and Yonemori K.
S06P03	Cross-pollination and control of seed formation in 'Afourer' mandarin Gravina A., Gambetta G., Fasiolo C., Espino M., and Rivas F.
S06P04	Changes of nutrient element contents and accumulation in fruit of navel orange during fruit growth and development Peng L.Z., Fa X., Cailun J., Li C., and Lili L.
S06P05	The effects of source-sink ratio on leaf photosynthetic characteristics of 'Newhall' navel orange Chun C., Wenwen Z., Liangzhi P., Lili L., Xingzheng F., Li C., Xiaohuan M., Fa X., Cai L., and Cailun J.
S06P06	Anatomy of abnormal 'HB' pummelo caused by boron deficiency Li S., Liu Y.Z., Yang C.Q., and Peng S.A.
S06P07	Pre-harvest rind breakdown in New Zealand and a comparison with similar problems reported from other countries Pyle K.R.

Poster ID	Title
S06P08	The effect of combined spraying of gibberellin and prohydrojasmon on peel puffing in satsuma mandarin Ikoma Y., Matsumoto H.M., and Sato K.S.
S06P09	Production cost reduction technical development of 'Shiranuhi' mandarin hybrid in plastic house cultivation used underground air and air mixer Han S.H.
S06P10	Abscission study during citrus fruit maturation in Corsica: unfavorable environmental conditions for fruit shedding Khefifi H., Ben Mimoun M., Luro F., and Morillon R.
S06P11	Fruit quality characteristics of very early satsuma mandarin by soil mulching with polyporous reflective sheet Han S.G., Kim Y.H., and Moon Y.E.
S06P12	Fruit growth and sugar and acid characteristics in leafy and leafless fruits of satsuma mandarin Kim S.B., Oh E.U., Yun S.H., Oh H.W., and Song K.J.
S06P13	Physiological investigation of June drop in 'Thomson' navel orange (*Citrus sinensis*) in north of Iran Akhlaghi Amiri N., Asadi Kangarshahi A., and Arzani K.
S06P14	Reducing alternate bearing index in satsuma mandarin by nitrogen management and pruning in north of Iran Asadi Kangarshahi A., Akhlaghi Amiri N., and Savaghebi Gh.R.
S06P15	Possibility of adjusting alternate bearing cycle in satsuma mandarin (*Citrus unshiu*) in north of Iran Asadi Kangarshahi A., and Akhlaghi Amiri N.
S06P16	Reproductive behavior of plants of 'Valencia' sweet orange under two soil moisture conditions Laskowski L.L.

SESSION 07: Regulation of growth and development

Oral presentations at Auditorium 3, on Monday 19th from 15:15 to 17:15.
Poster session on Monday 19th

Conveners: Dr. Domingo IGLESIAS (Instituto Valenciano Investigaciones Agrarias, Spain) and Dr. James SYVERTSEN (Citrus Research and Education Center, USA).

Oral ID	Title
S07O01	Citrus developmental research: a historic, conceptual perspective Goldschmidt E.
S07O02	Stock-scion interactions in grafted citrus: a role for microRNA Tzarfati R., Ben-Dor S., Sela I., and Goldschmidt E.E.
S07O03	Alternate bearing in *Citrus reticulata* - An overview Verreynne J.S., Faber B.A., and Lovatt C.J.
S07O04	Relationship between a citrus *FLOWERING LOCUS T* expression and fruit bearing Nishikawa F., Iwasaki M., Fukamachi H., and Endo T.
S07O05	Managing drought stress of oranges under Sao Paulo-Mina Gerais, Brazil conditions to optimize flower bud induction and productivity Albrigo L.G., and Carrera R.R.
S07O06	Exploring microRNA-target-modulation in citrus somatic embryogenesis by high-throughput small RNA and degradome sequencing Wu X.M., Liu M.Y., Xu Q., Deng X.X., and Guo W.W.

Poster ID	Title
S07P01	Periods of normal and induced vegetative and reproductive shoots of Persian lime (*Citrus latifolia*) Medina-Urrutia V.M., Durán-Martínez C.M., Virgen-Calleros G., Robles-González M.M., and Rendon-Salcido L.A.
S07P02	Daily temperature amplitude affects the vegetative growth and carbon metabolism of orange trees in a rootstock-dependent manner Bueno A.C.R., Prudente D.A., Machado E.C., and Ribeiro R.V.
S07P03	The effects of paclobutrazol and fruit on flowering and carbohydrate accumulation in roots, branches and leaves of alternate bearing clementine mandarin Martínez-Fuentes A., Mesejo C., Muñoz-Fambuena N., Reig C., González-Mas M.C., Iglesias D.J., Primo-Millo E., and Agustí M.
S07P04	Fruit set in orange with phytohormones and its relation to endogeneous levels of GA_3 Galván J.J., Martínez A., López N., and Salazar O.
S07P05	Analysis of expressed proteins in the pollen tube occurring in the self-incompatible response using mass- or single-culture system of mature pollen in citrus Abe A., Uchida A., Hoshino Y., Sakakibara Y., Suiko M., and Kunitake H.
S07P06	Hormone complex and micronutrients in production and fruit quality of 'Valencia' sweet orange Galván J.J., López N., and Tolentino A.
S07P07	Effects of Triclopyr (3,5,6-trichloro-2-pyridyloxyactetic acid) applications on fruit quality of 'SRA63', 'SRA85', 'SRA88' and 'SRA92' clementine Zurru R., Deidda B., Dessena L., and Mulas M.
S07P08	Foliar application effect of the 3,5,6-TPA on 'Tarocco' red orange yield and fruit size in on years of Eastern Sicily orchard Tumminelli R., Marano G., Carta Cerella D., and Sinatra V.
S07P09	Fruit thinning of 'Okitsu' satsuma mandarin Rivadeneira M.F., Gómez C., and Silva Muller W.
S07P10	Transcript accumulation of flowering genes in response to water deficit and gibberellins during floral induction in *Citrus sinensis* Chica E.J., and Albrigo L.G.
S07P11	The effect of ethyclozate on tree water stress and fruit quality of satsuma mandarin Okuda H.O., Ichinokiyama H.I., and Noda K.N.
S07P12	Relationship between flower intensity, oxidative damage and protection in citrus under water stress conditions Manzi M., Borsani O., Díaz P., and Rivas F.
S07P13	The influence of climate on the components of yield and quality for Cuban citrus fruits. Adaptation to climate change Pérez M.C., Almenares G., Betancourt M., Aranguren M., García M.E., Sistachs V., Núñez M., Torres W., Oliva H., Noriega C., and Llauger R.E.

SESSION 08: Abiotic stress

Oral presentations at Auditorium 3, on Friday 23rd from 14:15 to 16:45
Poster session on Monday 19th

Conveners: Dr. Aurelio GÓMEZ CADENAS (Universitat Jaume I, Spain) and Dr. Ji-Hong LIU (Huazhong Agricultural University, China)

Oral ID	Title
S08O01	Polyamines function as a key player in stress tolerance: physiology and molecular aspects Liu J.-H.
S08O02	*Omics*' and chemical approaches used to monitor iron-deficiency in citrus rootstocks Licciardello C., Muccilli V., Torrisi B., Tononi P., Fontanini D., Allegra M., Sciacca F., Foti S., Delledonne M., Intrigliolo F., and Reforgiato Recupero G.
S08O03	Root protein interatomic network obtained from citrus seedlings subjected to water deficit Magalhães De Oliveira T., Silva F., Morillon R., Coelho Filho M.A., Neves D.M., Costa M.G.C., Pirovani C.P., and Bonatto D.
S08O04	Hormone and metabolite traits related to abiotic stress tolerance in citrus Arbona V., de Ollas C., Argamasilla R., López-Climent M.F., and Gómez-Cadenas A.
S08O05	The adaptation of 'Sunki Maravilha' mandarin to drought depends on the ABA accumulation balance between roots and canopy Neves D.M., Coelho Filho M.A., Bellete B.S., Silva M.F.G.F., Souza D.T., Soares Filho W.S., Costa M.G.C. , and Gesteira A.S.
S08O06	Roots are necessary for the responses of *in vitro*-cultured citrus plants to high salinity but not to osmotic stress Pérez Clemente R.M., Montoliu A., Vives-Peris V., Espinoza V., Zandalinas S.I., and Gómez-Cadenas A.
S08O07	Physiological analysis of salt stress behaviour of citrus species and genera: low chloride accumulation as an indicator of salt tolerance Hussain S., Luro F., Costantino G., Ollitrault P., and Morillon R.
S08O08	Microsprinkler irrigation for frost protection of citrus in Florida Parsons L.R.

Poster ID	Title
S08P01	Response of 'Washington' navel orange plants to application of Helpstar and Saltrad as saline correctors El-SHazly S.M., Eisa A.M., and Sarhan Z.H.
S08P02	Effects of salinity on some citrus rootstock Yeşiloğlu T., İncesu M., Yılmaz B., Tuzcu Ö., Uysal Kamiloğlu M., and Çimen B.
S08P03	Salt stress tolerance in acidic and sweet mandarins Ben Yahmed J., Ben Mimoun M., Talon M., Ollitrault P., and Morillon R.
S08P04	Changes in transcriptional profiles of mature and immature citrus leaves acclimated to salinity Pérez-Pérez J.G., Talón M., Brumós J., Botía P., and Colmenero J.M.
S08P05	Role of ammonium nutrition on salt-induced oxidative stress in 'Carrizo' citrange plants Fernández-Crespo E., Gómez-Pastor R., Matallana E., Llorens E., Lapeña L., Scalschi L., Camañes G., and García-Agustín P.
S08P06	Characterization of the Arum-type mycorrhiza in *Citrus macrophylla* rootstock under salinity stress Rodríguez Morán M., Navarro J.M., and Morte A.

Poster ID	Title
S08P07	*In vitro* screening of four genotypes of citrus for salt tolerance Chetto O., Dambier D., Fadli A., Talha T.A., Benkirane B., and Benyahia B.H.
S08P08	Screening of ten citrus rootstocks for salt tolerance at seedling stage Fadli A., Chetto O., Talha A., Beniken L., Benkirane R., And Benyahia H.
S08P09	Physiological response of *Citrus macrophylla* inoculated with arbuscular mycorrhizal fungi under salt stress Navarro J.M., Morte A., Rodríguez Morán M., and Pérez-Tornero O.
S08P10	Cloning and functional analysis of stress-responsive genes in *Poncirus trifoliata* Liu J.H., Wang J., Huang X.S., and Sun P.P.
S08P11	Characterization of water deficit tolerance of *Poncirus trifoliata* genotypes as related diversity Ben Yahmed J., Costantino G., Ben Mimoun M., Talon M., Ollitrault P., Morillon R., and Luro F.
S08P12	Screening nine citrumelo rootstocks for tolerance to drought conditions Fadli A., Beniken L., Omari F.E., Benkirane R., and Benyahia H.
S08P13	The effect of water stress on ABA, JA and physiological characteristic of citrus Xie S.X., Lu X.P., Nie Q., and Zhao X.L.
S08P14	Physiological responses of diploid and doubled diploid 'Rangpur' lime and 'Carrizo' citrange -under water deficit Magalhães de Oliveira T., Micheli F., Maserti E., Navarro L., Talon M., Ollitrault P., Gesteira A. S., and Morillon R.
S08P15	Flooding and soil temperature affect photosynthesis of citrus rootstock leaves Otero A., Goñi Altuna C.T., and Syvertsen J.P.
S08P16	Flooding affects fruitlet abscission in satsuma mandarin Goñi Altuna C.T., Otero A., and Syvertsen J.P.
S08P17	Frost damage in lemon orchards in the region of Murcia Conesa A., Martinez-Nicolas J., Manera J., and Porras I.
S08P18	Physiological investigation of tolerance to iron chlorosis of 'Navelina' orange budded on different citrus rootstocks Çimen B., Yeşiloğlu T., İncesu M., and Yılmaz B.
S08P19	Differential tolerance to iron deficiency of citrus rootstocks grown in calcareous soil İncesu M., Yeşiloğlu T., Tuzcu Ö., and Çimen B.
S08P20	Orange varieties as interstock in 'Verna' lemon trees increase the salt tolerance but not the drought or flooding tolerance Gimeno V., Simón I., Martínez V., Nieves M., Balal R.M., and Garcia-Sanchez F.
S08P21	Environmental effects on citrus growth and yield in the arid and semi-arid conditions of kingdom of Saudi Arabia Aljaleel A.A.
S08P22	Micromorphological studies on the relation between fruit development and creasing in orange (*Citrus sinensis* cv.'Moro' sanguine) Rajaei H.
S08P23	Behavior of diploid and tetraploid genotypes of 'Carrizo' citrange under abiotic stresses Ruiz M., Pina J. A., Alcayde E., Morillon R., Navarro L., and Primo-Millo E.
S08P24	Comparison of photosynthesis and antioxidant performance of several *Citrus* and *Fortunella* species under natural chilling stress Santini J., Giannettini J., Pailly O., Herbette S., Ollitrault P., Berti L., and Luro F.
S08P25	'Flying Dragon' as a very cold hardy citrus rootstock in Northern Iran Ebrahimi Y., Joshari H., Tavakoli A., Ghasemi S., and Rajabi A.

SESSION 09: Postharvest physiology and pathology

Oral presentations at Auditorium 3, on Thursday 22nd from 11:30 to 17:00.
Poster session on Tuesday 20th

Conveners: Dr. Lorenzo ZACARIAS (Instituto de Agroquímica y Tecnología de Alimentos del CSIC, Spain), Dr. Jacqueline J. BURNS (Citrus Research and Education Center, USA) and Dr. M.T. LAFUENTE (Instituto de Agroquímica y Tecnología de Alimentos del CSIC, Spain)

Oral ID	Title
S09O01	Genome sequence of the necrotrophic fungus *Penicillium digitatum*, the main postharvest pathogen of citrus Ballester A.R., Marcet-Houben M., de la Fuente B., Harries E., Marcos J.F., Gabaldón T., and González-Candelas L.
S09O02	RNA-Seq analysis of yeast antagonist *Metschnikowia fructicola* during interactions with *Penicillium digitatum* and grapefruit peel reveals specific transcriptional responses Hershkovitz V., Sela N., Taha L., Rafael G., BenDayan C., Feygenberg O., Aly R., Wisniewski M., and Droby S.
S09O03	The mitogen-activated protein kinase *PdSLT2* of *Penicillium digitatum* is required for fungal pathogenesis/virulence during citrus infection De Ramón-Carbonell M., and Sánchez-Torres P.
S09O04	Function of *PdCrz1*, a calcineurin-responsive transcription factor, in *Penicillium digitatum* in conidiation, virulence and responses to abiotic stresses Zhang T., and Li H.
S09O05	The protein O-Mannosyltransferase PMT2 of the citrus-specific postharvest pathogen *Penicillium digitatum* is involved in conidiogenesis, virulence and sensitivity to the antifungal peptide PAF26 Harries E., Gandía M., Carmona L., and Marcos J.F.
S09O06	*PdCYP51B*, a new sterol 14α-demethylase gene of *Penicillium digitatum* involved in resistance to imazalil and other fungicides inhibiting ergosterol synthesis Sun X.P., Wang J.Y., Feng D., and Li H.Y.
S09O07	Current and emerging strategies for Sour Rot management of citrus in Australia Taverner P.D., and Cunningham N.M.
S09O08	A new perspective in controlling postharvest citrus rots Fallanaj F., Sanzani S.M., and Ippolito A.
S09O09	Global solution for preserving citrus fruit using natural treatments Conesa E.
S09O10	Could ethylene influence Peteca Spot incidence of lemon fruit? Cronjé P.J.R.
S09O11	´Tahiti´ lime post-harvest evaluation and non-destructive assessment of essential oils by NIR spectroscopy Diaz-Candelas C.C., Morales-Payán J.P., and Romañach R.
S09O12	Postharvest blue light treatments affect citrus fruit susceptibility to disease by altering oxylipin biosynthesis Alferez F., Liao H.-L., and Burns J.K.
S09O13	Potassium sorbate increases citrus weight loss in postharvest treatments but it does not provide good decay control in wax Parra J.P.

Poster ID	Title
S09P01	Transcriptional regulation of the *Citrus sinensis* ABA-signalosome during fruit dehydration Romero P., Rodrigo M.J., and Lafuente M.T.

Poster ID	Title
S09P02	Delayed color break in 'Tardivo', a late ripening mandarin mutant, is related to a defective ethylene response Alos E., Distefano G., Rodrigo M.J., Gentile A., and Zacarias L.
S09P03	Comparative proteome and metabolome profiling analysis heat treatment induced fruit resistibility during postharvest storage Yun Z., Liu P., Liu S.Z., Luo T., Jin S., Gao H.J., Xu Q., Xu J., Cheng Y.J., and Deng X.X.
S09P04	Postharvest water stress leading to peel disorders in citrus fruit involves regulation of phospholipases by ABA Romero P., Gandia M., and Alférez F.
S09P05	Quality attributes of 'Valencia' orange (*Citrus sinensis*) fruit coated with different edible covers Mohammadi Shamloo M., Sharifani M. M., Daraei Garmakhany A., and Seifi E.
S09P06	Influence of maturity stage and intermittent warming on storage of 'Nagpur' mandarin fruit Ladaniya M.
S09P07	Effect of postharvest temperature on accumulation of primary metabolites in juice sacs of satsuma mandarin (*Citrus unshiu*) fruit Matsumoto H.M., and Ikoma Y.
S09P08	Stem End Rind Breakdown of citrus fruit a new postharvest physiological disorder of lemon fruit in Tucuman (Argentina) Torres Leal G.J., Velazquez P.D., Carbajo M.S., Farias M.F., and Pedraza J.R.
S09P09	The effects of some postharvest applications on fruit quality of 'Valencia-Late' oranges Dal B.
S09P10	Influence of light on carotenoid accumulation in 'Star Ruby' grapefruit Lado Lindner J., Lado Lindner J., Cronje P.J.R., Rodrigo M.J., and Zacarías L.
S09P11	Assessment of antioxidant compounds during storage period of 'Valencia' orange fruit Sharifani M.M., Mohamadi Shamlou M., Daraei A., and Seifie E.
S09P12	Postharvest ethylene treatment reduces quality loss of stored mature 'Navelate' sweet orange Lafuente M.T., Alférez F., and Romero P.
S09P13	Organoleptic quality and preservation of tangerine fruits during cold storage in relation to application of waxes for postharvest Nascimento L.M., Machado M.A., Brito M.C.R., Moreira J., Brito P.C.N., and Sanches J.M.
S09P14	Effect of storage temperature on pitting development in satsuma 'Okitsu' Nuñez F.N., del Campo R.d.C., Pascale D.P., Díaz L.D., Solari J.S., Larrechart L.L., and Mara H.M.
S09P15	RNA-seq and phenylpropanoid metabolism of orange-*Penicillium digitatum* interaction Ballester A.R., Marcet-Houben M., Gabaldón T., Lafuente M.T., and González-Candelas L.
S09P16	Disruption of the chitin synthase gene *PdigChsVII* in the citrus postharvest pathogen *Penicillium digitatum* Gandia M., Harries E., and Marcos J.F.
S09P17	Proteins contributing to the pathogenicity of *Penicillium digitatum* towards citrus fruit Yamashita Y.K., Arimoto Y.A., Makino M.H., Annaka A.H., and Iida I.A.
S09P18	Identification and expression analysis of *Penicillium digitatum* genes involved in fungal virulence during citrus fruit infection de Ramón-Carbonell M., Ballester A.R., González-Candelas L., and Sánchez-Torres P.
S09P19	Metabolism of flavonoids and mycotoxins in citrus fruits infected by *Alternaria alternata* Del Río J. A., Díaz L., Ortuño A., García-Lidón A., and Porras I.

Poster ID	Title
S09P20	Modulation of host responses by the biocontrol yeast *Metschnikowia fructicola* Hershkovitz V., Sela N., Rafael G., BenDayan C., Wisniewski M., and Droby S.
S09P21	Protein hydrolysates as resistance inducers for controlling Green Mould of citrus fruits Lachheb N., Sanzani S. M., Nigro F., Boselli M., and Ippolito A.
S09P22	Biochemical and transcriptomic changes associated with induced resistance in citrus fruit treated with sodium salts Youssef K., Sanzani S.M., Ligorio A., Fallanaj F., Nigro F., and Ippolito A.
S09P23	*In vitro* and *in vivo* antifungal activity of eight medicinal plants against Citrus Sour Rot agent *Geotrichum candidum* Talibi I., Askarne L., Boubaker H., Boudyach E.H., and Ait Ben Aoumar A.
S09P24	*In vitro* and *in vivo* antifungal activity of several Moroccan medicinal plants against *Penicillium italicum*, the causal agent of citrus Blue Mold Askarne L., Talibi I., Boubaker H., Boudyach E.H., Msanda F., Saadi B., Serghini M.A., and Ait Ben Aoumar A.
S09P25	Effects of fluorescent *Pseudomonas* and *Bacillus* sp. on Blue Mold of citrus caused by *Penicillium italicum* Askarne L., Talibi I., Boubaker H., Boudyach E.H., Msanda F., Saadi B., Serghini M.A., and Ait Ben Aoumar A.
S09P26	Practical impact of imazalil resistance on control of postharvest citrus Green and Blue Mould Erasmus A., Rikhotso V., Lesar K.H., Lennox C.L., and Fourie P.H.
S09P27	Effectiveness of the fungicide application system in the control of *Penicillium digitatum* in orange in Tucuman, Argentina Torres Leal G.J., Kamiya N., Carbajo M.S., Lacina M., and Farías M.F.
S09P28	Resistant strains to postharvest fungicides in Algarve citrus companies. Packinghouse sampling and *in vivo* assays Salazar M., Mendes S., and Nunes C.
S09P29	Influence of paraben concentration on the development of Green and Blue Molds on 'Valencia' orange fruit Moscoso-Ramírez P.A., Montesinos-Herrero C., and Palou L.
S09P30	Selection and *in vitro* evaluation of yeasts for control of *Geotrichum candidum* Cunha T., Tosin E.S., And Kupper K.C.
S09P31	*In vitro* and *in vivo* evaluation of yeast isolates for biocontrol of *Penicillium digitatum*, causal agent of Green Mold Kupper K.C., Cunha T., And Tosin E.S.
S09P32	Optimal concentration of inoculum, types of injuries and control of *Penicillium digitatum* in orange fruits González-Fierro P., Nieto-Ángel D., Téliz-Ortiz D., Lara-Viveros F.M., and Orozco-Santos M.
S09P33	*In vitro* comparison of the effectiveness of disinfectants and a fungicide on the viability of spores of *Geotrichum citri-aurantii*. Diaz L.D., del Campo R.d.C., Peyrou M.P., and Mara H.M.
S09P34	Alternative treatments for postharvest decay control on 'Ponkan' tangerines Montero C.R.S., dos Santos L.C., Andreazza C.S., Schwarz L.L., and Bender R.J.
S09P35	Curative and protective control of *Penicillium digitatum* following imazalil application in aqueous dip and wax coating Njombolwana N.S., Erasmus A., and Fourie P.H.
S09P36	Fruit quality and storage potential of 'Kinnow' mandarin (*Citrus reticulata*) in relation to different geographical locations of Pakistan Khalid M.S., Malik A.U., Amin M., Khalid S., Asad H.U., Raza S.A., and Malik O.H.

Poster ID	Title
S09P37	Effect of organic and inorganic salts as alternative strategy for the control of the postharvest Citrus Sour Rot agent *Geotrichum candidum* Talibi I., Askarne L., Boubaker H., Boudyach E.H., and Ait Ben Aoumar A.
S09P38	Lemon fruits from endochitinase transgenic plants exhibit resistance against postharvest fungal pathogens Oliveri C., Distefano G., La Malfa S., La Rosa R., Deng Z., and Gentile A.

SESSION 10: Watering and nutrition

Oral presentations at Auditorium 3, on Friday 23rd from 10:30 to 13:00.
Poster session on Thursday 22nd

Conveners: Dr. Diego INTRIGLIOLO (Instituto Valenciano Investigaciones Agrarias, Spain) and Dr. Dirceu DE MATTOS Jr. (Centro de Citricultura Sylvio Moreira, Brazil)

Oral ID	Title
S10O01	Open hydroponics of citrus compared to conventional drip irrigation best practice: first three years of trialling and Australian experience Falivene S., Navarro Acosta J.M., and Connolly K.
S10O02	Deficit irrigation strategies: preliminary assessment on a Sicilian young orange orchard Stagno F., Parisi R., Cirelli G., Consoli S., Roccuzzo G., Barbagallo S., and Intrigliolo F.
S10O03	Citrus water use in South Africa Vahrmeijer J.T., Annandale J.G., Gush M.B., and Taylor N.J.
S10O04	Evapotranspiration over an irrigated orange orchard using micrometeorological techniques and sap flow measurements Consoli S., Continella A., Motisi A., Papa R., and Pasotti L.
S10O05	Partial root-zone drying effects under different rootstocks and irrigation systems in Valencia, Spain Ortega J., Forner-Giner M.A., Quiñones A., Legaz F., and Intrigliolo D.S.
S10O06	A critical evaluation of citrus leaf mineral status guidelines for optimal yield in Israel Raveh E.
S10O07	What limits nitrogen fertilization responses of fertigated citrus orchards under tropical conditions? Mattos Jr. D., Quaggio J.A., Boaretto R.M., Souza T.R., and Zambrosi F.C.B.
S10O08	Uptake of ^{44}Ca and ^{15}N by young citrus trees Quiñones A., Martínez-Alcántara B., Alcayde E., and Legaz F.
S10O09	A new approach to front citrus iron chlorosis: organo-mineral fertilizers from glass-matrix and organic biomasses Torrisi B., Trinchera A., Allegra M., Epifani R., Marcucci A., Roccuzzo G., Intrigliolo F., and Rea E.
S10O10	Nitrogen and calcium equilibrium on citrus nutrition Quaggio J.A., Souza T.R., Boaretto R.M., Zambrosi F.C.B., and Mattos Jr. D.

Poster ID	Title
S10P01	Physiological and nutritional responses of navel orange trees to different irrigation and fertigation practices Navarro J.M., and Falivene S.
S10P02	Influence of precipitation in the fruit growing season on sugar accumulation in satsuma mandarin fruit juice Takishita F., Uchida M., Hiraoka K., and Nesumi H.
S10P03	Recover of plugged drippers in orange grove irrigation system using mechanical and chemical techniques Fachini E., Junqueira Franco A.C.B., and Galbiatti J.A.
S10P04	Effect of Sunred metabolic promoter and deficit irrigation on fruit quality of Valencia oranges Massenti R., Barone F., Farina V., and Lo Bianco R.
S10P05	Foliar and root application of potassium nitrate and calcium nitrate to *Citrus macrophylla* seedlings under NaCl stress Navarro J.M., Andujar S., and Rodríguez Morán M.
S10P06	Effect of mulching and drip irrigation on satsuma mandarin fruit quality in a volcanic ash soil Han S.G., Kim Y.H., Moon Y.E., and Kang S.B.
S10P07	Tree-to-tree variation in plant-based measurements as indicators of orange water status Motisi A., Continella A., Massenti R., and Romolo F.
S10P08	Predicting the impact of the climatic variability in the necessities of watering of the grapefruit in the Island of the Youth in Cuba Cueto J.C., Pardo A.P., and Durán G.D.
S10P09	Effect of regulated deficit irrigation on vegetative growth, fruiting, stomatal conductance, leaf proline content and water use efficiency for Nules clementine grown in the Souss valley of Morocco Bagayogo S., El-Otmani M., El-Fadl A., and Benismail M.C.
S10P10	Response of Star Ruby grapefruit trees to deficit irrigation during different stages of fruit growth: effects on water relations, yield and fruit quality Pérez-Pérez J.G., Robles J.M., García-Oller M.I., and Botía P.
S10P11	Water relations and productivity of Natal orange plants under controlled deficit irrigation in São Paulo State, Brazil Pires R.C.M., Silva A.L.B.O., Ribeiro R.V., Quaggio J.A., Lima M.A.F., and Zani M.L.
S10P12	Responses of Nules clementine trees to deficit irrigation under different rootstocks. Tree performance and fruit quality Ortega J., Molina M.D., Intrigliolo D.S., De Miguel A., Bonafé C., Valero J.L., Peiró E., and Forner-Giner M.A.
S10P13	Root distribution of orange trees under two different drip irrigation systems Fachini E., and Galbiatti J.A.
S10P14	The effect of herbicide treatments on soil nutrient availability and plant nutritional status in a citrus garden Wu C.Q., Liao C.X., Ou Y., and Xie Y.H.
S10P15	Efficiency of zinc (68Zn) fertilizers applied to the soil with citrus Boaretto R.M., Hippler F.W.R., Quaggio J.A., Boaretto A.E., Abreu Jr. C.H., and Mattos Jr. D.
S10P16	Interaction of magnesium in calcium uptake in young citrus trees Martínez-Alcántara B., Quiñones A., Alcayde E., and Legaz F.
S10P17	Study on the relationship between fruit quality, soil nutrient availability and tree nutritional status Lu X.P., Huang C.N., Xiao Y.M., Nie Q., Zhao X.L., and Xie S.X

Poster ID	Title
S10P18	Boron supply affects growth of citrus and correlates with hydraulic conductivity and gas exchange of rootstock varieties Mesquita G.L., Mattos Jr. D., Ribeiro R.V., Boaretto R.M., and Quaggio J.A.
S10P19	Silicon uptake in citrus and the validation of an analytical method Vahrmeijer J.T., Asanzi N.M., and Taylor N.J.
S10P20	Phosphorus deficiency decreases nutrient use efficiency of citrus Zambrosi F.C.B., Mattos Jr. D., Quaggio J.A., and Boaretto R.M.
S10P21	Enhancement yield, fruit quality and nutritional status of 'Washington' navel orange trees by application of some biostimulants El-Shazly S.M., and Mustafa N.S.
S10P22	Calcium, magnesium and potassium levels in the soil and in the leaf of orange trees on different rootstocks and soil conservation systems in the northwest of Paraná State, Brazil Gil L. G., Auler P. A. M., and Pavan M.A.
S10P23	Response of clementine citrus to foliar potassium fertilization: effects on fruit production and quality Hamza A., El Guilli M., Bamouh A., Zouahri A., Bouabid R., and Lfadili R.
S10P24	Impact of biostimulant substances on growth of 'Washington' navel orange trees Mustafa N.S., and El-Shazly S. M.
S10P25	Foliar nutrition with macronutrients for 'Valencia' orange and 'Murcott' tangor trees Rodríguez V.A., Alayón Luaces P., Píccoli A.B., Chabbal M.D., Giménez L.I., and Martínez G.C.
S10P26	Effects of nutritional trunk injections on sweet orange production Martínez G.C., Alayón Luaces P., Yfran Elvira M.M., Chabbal M.D., Mazza S.M., and Rodríguez V.A.
S10P27	Influence of reduced rate of application of phosphorus and potassium fertilizers on soil and fruit quality in a Satsuma mandarin orchard Yoshikawa K.Y., Nakamura A.N., Baba A.B., and Kusaba S.K.
S10P28	Potassium leaching from young lysimeter-grown orange trees Boman B. J., and Battikhi A.M.
S10P29	Effect of organic fertilization on soil organic matter and on root apparatus of citrus trees Trinchera A., Torrisi B., Allegra M., Rinaldi S., Rea E., Intrigliolo F., and Roccuzzo G.
S10P30	Use of plant-soil-atmosphere sensors on a young clementine mandarin orchard under regulated deficit irrigation to monitor changes in water relations for better irrigation scheduling El-Otmani M., El-Fadl A., and Benismail M.C.

SESSION 11: Cultural practices and mechanization

Oral presentations at Auditorium 2, on Tuesday 20th from 16:15 to 18:15.
Poster session on Monday 19th

Conveners: Dr. Enrique MOLTÓ (Instituto Valenciano Investigaciones Agrarias, Spain) and Dr. Mohamed EL-OTMANI (Complexe Horticole d'Agadir, Morocco)

Oral ID	Title
S11O01	New method of citrus grafting by the use of laboratory tape (top budding) Kitamura M., Caisley J., Sakaki H., Fujita K., and Takahara T.
S11O02	Performance of citrus nursery trees and two-year older trees after transplantation in the field Zekri M.

Oral ID	Title
S11O03	Effect of inarched, two-rootstock trees on development and yield of 'Valencia' orange orchards De Lima J. E. O., and de Lima J. E. P.
S11O04	Morphological and yield characteristics of 'Washington' navel orange and 'Tahiti' lime trees produced with buds from floral *versus* vegetative mother shoots Lovatt C.J., and Krueger R.R.
S11O05	Effect of different pruning severities on incidence of creasing and fruit quality in sweet orange Hussain Z., Singh Z., and Mandel R.
S11O06	Reduction of fruit splitting and the incidence of creasing of 'Nova' mandarin and 'Washington' navel orange by NAA sprays Greenberg J., Kaplan I., Tagari E., Fainzack M., Egozi Y., and Giladi B.
S11O07	On-farm citrus trials in Australia: Effective extension for commercial assessment Falivene S., Connolly K., and Cannard M.
S11O08	Evolution of citrus mechanical harvesting in Florida – Lessons for the future Roka F.M.

Poster ID	Title
S11P01	Study on mushroom dregs application in citrus nursery Guan B., Chen K.L., He J., and Liu J.J.
S11P02	Effect of soil preparation systems, mulching and rootstocks on 'Pera' orange (*Citrus sinensis*) performance in the northwest of Parana State, Brazil Auler P.A.M., Fidalski J., Gil L.G., and Pavan M.A.
S11P03	Evaluation of rootstocks for the 'Tahiti' lime in the municipality of Jaíba, Brazil Alves R.R., Machado D.L.M., Salomão L.C.C., Siqueira D.L., and Silva S.D.R.
S11P04	Development and production of 'Tahiti' lime IAC-5 grafted on 'Flying Dragon' (*Poncirus trifoliata* var. *monstrosa*), grown in high planting densities Machado D.L.M., Alves R.R., Siqueira D.L., Salomão L.C.C., and Silva S.D.R.
S11P05	Effect of shade screen on production, fruit quality and growth parameters in 'Fino 49' lemon trees grafted on *Citrus macrophylla* and sour orange Gimeno V., Simón I., Martínez V., Lidón V., Shahid M.A., and García-Sánchez F.
S11P06	Effect of liquid fertilizer applied by injection into the soil on quality of 'Tarocco' blood orange He J., Chen K.L., Guan B., and Liu J.J.
S11P07	Effect of branch girdling and ringing on carbohydrates, chlorophyll fluorescence and SPAD index in leaves of 'Tahiti' acid lime Santos D., Siqueira D.L., Salomão L.C.C., and Cecon P.R.
S11P08	Production of 'Tahiti' acid lime after branch girdling or ringing Santos D., Siqueira D.L., Salomao L.C.C., and Cecon P.R.
S11P09	Horticultural package for high quality 'Tahiti' lime production Mourão Filho F.A.A., Stuchi E.S., Cantuarias-Avilés T., Espinoza-Núñez E., Bremer Neto H., Bassan M.M., and Silva S.R.
S11P10	Field evaluation of two canopy shake systems, OXBO 3210 and OXBO 3220, on citrus orchards in Andalusia (Spain) Arenas Arenas F.J., Salguero A., and Hervalejo A.
S11P11	Production seasonality of 'Tahiti' lime in three consecutive harvests under semiarid climatic conditions Maia V.M., Álvarez J.M., Oliveira F.S., Gomes F.H.C., Azpiazú I., and Salles B.P.A.

Poster ID	Title
S11P12	Enhancement of citrus value chain production in Pakistan and Australia under the AusAid Program Khurshid T., Ahmad I., Jaskani M.J., Nabi G., Tahir T., Ali W., Rahman A., Khan M.A., and Rahman H.
S11P13	Technology and production costs for organic versus conventional 'Navelina' citrus orchards Reis D., Marreiros A., Fernandes M.M., Mourão I., and Duarte A.
S11P14	Prediction of moving northward of favorable regions to cultivate subtropical citrus by estimation of changes in annual mean and minimum air temperature in Japan Sugiura T., Sakamoto D., Sugiura H., Koshita Y., and Asakura T.
S11P15	INTA´s Citrus Improvement Program Costa N., Plata M.I., and Anderson C.
S11P16	Labor-saving practices for 'Shatangju' mandarins in China Ma P.Q., and Wu W.
S11P17	FruTIC: An information and communication tool for an integrated health management of citrus groves Garrán S.M., Stablum A., Franco S., Ibarrola S., Mika R., Milera S., and Marnetto S.
S11P18	A phytosanitary evaluation method (MEF) for commercial citrus groves Garrán S.M., Mika R., Boca R.T., Beribe M.J., and Pérez A.
S11P19	Micro budding of commercial citrus cultivars of India Vijayakumari N.
S11P20	Evaluation of organic growing media and bio-control agents in the production of certified citrus rootstocks Yaseen T., Dongiovanni C., Roccuzzo G., Ippolito A., and D´Onghia A.M.
S11P21	The diversification of agriculture in the new Cuban citriculture Cueto J.C., Piñero J.P., Ortega I.O., and Torres L.T.
S11P22	Presence of weed biotypes with suspected resistance to glyphosate in agroecosystem citrus orchards in Cuba: a latent threat Otero L., and De Pardo R.

SESSION 12: Citrus HLB and other bacterial diseases

Oral presentations at Auditorium 1, on Thursday 22nd from 11:30 to 17:00.
Poster session on Tuesday 20th

Conveners: Dr. María M. LÓPEZ (Instituto Valenciano Investigaciones Agrarias, Spain), Dr. Olivier PRUVOST (Centre de cooperation Internationale en Recherche Agronomique pour le Développement, France) and Dr. Yongping DUAN (U. S. Department of Agriculture - Agricultural Research Service, USA).

Oral ID	Title
S12O01	New insights into the Citrus Huanglongbing complex and potential solutions to this devastating disease Duan Y.P., Zhou L.J., Zhang M.Q., Vahling-Armstrong C., Hao G.X., Zou H.S., and Hoffman M.
S12O02	Tissue-print and squash real-time PCR for direct detection of *Candidatus* Liberibacter spp. in citrus plants and insect vectors Bertolini E., Lopes S., Felipe R.T.A., Teresani G., Mourão F.A.A., Colomer M., Bové J.M., López M.M., and Cambra M.
S12O03	A new insect vector of *Candidatus* Liberibacter asiaticus, *Cacopsylla* (*Psylla*) *citrisuga* (Hemiptera: Psyllidae) Cen Y., Gao J., Deng X., Xia Y., Chen J., Zhang L., Guo J., Gao W., Zhou W., and Wang Z.

Oral ID	Title
S12O04	On the identity of orange jasmine and its relevance to Huanglongbing and *Diaphorina citri* Holford P., Nguyen C.H., Beattie G.A.C., Haigh A.M., Hasick N.J., Mabberley D.J., Weston P.H.
S12O05	Effect of HLB on the expression of calcium signals related genes Parra C.C., Kunta M., and Louzada E.S.
S12O06	Transcriptional genomics and proteomics in citrus roots infected by *Candidatus* Liberibacter asiaticus Zhong Y.
S12O07	Resistance and tolerance to Huanglongbing in *Citrus* Stover E., McCollum G., Driggers R., Duan Y.P., Shatters Jr. R., and Ritenour M.
S12O08	A comparison of different methods to evaluate host resistance or tolerance to Huanglongbing, caused by *Candidatus* Liberibacter asiaticus Bowman K.D., and Albrecht U.
S12O09	Evaluation of transgenic citrus for disease resistance to HLB and Canker Dutt M., Barthe G.A., Orbovic V., Irey M., and Grosser J.W.
S12O10	California's response to the first detection of HLB Luque-Williams M.J.
S12O11	Comparative study of different host range strains of *Xanthomonas citri* subsp. *citri*: Chemotaxis and biofilm formation Sena M., Ferragud E., Redondo C., Johnson E.G., Graham J.H., Girón J.A., and Cubero J.
S12O12	A new minisatellite-based scheme for the global surveillance of *Xanthomonas citri* subsp.-*citri*, the causal agent of Asiatic Citrus Canker Magne M., Leduc C., Vernière C., Vital K, Gordon J., Escalon A., Guérin F., Gagnevin L., and Pruvost O.
S12O13	Analysis of microRNAome of Chinese citron 'C-05' resistant to Citrus Canker disease Dai S.M., Chen Y.M., Li D.Z., Li F., Yan J.W., and Deng Z.N.
S12O14	The role of auxin in the citrus defense to early infection by *Xylella fastidiosa* Rodrigues C.M., Souza A.A., Takita M.A., and Machado M.A.

Poster ID	Title
S12P01	Yield loss modeling of *Candidatus* Liberibacter asiaticus on Persian lime (*Citrus latifolia*) in southern Mexico Flores Sanchez J.L., Mora Aguilera G., Loeza Kuk E., Domínguez Monge S., Acevedo Sánchez G., and López Arroyo J.I.
S12P02	Seasonal detection of Huanglongbing symptomatic trees and *Diaphorina citri* in citrus groves in Sao Paulo State, Brazil Bassanezi R.B.
S12P03	Identification of *Candidatus* Liberibacter and phytoplasma and evaluation of their association with Citrus Huanglongbing in China Su H.N., Wang X.F., Tang K.Z., Li Z.A., and Zhou C.Y.
S12P04	Rapid on site detection of the HLB/Citrus Greening causal agent *Candidatus* Liberibacter asiaticus by AmplifyRP™, a novel rapid isothermal nucleic acid amplification platform Russel P., Amato M.A., and Bohannon R.
S12P05	Diversity of *Candidatus Liberibacter asiaticus, Candidatus* Liberibacter africanus and *Candidatus Liberibacter americanus* based on 23S/5S rDNA sequences Liao H., Bai X., Li Y., Chen C., Yang L., Xu N., Huang H., and Wang X.
S12P06	Phloem anatomy of citrus trees: healthy vs. Greening Etxeberria E., and Narciso C.

Poster ID	Title
S12P07	Anatomical comparison of HLB-affected sweet orange and rough lemon Fan J., Chen C., Gmitter Jr. F.G., Achor D.S., Brlansky R.H., and Li Z.G.
S12P08	The significance of early root infection in Huanglongbing disease development and crop loss Johnson E.G., Bright D.B., and Graham J.H.
S12P09	Monitoring the *Candidatus* Liberibacter asiaticus in a commercial orchard of sweet orange and Tahiti lime in the northwest of Paraná State Sauer A.V., Nocchi P.T.R., Zanutto C.A., Rinald D.A.M.F., and Nunes W.M.C.
S12P10	Multiplication of *Candidatus* Liberibacter asiaticus in *Citrus sinensis, Citrus sunki, Poncirus trifoliata* and hybrids obtained from crosses between *Citrus sunki* and *Poncirus trifoliata* Boava L.P., Cristofani-Yaly M., Coletta Filho H.D., Bastianel M., and Machado M.A.
S12P11	Low titers of *Candidatus* Liberibacter asiaticus in citrus plants exposed to high temperatures result in low acquisition rates of the bacterium by the insect vector *Diaphorina citri* Lopes S.A., Luiz F.Q.B.Q., Martins E.C., Fassini C.G., and Sousa M.C.
S12P12	Pre-inoculacion with virus and citrus viroids in *Candidatus* Liberibacter infection and HLB symptoms Carvalho S.A., Silva L.F.C., Souza M.C.S., Francisco, C.S., and Coletta-Filho H.D.
S12P13	The efficient transmission of *Candidatus* Liberibacter americanus from periwinkle (*Catharanthus roseus*) to different citrus genotypes by dodder Francisco C.S., Locali E.C., Machado M.A., and Coletta-Filho H.D.
S12P14	Population dynamics of *Diaphorina citri* Kuwayama (hemiptera: psyllidae) in orchards of valencia sweet orange, Ponkan mandarin and Murcott Tangor Trees Beloti V.H., Felippe M.R., Rugno G.R., Carmo-Uehara A. do, Garbim L.F., Godoy W.A.C., and Yamamoto P.T.
S12P15	Systemic insecticides are effective in preventing the transmission of *Candidatus Liberibacter asiaticus* by *Diaphorina citri* Kuwayama? Yamamoto P.T., Miranda M.P de, and Felippe M.R.
S12P16	Vector control and foliar nutrition for management of huanglongbing in Florida citrus Stansly P.A., Arevalo H.A., Qureshi J.A., Jones M., Hendricks K., Roberts P.D., and Roka F.M.
S12P17	Foliar spray of nutrients and growth regulators on the management of citrus affected by HLB – Is it possible? Medina C.L., Bataglia O.C., Furlani P.R., Machado R.S., and Creste A.
S12P18	Antibiotic therapy of citrus bud wood for the management of Huanglongbing disease Yaqub M.S., and Khan I.A.
S12P19	Reduction of Huanglongbing in the project of appropriate techniques expansion for the cultivation of king mandarin (Citrus nobilis) in Southern Vietnam Yuasa K.Y., Nguyen V.H.N., and Ichinose K.I.
S12P20	Heat treatment of Huanglongbing–affected citrus trees in field for reduction of *Candidatus* Liberibacter asiaticus Deng X.L., Guan L. , Liang M.D. , Xu M. R., Xia Y. L. , Sequeira R. , and Chen J.C.
S12P21	Gas exchanged and water relations of 'Valencia' oranges trees infected with Huanglongbing (HLB, ex greening) Saccini V.A.V., Medina C.L., Santos D.M.M., Machado R.S., and Silva J.
S12P22	Assessing plant health risk in relation to *Xanthomonas citri* strains causing citrus bacterial canker and evaluating measures for managing this risk Holeva M., Olivier V., Hostachy B., Smith J., MacLeod A., and Pruvost O.

Poster ID	Title
S12P23	Characterization of a new *Xanthomonas citri* subsp. *citri* isolate which triggers a host-specific response Roeschlin R.A., Chiesa M.A., Enrique R., Favaro M.A., Torres P.S., Filippone M.P., Gmitter Jr. F.G., Vojnov A.A., Castagnaro A.P., Marano M.R., and Gadea J.
S12P24	Analysis of genetic diversity of *Xanthomonas citri* subsp *citri* strains. Characterization of a new isolate which triggers a host-specific response Siciliano M.F., Chiesa M.A., Ornella L., Pino Delgado N., Favaro M.A., Sendín L.N., Roeschlin R.A., Orce I.G., Ploper L.D., Vojnov A.A., Filippone M.P., Castagnaro A.P., and Marano M.R.
S12P25	The causal agent of Citrus Canker, *Xanthomonas citri* subsp. *citri*, enters in a reversible viable but non culturable state induced by copper, that can be reverted by citrus leaf extract Golmohammadi M.G., Llop P-Ll., Cubero J.C., and Lopez M.M.L.
S12P26	Effect of N-acetyl-L-cysteine (NAC) on the epiphytic fitness of *Xanthomonas citri* subsp. *citri* (Xcc) in sweet orange Picchi S.C., Inui-Kishi R.N., Takita M.A., Machado M.A., and De Souza A.A.
S12P27	Disruption of ParB alters the virulence of *Xanthomonas citri* subsp. *citri* Ucci A.P.U., and Ferreira H.F.
S12P28	Analysis of microRNAs involved in defense response signaling against *Xanthomonas* spp. in *Citrus limon* Chiesa M.A., Torres P.S., Campos L., Roeschlin R.A., Gerhardt N., Filippone M.P., Conejero V., Castagnaro A.P., Vojnov A.A., Gmitter Jr. F.G., Gadea J., and Marano M.R.
S12P29	Influence of different copper hydroxide spray volumes and doses to control Citrus Canker in lemon in Tucumán, Argentina Salas López H., Mansilla C., Stein B., Rojas A., and Figueroa D.
S12P30	Control of Citrus Canker (*Xanthomonas citri subsp. citri*) mediated by neonicotinoids in combination with acibenzolar-S-methyl and copper Barreto T.P., Pozzan M., and Leite R.
S12P31	Activity of antimicrobial peptides (AMPs) against *Xanthomonas citri* subsp. *citri* (Xcc) in *Citrus sinensis* 'Baía' Inui-Kishi R.N., Freitas-Astua J., Picchi S.C., Brand G.D., Bloch Jr C., Falcao A.O., Stach-Machado D., Cilli E.M., and Machado M.A.
S12P32	Bacteriostatic and bactericidal activity of different essential oils to alternative control in vitro of *Xanthomonas citri* subsp. *citri* Sauer A.V., Santos E.M., Zuliani-Gonçalves A.M.O., Nocchi P.T.R., Nunes W.M.C., and Bonato C.M.
S12P33	Canker control by the siderophore Pyochelin from *Pseudomonas fluorescens* Adler C., Lami MJ., de Cristobal RE., Filippone MP., Castagnaro AP., and Vincent P.A.
S12P34	Genetic response of sweet orange treated with chitosan to the infection of *Xylella fastidiosa* Coqueiro D.S.O., Rodrigues C.M., Souza A.A., Takita M.A., Kishi L.T., and Machado M.A.
S12P35	Genetic variation of California *Spiroplasma citri* populations revealed by two genetic loci Wang X., Doddapaneni H., Chen J. C., and Yokomi R. K.
S12P36	Serological detection of *Spiroplasma citri* using a bacterial secreted protein as the detection marker Shi J.S., Pagliaccia P.D., Morgan M.R., Ma W.M., and Vidalakis G.
S12P37	Witches Broom Disease of Lime, a serious disease of acid lime (*Citrus aurantifolia*) in the Arabian Peninsula Al-Sadi A.M., AL-Yahyai R.A., and Al-Said F.A.
S12P38	Portable chemical sensors for monitoring infection-specific volatiles in asymptomatic citrus Fink R.L., Aksenov A.A., Thuesen L.H., Pasamontes A., Cheung W.H.K., Peirano D.J., and Davis C.E.

Poster ID	Title
S12P39	Unforbidden fruits: preventing citrus smuggling by introducing varieties culturally significant to ethnic communities Karp D., Siebert T., Vidalakis G., Krueger R.R., Lee R.F., and Kahn T.
S12P40	Introduction of control on main citrus disease in P.R. China Zhou C.Y.
S12P41	Gaining experience with exotic citrus pest and disease threats to Australia through collaborative work in Bhutan Sanderson G., Hardy S., Donovan N., Beattie A., Holford P., Dorjee D., Wangdi P., Thinlay T., and Om N.
S12P42	Nucleotide sequence analysis of ribosomal protein gene sub-units (*rplA-rplJ*) of *Candidatus* Liberibacter asiaticus infecting different citrus cultivars in Maharastra, India Ghosh D.K., Bhose Sumit, and Gowda S.
S12P43	Analysis of Citrus Huanglonbing spatial dynamics in non-intervened groves of Cuba by Spatial Analysis by Distance Indices (SADIE) Batista L., Peña I., Luis M., Acosta I., Hernández L., Llauger R., Casín J.C., and Laranjeira F.F.
S12P44	Identification of tolerance in citrus germplasm for Huanglongbing Yaqub M.S., and Khan I.A.

SESSION 13: Fruit flies

Oral presentations at Auditorium 1, on Tuesday 20th from 16:15 to 18:15.
Poster session on Monday 19th

Conveners: Dr. Beatriz SABATER-MUÑOZ (Instituto Valenciano Investigaciones Agrarias, Spain) and Dr. Jorge HENDRICHS (International Atomic Energy Agency – Food and Agriculture Organization, Austria)

Oral ID	Title
S13O01	Application of the Sterile Insect Technique: an effective biological control method against fruit fly pests and its contribution to food security, the environment and trade Hendrichs J., Pereira R., and Reyes J.
S13O02	Assessing the effectiveness of sterile males in Mediterranean fruit fly population reduction by molecular techniques Juan-Blasco M., Sabater-Muñoz B., Argiles R., Jacas J.A., Castañera P., and Urbaneja A.
S13O03	Multiple insecticide resistance traits in a field derived population of the Mediterranean fruit fly, *Ceratitis capitata* Ortego F., Couso-Ferrer F., Arouri R., Huertas-Rosales O., Beroiz B., Perera N., Cervera A., Hernández-Crespo P., and Castañera P.
S13O04	Citrus fruits and the Mediterranean fruit fly Papadopoulos N.T., Papachristos D.P., and Ioannou C.S.
S13O05	Looking inside the chemosensory system of the medfly *Ceratitis capitata* Malacrida A.R., Gomulski L.M., Scolari F., Falchetto M., Siciliano P., Manni M., and Gasperi G.
S13O06	Field infestation and suppression of the invasive fruit fly *Bactrocera invadens* on citrus in Kenya Ekesi S
S13O07	Quarantine mitigation for Tephritid fruit fly pests in citrus Liquido N. J., and Griffin R. L.

Poster ID	Title
S13P01	The transcriptome and protein baits of *Bactrocera dorsalis* (Diptera: Tephritidae) Zheng W., Zhang W., and Zhang H.
S13P02	The potential benefits of using engineered Medfly to improve the efficacy and to reduce the cost of the Sterile Insect Technique: RIDL strain OX3864A Slade G., Koukidou M., Leftwich PT., Rempoulakis P., Economopoulos A., Vontas J., and Alphey L.
S13P03	An early step toward the development of a method to predict Mediterranean fruit fly adult emergence under different soil moisture and temperature regimes Garrido-Jurado I., Valverde-García P., and Quesada-Moraga E.
S13P04	Fruit flies in orange plantations of TicoFrut - Costa Rica Camacho H.
S13P05	Role of phytosanitary surveillance of *Anastrepha* spp fruit flies (Diptera: Tephritidae) in the context of the citrus industry of Cuba Borges Soto M., Beltrán Castillo A., Avalos Rodriguez Y., Hernandez D., Sabater-Muñoz B., and Rodriguez Rubial M.
S13P06	DECIS TRAP MedFly®: innovative platform technology for the sustainable management of the Mediterranean fruit fly (*Ceratitis capitata*) Wirtz K., Ramos E., and Fullana J.
S13P07	Potential of secondary metabolites secreted by the entomopathogenic mitosporic ascomycetes *Beauveria* sp. and *Metarhizium* spp. for medfly *Ceratitis capitata* adult control Lozano-Tovar M.D., Garrido-Jurado I., and Quesada-Moraga E.
S13P08	Comparison of different food attractants for fruit fly capture (Diptera: Tephritidae) in citrus orchards in the state of São Paulo, Brazil Rodrigues M.D.A., Raga A., Maldonado Jr. W., and Barbosa J.C.
S13P09	Evaluation of protein bait laced with various insecticides on the Queensland Fruit Fly (Diptera: Tephritidae): Attraction, feeding, mortality and bait persistence Mahat K., and Drew R.A.I
S13P10	Effectiveness evaluation of two attractants, CeraTrap® and Tripack®, for the mass trapping of *Ceratitis capitata* (Diptera: Tephritidae) in citrus orchards in different regions of Tunisia Hafsi A., Rahmouni R., Ben Jannet M., Harbi A., and Chermiti B.
S13P11	Field releases of the larval parasitoid *Diachasmimorpha longicaudata* in Spain: first results on dispersal pattern Harbi A., Beitia F., Tur C., Chermiti B., Verdú M.J., and Sabater-Muñoz B.
S13P12	Magnet® MED: A new long-life, ready-to-use Attract and kill system to control Mediterranean fruit fly, *Ceratitis capitata* Wiedemann Colás C., Dominguez M., Marti S., and Alfaro C.
S13P13	Assessment of the efficacy of lure and kill devices for the control of Mediterranean fruit fly in citrus orchards Navarro-Llopis, V., Primo Millo, J., Vacas, S.
S13P14	Evaluation of mass trapping technique for control of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) in citrus orchards of Northern Iran Mafi Pashakolaei S.
S13P15	The seasonal population dynamics of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) and fruit damage on horticultural crops in Northern Iran Mafi Pashakolaei S.

SESSION 14: Virus and virus like diseases

Oral presentations at Room 4, on Tuesday 20th from 10:30 to 13:30

Poster session on Monday 19th

Conveners: Dr. Pedro MORENO (Instituto Valenciano Investigaciones Agrarias, Spain) and Dr. William O. DAWSON (Citrus Research and Education Center, USA).

Oral ID	Title
S14O01	Recent developments on *Citrus tristeza virus* research Folimonova S.
S14O02	Large scale survey of *Citrus tristeza virus* (CTV) and its aphid vectors in Morocco Afechtal M., Djelouah K., Cocuzza G., and D'Onghia A.M.
S14O03	Capillary electrophoresis-single–strand conformation polymorphisms and multiple molecular marker genotyping allow a rapid differentiation of CTV isolates Licciardello G., Russo M., Daden M., Bar-Joseph M., and Catara A.
S14O04	Genetic and biological stability of *Citrus tristeza virus* (CTV) infecting the non-natural host *Nicotiana benthamiana* Navarro-López J., Ruiz-Ruiz S., Moreno P., and Ambrós S.
S14O05	*Citrus tristeza virus* enhances the titer of *Citrus dwarfing viroid* in co-infected 'Mexican' lime plants through p23 expression Serra P., Bani Hashemian S.M., Fagoaga C., Romero J., Bertolini E., Ruiz-Ruiz S., Gorris M.T., and Durán-Vila N.
S14O06	The p23 protein encoded by *Citrus tristeza virus:* fine dissection of its determinants for nucleolar localization and for suppression of RNA silencing and pathogenesis Ruiz Ruiz S., Soler N., Sánchez-Navarro J., Fagoaga C., López C., Navarro L., Moreno P., Peña L., and Flores R.
S14O07	Viruliferous *Brevipalpus phoenicis* gradient from Citrus Leprosis trees Bassanezi R.B., Montesino L.H., and Novelli V.M.
S14O08	New generation sequencing platforms for detection and characterization of viruses and viroids in citrus Olmos A., Bertolini E., Varveri C., Candresse T., Martínez M.C., Pina J.A., and Cambra M.
S14O09	Deep sequencing of viroid-derived small RNAs from citron Cao M.J., Su H.N., Yang F.Y., Chen H.M., and Zhou C.Y.
S14O10	The complete genome sequence of *Citrus vein enation virus* (CVEV) obtained through deep sequencing of small RNAs Vives M.C., Velázquez K., Pina J.A., Moreno P., Guerri J., and Navarro L.
S14O11	Deep sequencing of citrus affected by graft-transmissible diseases of unknown aetiology leads to discovery of two novel viruses Loconsole G., Giampetruzzi A., Saldarelli P., Onelge N., Yokomi R.K., and Saponari M.

Poster ID	Title
S14P01	Genetic variation of *Citrus tristeza virus* (CTV) isolates from Calabria, Italy Fontana A., Debreczeni D., Albanese G., Davino S., Flores R., and Rubio L.
S14P02	Incidence, distribution and first identification of *Citrus tristeza virus* by RT-PCR in citrus orchards in South Western Nigeria Adediji A.O., Atiri G.I., and Kumar P.L.
S14P03	A rapid procedure to evaluate the protecting ability of *Citrus tristeza virus* mild isolates against severe isolates Ruiz Ruiz S., Navarro-López J., Moreno P., and Ambrós S.

Poster ID	Title
S14P04	Expression of different proteins in sweet orange induced by severe and mild *Citrus tristeza virus* (CTV) isolates Yang F.Y., Li Z.A., Zhou C.Y., and Zhou Y.
S14P05	Screening of *Citrus tristeza virus* in sweet oranges and limes of the Colombian Citrus Collection (Corpoica - Meta) Guzmán-Barney M.M., Rodríguez P., Ordúz J., and Martínez J.
S14P06	Molecular diversity of *Citrus tristeza virus* strains collected over the past 50 years and maintained *in planta* collections in California Wang J.W., Bozan O.B., Kwon S.J., Rucker T.R., Thomas C.T., Yokomi R.K., Lee R.F., Folimonova S., and Vidalakis G.
S14P07	Implication of *Toxoptera citricida* on temporal and spatial dispersion of *Citrus tristeza virus* in Southern Mexico Domínguez Monge S., Mora Aguilera G., Loeza Kuk E., Flores Sánchez J.L., Acevedo Sánchez G., and Robles García P.
S14P08	Population variation of *Citrus tristeza virus* Qing L., Ruan T., Xiong Y., Zhou Y., Song Z., Sun X.C., Li Z.A., and Zhou C.Y.
S14P09	Nucleotide sequence of three genes of *Citrus tristeza virus* from selected isolates in a program of preimmunization Zanutto C.A., Muller G.W., Corazza M.J., and Nunes W.M.C.
S14P10	A comparative study and vector transmissibility of relevant *Citrus tristeza virus* (CTV) populations from Italy indicates a risk for further epidemics Yahiaoui D., Djelouah K., D´Onghia A.M., and Catara A.
S14P11	Prevalence and epidemiology of *Citrus tristeza virus* in Andalucía and Murcia regions of Spain Gorris M.T., Muñoz C., Cano A., Hermoso de Mendoza A., Martínez M.C., Hermosilla A., Fuentes F., Bertolini E., Collado C., López A., and Cambra M.
S14P12	Epidemiology of *Citrus tristeza virus* in experimental nursery blocks of citrus rootstock species in Spain Gorris M.T., Rodríguez A., Martínez M.C., Bertolini E., Collado C., Botella P., López A., and Cambra M.
S14P13	Monitoring of *Citrus tristeza virus* (CTV) and characterization of local isolates in Algeria Larbi D., Belkahla H., Djelouah K., and D'Onghia A.M.
S14P14	Evaluation of the sampling method of *Citrus tristeza virus* (CTV) in Apulia Region, Italy D´Onghia A.M., Al Naasan Y., Santoro F., Figorito B., and Gualano S.
S14P15	Integrated *Citrus tristeza virus* complex management in Sicily Tumminelli R., Rotolo M.C., Saraceno F., Patti M.R., Saitta R., Conti F., Fisicaro R., Cavallaro A., Cavallaro G., Cutuli A., Greco G., Di Natale A., Garozzo M., Gullotta S., Privitera S., Quattrocchi S., Ricca G., Bertolami E., Calderone G., Maimone F., Schillaci G., Trifiletti A., Corno G., Bono G., Federico R., Lo Grasso F., Vicari M., Lo Presti P., Truncali S., Sicilia L., Giacalone A., Adragna V., Vecchio S., Filiddani R., Fascetto G.T., Marano G., Spadafora A., D'Anna R., Carta Cerella D., and Sinatra V.
S14P16	Biological and molecular characterization of a *Citrus tristeza virus* isolate inducing seedling yellows and stem pitting detected in Hunan province, China Licciardello G., Russo M., Daden M., Dai S., Xiao C., Deng Z., and Catara A.
S14P17	Evaluation of productive and physiological parameters on 'Tarocco' orange infected by severe isolate of *Citrus tristeza virus* Sorrentino G., Guardo M., Russo M.P., Davino S., and Caruso A.
S14P18	Eradicating *Citrus tristeza virus* and *Citrus tatter leaf virus* from double infected citrus plants Qiao Q., and Jiang L.

Poster ID	Title
S14P19	Construction of an Infectious cDNA clone of *Citrus tatter leaf virus* from an infected sweet orange Song Z., Zhou C.Y., Liu K.H., and Li Z.A.
S14P20	The psorosis B syndrome in citrus is associated to a sequence variant of the *Citrus psorosis virus* RNA2 Velázquez K., Alba L., Pina J.A., Navarro L., Moreno P., and Guerri J.
S14P21	Sensitivity to *Citrus psorosis virus* of species and hybrids of the genus *Citrus* and relatives Velázquez K., Alba L., Zarza O., Vives M.C., Pina J.A., Juárez J., Navarro L., Moreno P., and Guerri J.
S14P22	Detection and quantitation of *Citrus psorosis virus* by real time RT-PCR Velázquez K., Alba L., Guerri J., Moreno P., Navarro L., and Vives M.C.
S14P23	Improvement in diagnosis for Citrus Psorosis in Argentina by qRT-PCR De Francesco A., Reyes C.A., Costa N., and Garcia M.L.
S14P24	Cloning and sequence analysis of the large coat protein of *Satsuma dwarf virus* Fengjie isolate Sun X.C., Qing L., Yang F.Y., and Zhou C.Y.
S14P25	Characterization and diffusion of *Citrus leaf blotch virus* (CLBV) in Southern Italy Guardo M., Sorrentino G., and Caruso A.
S14P26	*Citrus leaf blotch virus* (CLBV) invades meristematic regions in *Nicotiana benthamiana* and citrus Agüero J., Vives M.C., Velázquez K., Ruiz-Ruiz S., Juárez J., Navarro L., Moreno P., and Guerri J.
S14P27	Simultaneous detection of *Citrus exocortis viroid* and *Hop stunt viroid* in citrus plants by direct tissue-print duplex real-time RT-PCR Bertolini E., Martínez M.C., Serra P., Olmos A., Carbó C., Duran-Vila N., and Cambra M.
S14P28	Setting up and evaluation of new primer sets for the detection of *Spiroplasma citri* the causal agent of Citrus Stubborn disease Abdel Fattah A., Valentini F., Frasheri D., D'Onghia A.M., and Djelouah K.
S14P29	Biological characterization of a new lemon disease in China Chen H.M., Li Z.A., Zhou Y., Wang X.F., Tang K.Z., and Zhou C.Y.
S14P30	Virus and virus-like diseases of citrus in Oman Al-Sadi A.M., and Al-Harthi S.A.
S14P31	Recent trends in diagnosis, characterization and management of major virus and virus-like pathogens infecting citrus in India Ghosh D.K.
S14P32	The citrus nursery tree certification program in Spain Pina J.A., Chomé P., Vives M.C., and Navarro L.
S14P33	COST FA0806: Plant virus control employing RNA-based vaccines: A novel non-transgenic strategy Voloudakis A.E.
S14P34	Performance of 'Maltaise' demi-sanguine sweet orange on eight rootstocks inoculated with *Citrus exocortis viroid* (CEVd) and *Cachexia viroid* (CVIIb) Najar A., Homri N., Bouhlel R., Ben Mimoun M., and Duran-Vila N.
S14P35	Biological and molecular detection of *Citrus psorosis virus* in the Northwest region of Morocco Achachi A.A., and Ibriz M.

SESSION 15: Fungal diseases

Oral presentations at Auditorium 3, on Tuesday 20th from 10:30 to 13:30.

Poster session on Thursday 22nd

Conveners: Dr. Antonio VICENT (Instituto Valenciano Investigaciones Agrarias, Spain) and Dr. Tian SCHUTTE (Citrus Research International, South Africa).

Oral ID	Title
S15O01	The arrival of Citrus Black Spot (*Guignardia citricarpa*) in Florida and current research questions Dewdney M.M., Peres N.A., Schubert T.S., Mondal S.N., Hu J., and Hincapie Caputo M.
S15O02	Predictive model for ascospore release of *Guignardia citricarpa* using climatological data Dummel D.M., Agostini J.P., and Moschini R.
S15O03	Assessment of retention and persistence of copper fungicides on sweet orange fruit and leaves using fluorometry and copper residue analyses Schutte G.C., Kotze C., Van Zyl J.G., and Fourie P.H.
S15O04	*Phyllosticta* species associated with citrus diseases in China Wang X.H., Chen G.Q., Huang F., and Li H.Y.
S15O05	Development of an agrotransformation gene-silencing-system for *Phyllosticta citricarpa* and its use in functional analysis of the pathogenic genes Goulin E.H., Petters D.A. L., Figueiredo J.A.G., Senkiv C.C., Silva Jr. G.J., Kava-Cordeiro V., Galli-Terasawa L. V., Peña L., and Glienke C.
S15O06	Epidemiology of Alternaria Brown Spot of mandarins under semi-arid conditions in Spain Bassimba D.D.M., Mira J.L., and Vicent A.
S15O07	Spray deposition benchmarks for control of Alternaria Brown Spot and evaluation of adjuvants to improve fungicide spray deposition in citrus orchards van Zyl J.G., Schutte G.C., and Fourie P.H.
S15O08	Chemical control of *Colletotrichum acutatum* and *C. gloeosporioides*, causal agents of Citrus Postbloom Fruit Drop in Brazil Goes A., and Rinaldo D.
S15O09	Pathogenicity and genetic relationship of strains of *Elsinoë australis* causing Citrus Scab disease Hyun J.W., Yi P.H., Yun S.H., Hwang R.Y., and Levy L.
S15O10	Association and interaction of edaphic factors with root disease related citrus decline Pretorius M.C., Labuschagne N., Kotze C., and McLeod A.
S15O11	Searching for citrus rootstocks resistant to Mal Secco disease: a review Nigro F., Ippolito A., and Salerno M.G.

Poster ID	Title
S15P01	Endophytic actinomycetes for the biological control of *Phyllosticta citricarpa* Glienke C., Savi D.C., Goulin E.H., Kava-Cordeiro V., and Silva Jr G.J.
S15P02	Population genetics of *Guignardia citricarpa* in South Africa Carstens E., Linde C.C., Slabber R., Langenhoven S., Schutte G.C., Fourie P.H., and McLeod A.
S15P03	First report of false melanose symptoms of Citrus Black Spot on sweet orange leaves in Brazil Silva Jr G.J., Pereira R.G., Marin D.R., Wulff N.A., Scapin M.S., and Sala I.

Poster ID	Title
S15P04	Biocontrol of Citrus Black Spot disease: perspectives using fungal endophytes of citrus Jung L.F., Goulin E.H., Savi D.C., Schuh R., Galli-Terasawa L.V., Glienke C., and Kava-Cordeiro V.
S15P05	Modelling of *Guignardia* pseudothecium maturation and ascospore dispersal in citrus orchards Fourie P.H., Schutte G.C., Serfontein S., and Swart S.H.
S15P06	Infectious period of Citrus Black Spot on sweet orange Lourenço S.A., Gasparoto M.C.G., Spósito M.B., Amorim L., and Gottwald T.R.
S15P07	Characterization of *Guignardia* spp. from citrus fruit in Argentina Kornowski M.V., and Agostini J.P.
S15P08	Integrated approaches to minimize Alternaria Brown Spot of citrus in Italy Bella P., Russo M., Tomasello M., Catara A., Catara V., and La Rosa R.
S15P09	Varietal resistance and management of Alternaria Brown Spot in Brazil Azevedo F.A., Pacheco C.A., Martelli I.B., and Polydoro D.A.
S15P10	Outbreak and occurrence of Alternaria Brown Spot in China Huang F., and Li H.Y.
S15P11	Evolution of symptoms caused by *Alternaria alternata* on 'Nova' mandarin fruit and its schematic sequence Burdyn L., Garran S.M., and Avanza M.M.
S15P12	Commercial-scale Alternaria Brown Spot resistance screening as the first step in breeding new mandarins for Australia Miles A.K., Newman T.K., Gultzow D.L., Parfitt S.C., Drenth A., and Smith M.W.
S15P13	Difference in susceptibility between young and mature 'Minneola' tangelo leaves Liarzi O., Sela N., and Ezra D.
S15P14	Screening for Alternaria Brown Spot resistance in the triploid mandarin breeding programme in Spain Bassimba D.D.M., Vicent A., Cuenca J., Aleza P., and Navarro L.
S15P15	Enhancing of physiological parameters improves resistance against Alternaria Brown Spot of mandarins Llorens E., Fernandez-Crespo E., Camañes G., Lapeña L., and Garcia-Agustin P.
S15P16	Strobilurin resistance of *Alternaria alternata*, the causal agent of Alternaria Brown Spot, in Florida tangerine hybrid groves Vega B., and Dewdney M.M.
S15P17	Evaluation of fungicide mixtures as replacement for carbendazim for Citrus Postbloom Fruit Drop control in Brazil Silva Jr G.J., Spósito M.B., Marin D.R.,and Amorim L.
S15P18	Survival of *Colletotrichum acutatum* in citrus leaves Pereira W.V., Tanaka F.A.O., Rodrigues M.B.C., and Massola Junior N.S.
S15P19	Pollen exudate stimulates conidial germination of *Colletotrichum acutatum* Gasparoto M.C.G., Lourenço S.A., Marques J.P.R., Appezzato-da-Glória B., and Amorim L.
S15P20	Fungal diseases of citrus in Panama Aguilera-Cogley V., and Vicent A.
S15P21	Epidemiology and control of Citrus Greasy Spot in Panama Aguilera-Cogley V., and Vicent A.
S15P22	Biological control of Citrus Canker and Melanose using rhizobacteria in Korea Ko Y.J., Kang S.Y., and Jeun Y.C.
S15P23	Expression profiles of differentially regulated genes of citrus during infection by *Elsinoë fawcettii* Hyun J.W., Yi P.H., and Kim Y.J.

Poster ID	Title
S15P24	Biological control of *Phytophthora* sp. and *Fusarium* sp. in citrus nurseries in Egypt Ahmed Y., Ippolito A., El-Shimy H., D'onghia A.M., and Yaseen T.
S15P25	Dry Root Rot, an alliance between *Fusarium solani* and *Phytophthora* or other factors against citrus in California Adesemoye A.O., and Eskalen A.
S15P26	Efficacy of water soluble silicon in managing Fusarium Dry Root Rot of citrus Marais L.J.
S15P27	Application of artificial intelligence to the visual diagnosis of quarantine citrus diseases Burdyn L., Garran S.M., Stegmayer G., and Milone D.H.
S15P28	Optimization of copper application schedules for foliar citrus disease management based on fruit growth, historical rainfall patterns and copper residue decay Zortea T., Fraisse C.W., and Dewdney M.M.
S15P29	Phytosanitary situation of Chilean citrus industry Besoain X., Castro M., Camps R., and López E.
S15P30	Storage technology for conservation of seeds of citrus rootstocks Nascimento L.M., Moreira J., Brito M.C.R., Brito P.C.N., and Sanches J.M.
S15P31	Morphological and molecular diversity of *Phytophthora nicotianae* strains isolated from citrus plantations in Cuba Llauger R., Coto O., Peña M., Zamora V., and Collazo C.

SESSION 16: Entomology and pest control

Oral presentations at Auditorium 1, on Friday 23rd from 10:30 to 16:45.
Poster session on Monday 19th

Conveners: Dr. Alberto URBANEJA (Instituto Valenciano Investigaciones Agrarias, Spain), Dr. Josep A. JACAS (Universitat Jaume I, Spain), Dr. Phil A. STANSLY (University of Florida, USA) and Dr. Jorge E. PEÑA (University of Florida, USA).

Oral ID	Title
S16O01	The status of citrus IPM in California Grafton-Cardwell E. E.
S16O02	The status of citrus IPM in South Africa Grout T.G.
S16O03	IPM in Spanish citrus: current status of Biological Control Urbaneja A., Tena A., and Jacas J.A.
S16O04	Status of citrus IPM in the southern Mediterranean Basin Mazih A.
S16O05	Integrated and disease management in New Zealand. Progress, changes and challenges since 2004 Pyle K.R., and Jamieson L.E.
S16O06	Progress toward integrated management of Asian Citrus Psyllid in Florida Stansly P.A.
S16O07	Perspective of the Indonesian citriculture in the presence of Huanglongbing disease Supriyanto A.S., and Nurhadi N.
S16O08	Non-target effects of cultural practices to manage the bacterial disease HLB on soil food webs that affect the insect pest *Diaprepes abreviatus* Campos-Herrera R., El-Borai F.E., Schumann A., and Duncan L.W.

Oral ID	Title
S16O09	Protection of young trees from HLB through disruption of *Diaphorina citri* (Kuwayama) feeding behavior Rogers M.E., Ebert T.A., Kim K.D., and Weaver C.E.
S16O10	Effect of UV-blocking plastic films on plant location and spread of the Asian Citrus Psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) on citrus Miranda M.P., Marques R.N., Santos F.L., Felippe M.R., Moreno A., and Fereres A.
S16O11	Ontogenic variation in citrus flush shoots and its relation with host plant finding and acceptance by Asian Citrus Psyllid (Hemiptera: Psyllidae) Setamou M., and Patt J. M.
S16O12	Targeting juvenile hormone metabolic genes in the Asian Citrus Psyllid (*Diaphorina citri*) as a strategy to reduce the spread of Citrus Greening disease Van Ekert E., Borovsky D., Powell C.A., Cave R.D., Alessandro R.T., and Shatters R.G.
S16O13	Biological Control of red scale on citrus on the central coast of New South Wales Dao H.T., Beattie G.A.C., Holford P., Spooner-Hart R., Meats A., and Burgess L.
S16O14	Sugar subsidies improve the fitness and efficacy of the parasitoid *Aphytis melinus* in the field Tena A., Cano D., Pekas A., Wäckers F., and Urbaneja A.
S16O15	Ground cover management in citrus affects the Biological Control of aphids Gómez-Marco F., Tena A., Jacas J.A., and Urbaneja A.
S16O16	Field evaluation of some pesticides and biological control against citrus mealybug *Planococcus citri* Risso (Hemiptera: Pseudococcidae) Kararacoglu M., Kutuk H., Tufekli M., Satar G., and Yarpuzlu F.
S16O17	Can imidacloprid cause lepidopteran pest repercussions? Moore S.D., Van der Walt R., Kirkman W., and Du Preez D.
S16O18	Ecology and management of Kelly's citrus thrips in eastern Spain Navarro-Campos C., Pekas A. , Aguilar A., and Garcia-Marí F.
S16O19	Monitoring and management of *Brevipalpus chilensis* Baker (Acarina: Tenuipalpidae) in citrus Olivares N., Vargas R., and Ripa R.
S16O20	Host adaptation of *Tetranychus urticae* populations in clementine orchards with a *Festuca arundinacea* cover may contribute to its natural control Aguilar-Fenollosa E., Pina T., Gómez-Martínez M.A., Hurtado M.A., and Jacas J.A.

Poster ID	Title
S16P01	Analysis of population trends of citrus pests from an area-wide field survey and monitoring network established in eastern Spain Garcia-Mari F.
S16P02	Arthropod pest composition and farmers perceptions of pest and disease problems on citrus in Kenya Ekesi S.
S16P03	Thresholds for HLB vector control in infected commercial citrus and compatibility with Biological Control Monzó C., Stansly P.A., and Urbaneja A.
S16P04	Development of the Huaglongbing (HLB) vector, *Diaphorina citri* Kuwayama, 1908 (Hemiptera: Psyllidae), in different host plants Alves G.R., Diniz A.J.F., Lima A.A., Vieira J.M., and Parra J.R.P.
S16P05	Agreggation and sampling plans for *Diaphorina citri* (Hemiptera: Psyllidae) immatures in citrus Asplanato G., Amuedo S., and Franco J.

Poster ID	Title
S16P06	Use of oils to control the Asian Citrus Psyllid (*Diaphorina citri*) in Mexican lime under dry tropic conditions in Mexico Orozco-Santos M., Velázquez-Monreal J.J., García-Mariscal K., Manzanilla-Ramírez M.A., Carrillo-Medrano S.H., and Robles-González M.M.
S16P07	Seasonal patterns in the proportion of Asian Citrus Psyllid (*Diaphorina citri*) carrying *Candidatus* Liberibacter asiaticus Ebert T.A., Brlansky R.H., and Rogers M.E.
S16P08	Effect of Imidacloprid (Winner) on trunk application to psyllid (*Diaphorina citri* – Hemiptera: Psyllidae) control on citrus Lozano Leonel Jr F., Soares R.D.J., and Moraes J.P.
S16P09	Movement of Asian Citrus Psyllids in HLB-affected and healthy citrus trees Cen Y., Wu F., Liang G., Chen J., Deng X., and Xia Y.
S16P10	Effectivity of Huanglongbing vector (*Diaphorina citri* kuw.) control citrus grower group based in regency of Sambas, West Kalimantan, Indonesia Supriyanto A.S., Nurhadi N., Zuhran Z., and Purbiati P.
S16P11	Behavioural responses of *Diaphorina citri* to host plant volatiles Fancelli M., Birkett M.A., Pickett J.A., Moraes M.C.B., Laumann R.A., and Borges M.
S16P12	Efficacy of selected insecticides for the control of the California Red Scale in Southern Italy Campolo O., Grande S.B., Chiera E., and Palmeri V.
S16P13	Altea (Eastern Spain) area-wide project to control California Red Scale *Aonidiella aurantii* (Hemiptera: Diaspididae) based on conservation and release Biological Control agents Laborda R., Garcia-Mari F., Sanchez A., Xamani P., Garcia A., Punset C., Bernabeu P., Aznar M., and Bertomeu S.
S16P14	Hyperparasitism may prevent efficient regulation of *Aphis spiraecola* Patch. (Homoptera: Aphididae) in citrus orchards by primary parasitoids Gómez-Marco F., Tena, A., Jacas, J.A., and Urbaneja, A.
S16P15	Citrus mealybug biological control strategies and large scale implementation on citrus in Turkey Erkilic L.B., Demirbas H., and Guven B.
S16P16	Repellency and acceptability of several substances by ant species in citrus groves in Spain Campos Rivela J.M., Martínez-Ferrer M.T., Fibla J.M. , and Pla M.
S16P17	Taxonomic studies for classical Biological Control of *Delottococcus aberiae* (Hemiptera: Pseudococcidae) Beltrà A., Navarro-Campos C., Garcia-Marí F., Malausa T., Giliomee J.H., Addison P., and Soto A.
S16P18	Canibalism and intraguild predation in citrus mealybug natural enemies Gkounti V.T., Savvopoulou-Soultani M., Kontodimas D.C., and Milonas P.G.
S16P19	Biological Control of the citrus mealybug: dispersion pattern of the parasitoid *Anagyrus sp.* near *pseudococci*, during augmentative releases Franco J.C. , Zina V., Silva E.B. , Steinberg S., Belda J.E. , Branco M., Suma P., and Mendel Z.
S16P20	Study on Biological Control and trap techniques of *Thrips flavidulus* (Thysanoptera: Thripidae) in citrus orchards Yao H., Yu F., and Zhang H.
S16P21	Thysnoptera occurring in clementine mandarin under three ground cover management strategies: species composition, abundance and distribution Aguilar-Fenollosa E., and Jacas J.A.

Poster ID	Title
S16P22.	Effect of biotic and abiotic factors on the insurgence of fruit scars in Italian orange orchards Siscaro G., Zappalà L., Biondi A., Conti F., and Fisicaro R
S16P23	Thrips population dynamics, composition and first survey on ground-dwelling predatory mites in lemon orchards in Italy Biondi A., Zappalà L., Tropea Garzia G., Perrotta G., and Siscaro G.
S16P24	Pollen availability in the cover crop may affect the biological control of *Tetranychus urticae* in citrus Pina T., Argolo P.S., Urbaneja A.,and Jacas J. A.
S16P25	Assessment of trophic interactions among citrus mites by DNA-based gut content analysis Pérez-Sayas C., Pina T., Gómez-Martínez M.A., Jacas J.A., and Hurtado M.A.
S16P26	Effectiveness and economics of a novel proactive approach for managing the Citrus Rust Mite in Texas Setamou M., and Sekula D.
S16P27	Spider mite response in citrus rootstocks is mediated by the oxylipin pathway Agut B., Hurtado M.A., Jacas J.A., and Flors V.
S16P28	Impact of three ant species on pest populations in Mediterranean citrus orchards Calabuig A., Pekas A., and García Marí F.
S16P29	Ant nests distribution in citrus groves under different ground cover management strategies in Spain Martinez-Ferrer M. T., Campos Rivela J. M., Fibla J. M., Pla M.
S16P30	Composition and flying activity of Coleoptera staphylinids in citrus orchards Adorno A., Biondi A., Zappalà L., Siscaro G., and Sabella G
S16P31	Entomo-pathogenic nematodes to combat -infestations at Ticofrut Camacho H., Yglesias G., and Faerron P.
S16P32	Monitoring and Management of *Naupactus* (= *Asynonychus*) *cervinus* in citrus orchards in Chile Luppichini P., and Olivares N.
S16P33	Population dynamic of citrus blackfly in Tahiti lime in the state of São Paulo, Brazil Felippe N, and Raga A.
S16P34	Integrated strategies to monitor and control Citrus Flower Moth, *Prays citri* Mill. (Lepidoptera: Yponomeutidae) on nursery trees of the Mediterranean área Conti F., and Fisicaro R.
S16P35	Biology of Citrus Fruit Borer, *Citripestis sagittiferella* Moore and its control Srijuntra S., Manusmunkong B., Sahaya S., Jumroenma K., and Srikachar S.
S16P36	Spirotetramat (Movento®): a new tool for *Aonidiella aurantii* Maskell management in Spain Izquierdo J., and Fullana J.
S16P37	Carbendazim, mancozeb and pyraclostrobin residues resulting from the chemical control of Citrus Black Spot (*Guignardia citricarpa*) on Valencia orange in the Northeast of Argentina Waskowicz C., Sosa A., Becerra V., Navarro R., Burdyn L., Garran S.M., and Mousqués J.
S16P38	Evaluation of a novel insecticide, DPX-HGW86 10% OD (Benevia™) and DPX-HGW86 20% SC (Verimark™), for *Diaphorina citri* (Hemiptera: Psyllidae) control Miranda M.P., Felippe M.R., Garcia R.B, Caldeira R.E., and Noronha Jr N.C.
S16P39	The effect of foliar application of insecticides pyrethroids and neonicotinoids on the population of *Panonychus citri* (McGregor, 1916) (Acari: Tetranychidae) in laboratory Zanardi O.Z., Rugno G.R., Bordini G.P., Beloti V.H., and Yamamoto P.T.

Poster ID	Title
S16P40	Side effects of some pesticides under laboratory conditions on important parasitoids and predators in citrus ecosystem Simsek V.M., Uygun N., and Satar S.
S16P41	Side effects of some pesticides under laboratory conditions on important parasitoids and predators in citrus ecosystem Simsek V.M., Uygun N., and Satar S.
S16P42	New book on citrus pests: citrus pests. Integrated pest management in countries of Mediterranean climate (Plagas de los cítricos. Gestión integrada en países de clima mediterráneo) Garcia-Mari F.
S16P43	Impact of pesticides used in citrus orchards on the beneficial insects *Cryptolaemus montrouzieri* Mulsant Rahmouni R., and Chermiti B.
S16P44	Preliminary survey of the Green Lacewings (Neuroptera, Chrysopidae) in citrus orchards in Northern east of Tunisia (Cap Bon) Limem S. E., and Chermiti B.
S16P45	Mating disruption technique as a control method for California Red Scale: a review of doses, efficacy and date of application. Navarro-Llopis, V., Alfaro C., Vacas S., Primo Millo, J.
S16P46	Physiological selectivity of insecticides used for control of *Diaphorina citri* (Kuwayama, 1908) on larvae of *Ceraeochrysa cubana* (Hagen, 1861) Rugno G., Zanardi, Beloti, Cunha, Barbosa, Parra, and Yamamoto
S16P47	Distribution of the Asian Citrus Psyllid, *Diaphorina citri* (Hemiptera: Psyllidae) in Iran Parsi F., Manzari S., Askari M., Amin G., Ranjbar S., and Naseri M.

SESSION 17: Varieties

Oral presentations at Auditorium 1, on Monday 19th from 15:15 to 17:15
Poster session on Tuesday 20th

Conveners: Dr. Pablo ALEZA (Instituto Valenciano Investigaciones Agrarias, Spain) and Dr. Giuseppe REFORGIATO RECUPERO (Centro di Ricerca per l'Agrumicoltura, Italy).

Oral ID	Title
S17O01	New citrus variety evaluation in Australia 2005-2012 Sanderson G., Creek A., Lacey K., and Wallace M.
S17O02	'Sweet Sicily' and 'Early Sicily', two new triploids from the program of CRA-ACM, Centro di- Ricerca per lAgrumicoltura e le Colture Mediterranee, Acireale, Italy Russo G, Reforgiato Recupero G, Recupero S, and Pietro Paolo D.
S17O03	Comparison of performance of different late-maturing navel selections around the world Rabe E., Turner P., and Chavarria J.
S17O04	Three new varieties of clementine: 'Clemenverd', 'Nero' and 'Iviaman1957' López-García A., Terol J., Tadeo F.R., Herrero A., Ibáñez V., and Talón M.
S17O05	Relationship between sensory and physic-chemical quality parameters of 'Lanelate' with protected geographical indication Duarte A., Mendes S., Nunes S., Sustelo V., and Gomes C.
S17O06	New promising citrus hybrids for the ornamental use Recupero S., Russo G., and Reforgiato Recupero G.

Oral ID	Title
S17O07	'Meirav Seedless', a new promising Israeli mandarin: effects of rootstocks and growth regulators on yield and fruit quality Kanonich Y.K., Holsman S.H., Oren Y.O., Kaplan I., Tagari E., Fainzack M., and Giladi B.
S17P08	How many seeds are acceptable in commercial citrus varieties derived from irradiation? Williams T. E.

Poster ID	Title
S17P01	Plant growth, initial fruit production and yield efficiency of twelve early maturing sweet orange cultivars in São Paulo State, Brazil Caputo M.M., Mourão Filho F.A.A., Silva S.R, Stuchi E.S., and Bremer Neto H.
S17P02	Initial production of 17 mid-season sweet orange cultivars in Southwestern São Paulo State, Brazil Ramos Y. C., Mourão Filho F. A. A., Stuchi E. S., Silva S. R., Caputo M. M., Bremer Neto H., and Fadel A. L.
S17P03	Determination of yield and fruit quality characteristics of several local and foreign originated nucellar orange clones in Adana province of Turkey Polatöz S., and Tuzcu Ö.
S17P04	Yield and fruit quality of two early maturing orange cultivars, Navelina and Fukumoto, in Andalusia (Spain) Merino C., Hervalejo A., Salguero A., and Arenas Arenas F.J.
S17P05	Phenological growth stages of oranges and mandarines in Entre Ríos province (Argentina) Rivadeneira M. F.
S17P06	Fruit characteristics of some late maturing commercial mandarin cultivars in Spain Simón-Grao S., Simón I., Lidón V, Nieves M., Carbonel A.A., Manera J., Hernandez F., and Garcia-Sanchez F.
S17P07	Evaluations of early satsuma selections for California. Kahn T., and Siebert T.
S17P08	Yield and fruit quality of two satsuma cultivars, Okitsu and Iwasaki, in Andalusia (Spain) in 2010/11 season Merino C., Hervalejo A., Salguero A., and Arenas Arenas F.J.
S17P09	Behaviour of Parana grapefruit in the Southeastern region of Paraguay Wlosek Stañgret C.R., and Canteros B.I.
S17P10	Characterization of grapefruit varieties produced in Argentina and Paraguay Sgroppo S.C., Pereyra M.V., Wlosek Stañgret C.R., and Canteros B.I.
S17P11	Evaluation of lemon selections for the deserts of the United States Wright G.C., and Kahn T.
S17P12	Selection and field evaluation of three new cultivars of lemon in the South-east of Spain Porras I., Pérez-Pérez J. G., García-Lidón A., Sánchez-Baños M., and Pérez-Tornero O.
S17P13	Preselection of promising triploid mandarin varieties in Corsica Bouffin J., Froelicher Y., Luro F., and Ollitrault P.
S17P14	Two new IVIA triploid hybrids of mandarin; IVIA-592 and IVIA-599 Cuenca J., Aleza P., Juarez J., Pina J.A., and Navarro L.
S17P15	Selection of new Ponkan like mandarins Bastianel M., Cristofani-Yaly M., Schinor E.H., Simonetti L.M., Manente K.K.M., De Negri J.D., Azevedo F.A., and Machado M.A.
S17P16	New tangors for Brazilian citriculture Schinor E.H., Pacheco C.A., Bastianel M., Azevedo F.A., and Cristofani-Yaly M.

Poster ID	Title
S17P17	Three new easy peeler varieties bred by the agricultural research council released world wise. van Rensburg P.J., Sippel A.D., Combrink N.K., Maritz J.G.J, and Bijzet Z.
S17P18	A new potential elite citrus hybrid Cheng C.F., Wei Z.X., Hong L., Wu C.Q., and Qi J.R.
S17P19	Acceptability of Fremont mandarin in Brazil: a variety with agronomic performance and resistance to Alternaria Brown Spot Pacheco C.A., Schinor E.H., Moretti M.R., Bastianel M., Machado M.A., and Azevedo F.A.
S17P20	Scion and rootstock combinations for ornamental citrus in containers Fadini M., Girardi E.A., Santos M.G., Gesteira A.S., Passos O.S., Souza F.V.D., and Soares Filho W.S.
S17P21	Current situation of the Chilean citrus industry Castro M., Besoain X., Herrera V., and Cautin R.
S17P22	Influence of gamma irradiation on seedless citrus production: pollen germination and fruit quality Bermejo A., Pardo J., and Zaragoza S.
S17P23	Identification of new volatile compounds in citrus hybrid mandarinquat Indio (*Citrus japonica* Thunb. sp. × *Citrus reticulata* Blanco) Delort E., Decorzant E., Casilli A., and Jaquier A.
S17P24	Molecular characterization of Tahiti lime selections by RAPD analysis Bremer Neto H., Mourão Filho F.A.A.M.F., Stuchi E.S.S., Soriano L.S., Miyata L.M., and Camargo L.E.A.C.
S17P25	A natural mutant cultivar Zigui shatian pummelo (*Citrus grandis* (L.) Osbeck) showing self-sterility due to abnormal post-zygotic embryo development and not to self-incompatibility Chai L.J., Ge X.X., Biswas M.K., Xu Q., and Deng X.X.
S17P26	Relation between temperature and the colour coordinate a during the development of the external colour of lemon fruits Conesa A., Manera J., Brotons J., and Porras I.
S17P27	Determination of the geographical origin of navel orange by near infrared spectroscopy Su X.S., Zhang X.Y., Jiao B.N., and Cao W.Q.
S17P28	A new lemon genotype for ornamental use obtained by gamma irradiation Uzun A., Gulsen O., Kafa G., and Seday U.
S17P29	New mandarin triploid hybrids Aya and Hana selected in Morocco Handaji N., Benyahia H., Benaouda H., Arsalane N., and Srairi I.
S17P30	Mutation breeding for seedless cultivars in Argentina Garavello M., Anderson C., Prina A., and Martinez A.

SESSION 18: Rootstocks

Oral presentations at Auditorium 2, on Tuesday 20th from 10:30 to 13:30
Poster session on Thursday 22nd

Conveners: Dr. María A. FORNER (Instituto Valenciano Investigaciones Agrarias, Spain) and Dr. William S. CASTLE (Citrus Research and Education Center, USA)

Oral ID	Title
S18O01	Reflections on a career in citrus rootstock evaluation and commercialization Castle W.S.
S18O02	Citrus rootstocks in Morocco: present situation and future prospects El Guilli M., Belmahdi I., and Zemzami M.
S18O03	Seven hybrid citrus rootstocks released by USDA: comparison of characteristics and use Bowman K.D., and Albrecht U.
S18O04	The evaluation of Chinese rootstock for tree growth, yield and quality of 'Lane Late' oranges grown in Australia Khurshid T., Sanderson G., and Donovan N.
S18O05	Dwarfing rootstocks for 'Valencia' sweet orange Ramos Y.C., Stuchi E.S., Girardi E.A., Leão H.C. , Gesteira A.S., Passos O.S., and Soares Filho W.S.
S18O06	The development of improved tetraploid citrus rootstocks to facilitate advanced production systems and sustainable citriculture in Florida Grosser J.W., Barthe G.A., Gmitter Jr. F.G., and Castle W.S.
S18O07	*Citrus macrophylla* rootstock improves the performance of 'Mexican' lime trees on citrus interstocks in calcareous soils Medina-Urrutia V.M., Robles-González M.M., Virgen-Calleros G., and Manzanilla-Ramírez M.A.
S18O08	Performance of 'Valencia Late' orange on 14 rootstocks in the Gharb region in Morocco Benyahia H., Benazzouz A., Talha A., Beniken L., Omari F.E., Handaji N., Jacquemend E., and Srairi I.
S18O09	Results from a three years field trial planted at Concordia, Argentina using transgenic rootstocks potentially resistent to Citrus Tristeza Anderson C., Plata M.I., Garavello M., Costa N., and Peña L.
S18O10	'Forner-Alcaide 5': a citrus rootstock released in Spain Forner-Giner M.A., and Forner J.B.

Poster ID	Title
S18P01	Rootstocks affect fruit yield and some fruit quality traits of 'Star Ruby' grapefruit in Adana (Turkey) Yeşiloğlu T., Incesu M., Yılmaz B., Çimen B., and Tuzcu Ö.
S18P02	Rootstock effects on photosynthetic performance of young 'Valencia' orange trees Yeşiloğlu T., Çimen B., İncesu M., and Yılmaz B.
S18P03	Effect of the rootstock on some agronomic traits of 'Nules' and 'Nour' clementines in the Souss valey in south Morocco Benyahia H., Benazzouz A., Beniken L., Handaji N., Kabbaj T., Essajid A., Chahidi B., Jacquemend E., and Srairi I.
S18P04	Behavior of 'Navelina ISA 315' on 15 rootstocks in Metaponto area, Basilicata, Italy Mennone C., Ippolito A., and Reforgiato Recupero G.
S18P05	Influence of rootstock on the characteristics of clementine in the plain of Sibari (italy) Russo G., Reforgiato Recupero G., Recupero S., Pietro Paolo D., Di Leo A., and Filippelli S.

Poster ID	Title
S18P06	Is there a relationship between ferric-chelate reductase activity in roots of *Poncirus trifoliata* and leaf chlorophyll contents? Correia P.J., Lopes D., Duarte A., Gama F., Saavedra T., and Pestana M.
S18P07	Influence of six citrus rootstocks on fruit quality of 'Lane Late' Hervalejo A. , Cardeñosa V., Forner-Giner M.A., Salguero A., Pradas I., Moreno J.M., and Arenas Arenas F.J.
S18P08	Agronomic performance of citrus rootstocks 'F-A 5', 'F-A 13' and 'F-A 418' on 'Nules' clementine and 'Navelate' orange in northeastern Spain Fibla J.M., Pastor J., Martinez-Ferrer M.T., Campos Rivela J.M., Forner-Giner M.A., and Forner J.B.
S18P09	New rootstocks for the high density plantings in citrus Bordas M., Torrents J., and Forner-Giner M.A.
S18P10	Performance of twelve rootstocks grafted on 'Nules' clementine in ANECOOP del Pino A., Martínez M., and Forner-Giner M.A.
S18P11	Influence of rootstock hydraulic conductance in the response to water stress and its relationship to aquaporin expression in citrus Rodríguez-Gamir J., Ancillo G., Bordas M., Primo-Millo E., and Forner-Giner M.A.
S18P12	Micropropagation for evaluation of new citrus somatic hybrid rootstocks Bordas M., Torrents J., and Navarro L.
S18P13	Performance of 'Forner-Alcaide 5' and 'Forner-Alcaide 517' with 'Loretina' clementine Molina-Nadal M.D., De Miguel A., Valero J.L.., Ripoll F., and Forner-Giner M.A.
S18P14	Performance of three rootstocks with 'Clemenrubi' and 'Orogros' clementines cultivated under screen Molina-Nadal M.D., De Miguel A., Bonafé C., Valero J.L., Ripoll F., and Forner-Giner M.A.
S18P15	Rootstock influence on the incidence of fruit splitting in navel oranges Mesejo C., Reig C., Martínez-Fuentes A., and Agustí M.
S18P16	Rootstocks for 'Lane Late' orange in Valencia del Pino A., Martínez M., and Forner-Giner M.A.
S18P17	Citrus rootstock trials on calcareous soils in California Roose M.L., Kupper R.S., and Federici C.T.
S18P18	Potential of 'Tahiti' acid lime and 'Flame' grapefruit in the Brazilian semiarid Bastos D.C., Passos O.S., Nascimento F.S.S., and Nascimento S.S.
S18P19	Selection of new citrandarins for citrus rootstocks Cristofani-Yaly M., Schinor E.H., Bastianel M., Nascimento A.L., Stuchi E.S., and Machado M.A.
S18P20	Initial production of 'Valencia' sweet orange on 40 rootstocks in Northern São Paulo state, Brazil Fadel A.L, Mourão Filho F.A.A, Stuchi E.S, and Ramos Y.C.
S18P21	Crop production of 'Okitsu' satsuma mandarin on nine rootstocks in Southern Brazil Tazima Z.H., Neves C.S.V.J., Yada I. F.U., and Leite Junior R.P.
S18P22	Effect of rootstock on yield of 'Okitsu' satsuma mandarin on sandy soils in Southern Brazil Tazima Z.H., Neves, C.S.V.J., Yada I.F.U., Stenzel N.M.C., and Leite Junior R.P.
S18P23	Scion rooting and its discriminance in 'Shiranuhi' mandarin hybrid in plastic film house in Korea Moon Y.E., Kang S.B., Kim Y.H., and Han S.G.
S18P24	Effects of scion root on flowering, fruit quality and yield of 'Shiranuhi' mandarin hybrid in plastic film house in korea Moon Y.E., Kang S.B., Han S.G., Ko S.W., Chae C.W., Lee D.H., and Choi Y.H.

Poster ID	Title
S18P25	Evaluation on drought resistance traits of citrus rootstock seedlings based on multivariate statistics Xu X.B., Yang Y.L., Gu Q.Q., Huang C.H., and Qu X.Y.
S18P26	Performance of three sweet orange varieties grafted on different rootstocks Yan S.T., Cheng C.Z., Ma Y.Y., Wang X., and Zhong G.Y.
S18P27	Effects of six rootstocks on tree growth, fruit quality and N, P, K content of lemon Hong L, and Xie Y.H.
S18P28	Tree performance and fruit quality evaluation of 'Shatangju' mandarin grafted on four rootstocks in South China Liu X., Huang M., Li J., Che J., and He Y.
S18P29	Influence of different rootstock type on chlorophyll content and mineral elements concentration in scion of 'Kinnow' mandarin Hosseini Farahi M., Aboutalebi Jahromi A., and Hasanzada H.
S18P30	Preliminary studies on high density planting of some citrus cultivars to obtain a more rapid recovery of investment capital Bassal M.A.

SESSION 20: Citrus and Health

Oral presentations at Auditorium 2, on Monday 19th from 15:15 to 17:15.
Poster session on Tuesday 20th

Conveners: Dr. Francisco TOMÁS-BARBERÁN (Centro de Edafología y Biología aplicada del Segura del CSIC, Spain) and Dr. Christine MORAND (Institut National de la Recherche Agronomique, France).

Oral ID	Title
S20O01	Flavanones are involved in the cardiovascular protective effects of citrus fruits Morand C.
S20O02	Health benefits of citrus: recent advances and future outlook Patil B.S., Jayaprakasha G.K., and Uckoo R.M.
S20O03	Orange and grapefruit bioactive compounds, citrus consumption and health benefits Cancalon P., and King D.
S20O04	Effects of a long-term grapefruit juice consumption on vascular protection and bone metabolism: a controlled randomized cross-over study in post-menopausal women to determine the specific role of naringin Habauzit V., Verny M., Milenkovic D., Pickering G., Duale C., Bayle D., Thien S., Rambeau M., Mazur A., Horcajada M., Dubray C., and Morand C.
S20O05	Effect of technological treatments on the bioavailability of flavanones from orange Vallejo F., Tomás-Navarro M., Carbonell J.V., Navarro J.L., and Tomás-Barberán F.A.
S20O06	Evaluation of the anticancer activity on prostate cancer by low molecular weight citrus pectin Lu S., Chen J., Yin Y., Xia Q., Zhang J., and Yang Y.
S20O07	A molecular approach to characterize the accumulation of ascorbic acid in citrus fruits Alós E., Legaz P., Rodrigo M.J., and Zacarías L.

Poster ID	Title
S20P01	Protective effect of grapefruit naringin on atherosclerosis development and identification of the underlying mechanisms Milenkovic D., Chanet A., Habauzit V., Bennetau-Pelissero C., Berard Am., Mazur A., and Morand C.
S20P02	Effect of membrane processing on the radical scavenging activity of juice from IGP product *Citrus limon* from Rocca imperiale (South Italy) Loizzo M.R., Tundis R., Bonesi M., Pugliese A., Di Sanzo G., Balducchi R., Verardi A., and Calabro V.
S20P03	The effect of time and refrigeration on bioavailability of flavanones in healthy volunteers after fresh squeezed orange juice consumption Tomas-Navarro M., Vallejo F., and Tomás-Barberán F.A.
S20P04	The leaves of Mediterranean mandarin could be used as an important source of hesperidin, nobiletin and tangeretin Durand M.V.S., Fanciullino A-L., Jay-Allemand C., Ollitrault P., Froelicher Y., and Bidel L.P.R.
S20P05	Comparative study of fruit flavonoids in ten citrus hybrids Ran Y., Zhao Q.Y., Su X.S., Chen W.J., Jiang D., and Jiao B.N.
S20P06	Over-consumption of satsuma mandarins is associated with Shang Huo reaction: results from case-control studies Ma Z.C., and Deng X.X.
S20P07	Dissipation curves of cypermethrin in whole fruit and mandarin pulp in Argentina Kulczycki Waskowicz C., Sosa A., Beldoménico H., García S., and Repetti M.R.
S20P08	Dissipation and residue of forchlorfenuron in citrus fruits Chen W.J., Su X.S., Zhao Q.Y., and Jiao B.N.
S20P09	Dissipation and residue of 2,4-D in citrus fruits Chen W.J., Su X.S., Zhao Q.Y., and Jiao B.N.
S20P10	Effect of pesticide spray volumes and doses on the production of marketable lemons in Tucumán, Argentina Salas López H., Mansilla C., Figueroa D., Carrizo B., Rojas A., Campos A., and Goane L.

SESSION 21: Postharvest and juice processing technology

Oral presentations at Auditorium 3, on Monday 19th from 12:00 to 13:30
Poster session on Thursday 22nd

Conveners: Dr. Alejandra SALVADOR (Instituto Valenciano Investigaciones Agrarias, Spain) and Dr. Paolo RAPISARDA (Centro di Ricerca per l'Agumicultura e le Colture Mediterranee, CRA-ACM, Italy)

Oral ID	Title
S21O01	Biochemical and molecular mechanisms involved in mandarin flavor deterioration after harvest Tietel Z., and Porat R.
S21O02	Traceability of citrus fruit using isotopic and chemical markers Fabroni S., Amenta M., and Rapisarda P.
S21O03	Fluctuation of limonin and nomilin content in different tissues during fruit development of three sweet orange varieties Wu H., Tan A., Wang H., Ma Y., Sun Z., Huang X., and Guo L.
S21O04	Post-harvest management of citrus fruit in South Asian countries Ladaniya M.

Oral ID	Title
S21O05	A technological platform for the valorization and recovery of outsize and defective PGI citrus fruits from South Italy Mazzuca S., Spadafora A., Serra I.A., Bernardo L., Balducchi R., Di Sanzo G., Maccioni O., Verardi A., and Calabro V.
S21O06	Industrial orange juice (var. 'Salustiana') debittering: effects on sensory properties Fernández-Vázquez R., Stinco C.M., Hernanz D., Heredia F.J., and Vicario I.M.

Poster ID	Title
S21P01	Anthocyanins in citrus Fabroni S., Amenta M., Rizza G., and Rapisarda P.
S21P02	Organic acids metabolism in postharvest citrus fruit Cheng Y.J., Sun X.H., Zhu A.D., Xu J., and Deng X.X.
S21P03	Ethylene degreening treatment under commercial conditions does not cause loss of nutritional and aroma quality of citrus fruit Sdiri S., Navarro P., and Salvador A.
S21P04	Change in nonvolatile flavours of blood and common orange fruits during cold storage Fabroni S., Amenta M., Todaro A., and Rapisarda P.
S21P05	Antioxidant capacity and total phenolic contents of bergamot (*Citrus bergamia*) Yilmaz K.U., Uzun A., Ercisli S., Sengul M., Yildiz H., and Uysal O.
S21P06	Simulation of cold treatment during a cargo shipment of citrus fruits Tauriello A., Di Renzo G.C., Altieri G., Strano M.C., Genovese F., and Calandra M.
S21P07	Application of 3,5,6-TPA under commercial conditions to control calyx senescence associated to degreening treatment Tormo D., Conesa E., Sdiri S., Navarro P., and Salvador A.
S21P08	Postharvest behavior of new mandarins cultivars obtained at the IVIA Sdiri S., Navarro P., Cuenca J., Pardo J., and Salvador A.
S21P09	Characterization of fruits of four different lemon cultivars, collected in the Northern coast of Sicily Cupane M., Guarrasi V., Palazzolo E., San Biagio P. L., and Germanà M. A.
S21P10	Postharvest green mold control by hot water dips in kumquats Vázquez D., Cocco M., Bello F., Panozzo M., and Meier G.
S21P11	Fate of foodborne pathogens in minimal processed orange and reduction of their growth using UV-C illumination Lourenço A., Salazar M., Graça A., Quintas C., and Nunes C.
S21P12	Evaluation of the effect of ultrasonic variables at locally ultrasonic field on extraction of hesperidin from Penggan mandarin (*Citrus reticulata*) peels Ma Y.Q., Wu H.J., Ye X.Q., Wang H., and Sun Zh.G.
S21P13	Industrial orange (var. 'Salustiana') juice debittering: effects on the color and bioactive compounds Stinco C.M., Fernández-Vázquez R., Hernanz D., Heredia F.J., Meléndez-Martínez A.J., and Vicario I.M.
S21P14	The effect of fermentation on antioxidant content (ascorbic acid, carotenoids and flavonoids) and antioxidant activity (abts and dpph values) of orange juice Escudero B., Cerrillo I., Hornero-Méndez D., Herrero-Martín G., Berná G., Martín F., and Fernández-Pachón M.S.
S21P15	Study on microwave sterilization characteristics of NFC orange juice Wang H., Ma Y.Q, and Wu H.J.

Poster ID	Title
S21P16	Equipments automation in citrus canned processing line Zhang J., and Lu S.
S21P17	Extension of the project for obtaining bioethanol from citrus waste Conesa C., Fito P., and Fito P.J.
S21P18	Sensory analysis as an instrument for colour measurement in satsuma and 'Nova' mandarin fruits Bello F., Vázquez D., Almirón N., Meier G., and Cocco M.
S21P19	Discrimination of three *Citrus paradisi* cultivars by electronic nose Guarrasi V., Bernik D., Bulone D., San Biagio P.L., Germanà M.A., and Negri R.M.
S21P20	Effect of several clay content in chitosan-clay nanocomposite coating on chemical and mechanical properties of orange fruit comparison with conventional coating during storage Torabi A.R., Hashemi S.J., and Hashemi Z.S.
S21P21	Reducing decay and physiological disorders in oranges stored at suboptimal temperature by combined treatments Artes Calero F., Velázquez P., and Artés Hernández F.

SESSION 22: Citrus economics and trade

Oral presentations at Auditorium 3, on Tuesday 20th from 16:15 to 18:15.
Poster session on Thursday 22nd

Conveners: Dr. Elena MELIÁ MARTÍ (Universidad Politécnica de Valencia, Spain) and Dr. Orlando S. PASSOS (Embrapa Cassava and Fruit Crops Center, Nugene-Citros, Brazil)

Oral ID	Title
S22O01	The development of citrus industry in China during the past decade Deng X.X.
S22O02	Risk assessment of citrus yield based on yield minus deviate index and risk probability Huang S.
S22O03	Recent trends of the citrus industry in Chile Ortúzar J.E., and Valenzuela M.
S22O04	A critical economic analysis of advanced citrus production systems (ACPS) Roka F.M.
S22O05	Spanish citrus cooperatives: keys to success and challenges for the future Meliá Martí E., Server Izquierdo R.J.,and Lajara-Camilleri N.
S22O06	Evolution of development models for Italian organic citrus growing: economic and environmental aspects Scuderi A., and D'Amico M.
S22O07	The importance of research and technical services in the recent growth of the Southern African citrus export industry Hattingh V.

Poster ID	Title
S22P01	Trends in varietal composition in Spanish citrus-growing and underlying variables Caballero P., Fernandez-Zamudio M.A., Carmona B., Alcon F., and de Miguel Gomez M.D.
S22P02	Evolution of the international citrus trade in the Valencian region Aznar Puente J.L., García Martínez G., Silvestre Esteve E., and Orea Vega G.

Poster ID	Title
S22P03	The citrus chain in Entre Rios: characterization and economic impact of some technologies to increase competitiveness and equity Vera L.M.V., Díaz Vélez R.D.V., Banfi G.B., Garrán S.M., and Gómez C.G.
S22P04	The experience of the Entre Ríos Citrus Board, and its contribution to the citrus industry Vera L.M.V.
S22P05	Overview of organic citrus production in the Algarve (Portugal) Reis D., Fernandes M.M., Marreiros A., Mourão I., and Duarte A.
S22P06	Citrus and pomegranate cultivation: concurrence *vs* competition in eastern Spain Melian Navarro A., De Miguel Gómez M.D., and Fernández-Zamudio M.A.
S22P07	PDO and PGI citrus productions: state of the art and perspectives Scuderi A., and Pecorino B.
S22P08	Comparisons of fruits load estimation methods using geostatistical techniques Gimenez L.I., Cabrera Brunetti S.C., Avanza M.M., and Mazza S.M.
S22P09	Price evolution of 'Clemenules' and 'Navelina' in Spain in the period 2007-2012 Server Izquierdo R.J., García-Martínez G., Lajara-Camilleri N., and Orea-Vega G.
S22P10	São Francisco Valley as a new belt for the Brazilian citrus industry Passos O.S., Bastos D.C., Soares Filho W.S., Girardi E.A., and Leão H.C.

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Plenary Conferences

PC01 The Spanish citrus industry
Luis Navarro

PC02 Citrus and health
Gary Williamson

PC03 The importance of citrus for the juice and beverage industry
Ademerval Garcia

PC04 Food safety, social compliance and sustainability, in relation to commercial fruit and vegs strategies with special reference to citrus
Gé Happe

PC05 The experience of Huanglongbing control in Brazil
Antonio J. Ayres

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The Spanish citrus industry

Luis Navarro

Instituto Valenciano de Investigaciones Agrarias (IVIA). E-mail: lnavarro@ivia.es. Moncada, Valencia, Spain

Citrus have a long history in Spain. Citrons were introduced by the Romans during the V century, sour oranges, lemons, and pummelos by the Arabs during the X and XI centuries, the first sweet oranges by the Genovese traders during the XV century and then higher quality varieties by the Portuguese in the XVI century, the mandarins during the XIX century, and finally, grapefruits from U.S.A. in the early XX century. Initially citrus were used as ornamental plants and for medicinal purposes, and after introduction of sweet oranges also for direct consumption at very local scale. Commercial plantings and exports of fresh fruit to other European countries started at the end of the XVIII century.

In the past, the Spanish citrus industry has been conditioned by diseases. The first important problem appeared at the end of the XIX century, when about 4,000 ha of citrus were grown as seedlings or grafted on sweet orange, lemon, citron and sour orange rootstocks. Observations published in 1892 indicated that trees were dying from a disease, much later identified as *Phytophthora* root rot. In 10-15 years all trees in commercial plantings died, except those grafted on sour orange, which is tolerant to this pathogen. Consequently, all new plantings were established on sour orange rootstock, which has an excellent horticultural behavior and is tolerant to most biotic and abiotic stresses. Indeed most citrus growing countries followed the Spanish example and sour orange became the predominant citrus rootstock worldwide. Later this created a serious problem due to its high susceptibility to decline induced by *Citrus tristeza virus* (CTV).

In the period 1910-1930 new higher quality cultivars were imported from different countries that were used for new plantings and also to change old cultivars by topworking. Evidence indicates that many of the imported cultivars were affected by viruses, including CTV, and viroids that spread throughout the country.

The first CTV outbreak in Spain was recorded in 1957, and since then it has produced the death of more than 44 million trees of sweet oranges and mandarins grafted on sour orange. At the end of the 1960's the total surface of citrus was about 210,000 ha and citrus exports were the main source of foreign currency for the country. The risk of a socio-economic disaster caused by CTV was so high that the Government set up very severe rules to reduce losses produced by the virus. The use of sour orange was prohibited in new plantings and propagation of plants at citrus nurseries was strictly regulated, leading to a drastic reduction in its number from thousands to nine.

Early surveys and indexing disclosed that other virus and virus-like diseases were widespread in all Spanish cultivars. Psorosis, impietratura, and concave gum were producing important damage. Exocortis infected all cultivars and consequently, Troyer citrange, the best tristeza-tolerant rootstock under the Spanish growing conditions, could not be used for propagation. It was estimated that in addition to tristeza, other virus and virus-like diseases produced losses estimated at 10-25% of the total production.

In the early 1970s graft-transmissible pathogens were the major limitation for the development and even for maintenance the Spanish citrus industry. It became evident that control of virus and virus-like diseases required the use of pathogen-free cultivars grafted on tristeza tolerant rootstocks. Since all the Spanish cultivars were infected, the first step was to recover healthy plants from those cultivars. The development of shoot-tip grafting in vitro (STG) to recover healthy citrus plants allowed in 1975 to set up a program with the objectives of recovering pathogen-free plants from all cultivars grown in Spain, establishing a germplasm bank with pathogen-free plants, and releasing pathogen-free budwood to nurseries through a mandatory certification program. Later, in 1983, a new quarantine procedure based on STG was developed and legislation was changed to allow the safe importation of citrus budwood. Thus, introduction of genotypes for commercial and scientific purposes was included as an additional objective of the program.

Today the germplasm bank has more than 600 accessions protected in screenhouses that are the initial material used by all nurseries to produce plants under the certification program. Nurseries started to sell certified plants originated in the Program in 1982 and until now they have sold about 140 million plants, representing more than 97% of the Spanish citrus industry.

Drip irrigation introduced in the 80's allowed cultivation in hilly and relatively poor soils, thus allowing an important expansion of citrus to new areas. The availability of healthy plants, together with important improvements of cultural practices produced an increase in yields and fruit quality.

Today Spain has about 330.000 hectares of citrus, with an average production of 6.5 million tones/year, with sweet oranges representing 48%, mandarins 35% and lemons 16%, although the tendency in new plantings is to reduce sweet oranges and increase mandarins, according to the preferences of consumers. Spain is the first exporting country of fresh fruit, with more than 50% of its production being commercialized abroad. About 20% of the production is consumed in the internal market also as fresh fruit, 18% is processed and the rest is waste. Citrus plantings are mainly located along the Mediterranean coast in the provinces of Tarragona, Castellón, Valencia, Murcia, Almeria and Málaga, in the Guadalquivir river valley in the provinces of Córdoba and Sevilla and in the Atlantic coast in the province of Huelva.

Since the Spanish citrus industry has been completely renewed in the last 30 years with healthy certified nursery trees, graft and vector transmitted diseases do not pose significant problems any longer. Today, the only disease problem is caused by *Alternaria alternata*, which produces significant damage in susceptible mandarin varieties like Fortune that is being replaced by others.

Cultivars have always been a key aspect of the Spanish citrus industry due to the need to adapt production to the international market demands. The competitiveness is dramatically increasing in citrus international markets and economic survival of the Spanish citrus industry highly depends on availability of new improved cultivars that meet the increasing market demand for higher quality fruits. The main varieties selected in Spain or imported through the Quarantine Station are available for commercial propagation. However, there is still an important demand for new late maturing seedless mandarin cultivars. Recently two triploid cultivars with these characteristics, 'Garbí' and 'Safor', have been released and are being widely accepted by the growers.

Without significant technical constraints, the Spanish citrus industry faces several new problems. Production cost is very high compared to those of direct competitors' countries, mainly due to high labor costs but also to the small size of plantings in traditional citrus areas. The amount of different fruits reaching the markets in some periods is higher than the demand and this reduces the price, drastically diminishing benefits of the growers. To overcome this situation it is very important to increase research activities to obtain new products of higher quality at lower costs.

PC02

Citrus and health

Gary Williamson

School of Food Science and Nutrition. University of Leeds. Leeds, United Kingdom

Although diet clearly affects health and disease risk in many ways, the beneficial effects of fruit and vegetable consumption is now a well established concept. The effect of individual components of the diet on health is difficult to entangle, since the diet consists of many components and disease progression is a biologically and biochemically complex process. In general, citrus fruit contributes to the intake of "5-a-day". Specifically oranges and other citrus fruits contain several components which may affect health in a beneficial way. Of these, vitamin C, carotenoids and polyphenols have received the most intensive research efforts in recent years. These components are retained during gentle processing of citrus fruits into juice. Hesperidin and naringenin are the most abundant polyphenols, and are found at very high levels in oranges and grapefruit respectively, and are unique to citrus fruits. Orange juice can contain hundreds of milligrams per portion of hesperidin, and this intake can make a major contribution to the overall dietary intake of polyphenols in many countries. Hesperidin consumption helps preserve vascular function and reduces blood pressure in overweight individuals, and reduces endothelial dysfunction. Hesperidin is absorbed in the colon and is dependent upon the presence of colonic microflora. Vitamin C performs several functions in the body, is essential for prevention of scurvy and may have additional health benefits related to antioxidant action. The lipid-soluble carotenoids also have certain antioxidant health benefits and are beneficial for cardiovascular function. These different components from citrus fruits provide benefits to health which can help to reduce the risk of chronic disease and maintain the general healthiness of the population in many countries.

PC03

The importance of citrus for the juice and beverage industry

Ademerval Garcia

Grove 2 Glass Trading GmbH, a 'The Coca-Cola Company' subsidiary. Zürich, Switzerland

The Juice Industry - as any other industry – needs to manage the short-term while planning, executing and managing the long-term needs. Over-emphasis on the short-term leaves us exposed on those longer-term challenges that are quite specific to our Industry – nature does not operate on a quarterly basis - and many of the decisions that we are required to take to ensure the future prosperity of our business need a multi-year and multi-decade perspective and financial commitment. So while the short-term outlook for sales is both challenging and volatile, we need, as companies and as an Industry, to continue to plan for the future.

Our vision of operating in Juices is the result of a three pronged strategy applied consistently wherever we operate:

- Sustainable sourcing: We have set out to partner with growers, processors and juice suppliers to ensure long term supply of juice at competitive prices, while minimizing our environmental footprint, benefiting the communities where we operate and ensuring we receive juice that consistently meets our stringent quality standards.
- Implementing efficient operating practices: Marketing fruit juice demands a completely different approach when compared to other beverages: convert juice ingredients into packaged juice & deliver to customers through a "Fit for Purpose" juice operating model supported by the right processes, advanced analytics and sensory science.
- Delivering compelling consumer propositions led by a Global "Master Brand": We have united multiple local brand names under a single mainstream global umbrella brand that shares common Visual Identity System, brand architecture and integrated marketing communication and spans multiple juice product segments.
- A scientific approach to preserve and grow the business: It's our belief that the historical model of segmentation of the Industry in three isolated production chains is not valid anymore. Growing, Processing and Marketing are part of the same effort to secure the viability of the Industry in the long term. And all of those legs of the juice business demand continuous scientific support: to Grow (irrigation, fertilization, plant protection, better varieties); to Process (high yields, less waste, energy conservation, less water usage, quality) and to Market (quality, sensory, obesity, water and carbon footprints). And Social Responsibility covering every single step from the Grove to Glass.
- At the end, the business is supported by four legs instead of three: Grow, Process, Market and Science. As the rest, scientists are not expected to remain isolated on their fields of expertise. An integrated approach inside the various branches of Science is now necessary. Economics are necessary in the pos-greening era of planting; Sensory is necessary in processing; Environmental is necessary in the whole production chain. New scientific fields are a challenge to which the scientific community has to respond quickly.
- A dedicated juice procurement organization: If we are to succeed in building scale and driving growth we have to safeguard the essential fuel of our industry – a continuous supply of the highest quality juice at competitive costs. To ensure this, we set up a dedicated juice procurement arm based out of Zurich –G2G Trading Services GmbH–, with fruit and procurement experts embedded in the key growing regions around the world. Through this team we are building long term relationships with growers and processors.
- Developing new production areas: In some cases we are committed to significant investments in new fruit growing projects, via long term supply agreements thus safeguarding our supply and enabling future growth. We have reinvented our overall approach to juice from the Grove, where our fruit is grown, to the Glass where it is consumed. Our Grove to Glass philosophy is now embedded in everything we do in juice and provides us with both a strong business philosophy and a roadmap for our future growth. And again, Science is most needed to adapt varieties to other environments, to produce more convenient varieties, to increase yields.

Our industry faces many challenges:

Global warming and its impact on climate patterns are influencing the availability, cost and yield of fruit and the increasing impact of disease is a barrier to investment in groves despite high prices and Industry growth. Add to this the increasing consumer intolerance for agricultural chemical residue at any level and we have a set of challenges that are truly unique.

Juice is not immune from the Obesity challenge and the Industry is increasingly having to defend its role in the diet.

Given the rise of social media and social consciousness and the need to deliver on our commitments to sustainability we must as an industry and as individual companies preserve and nurture our license to operate in all of the communities that we touch in an extremely complex global supply chain.

Huanglongbing (HLB) is the greatest threat to the future of our industry, affecting not only oranges but all citrus. But, HLB is not the only threat. Citrus Canker remains a threat, CVC, Tristeza, Sudden Death, fruit flies. It is essential that our scientists, around the globe, remain committed to research on prevention and cure in order to safeguard our future. And it is essential that the governments, society and the industry continue to support the science applied to agriculture and, particularly, to citrus – the biggest item in the whole global juice Industry. And not only orange: the whole citrus. Science is complex, expensive and challenging but essential. Gathering such a high number of talented scientists in this International Citrus Congress shows that the scientific community understands and is up to the challenges – today and in the future.

We need to know where our fruit is coming from and that our agricultural practices are sustainable. Consumers, NGO's, governments and the industry will not accept agricultural practices that threaten water and other natural resources.

We need to work on dealing with our issues and we have to work on marketing our category and the unique benefits that only juice can offer. It is our responsibility to ensure that the generations to come can enjoy the benefits of this wonderful natural product.

PC04

Food safety, social compliance and sustainability, in relation to commercial fruit and vegs strategies with special reference to citrus

Gé Happe

European Sourcing Director Ahold. Zaandam, The Netherlands

Most times, growers blame retailers for their poor revenues (mostly discount chains). But we, as retailer, face a competitive world. Consumer continuously asks for better prices and higher quality. Now in this time of crisis, the price factor becomes really important. But to focus only on price can have serious undesirable consequences. When retailer sells cheaper, he has to buy cheaper. When grower receives lower revenues, he has to produce cheaper, usually paying less attention to quality as "nobody pays for it". But when finally consumer perceives lower quality at shops, he does not like it and does not buy. And all the money invested in the supply chain, even if lower, gets lost.

Therefore, the model AH has been developing in the latest 50 years is a model of partnership. Quality is a MUST. But at the same time, teamwork with all players in the supply chain, being directed by the same goal (consumer satisfaction), allows to look for all possible efficiencies in the chain, to reduce cost and transmit that effort to consumer. In that sense, consumer is receiving a better quality at a more reasonable price.

However, we are all living in a dynamic context. Citrus varieties evolute and logistics also improve. New shipping lines arise, more a modern frigo containers appear and new fruit terminals are built in new production areas. Then it is easier to sell citrus from wherever production area to wherever retailer in the world. That's globalization and nobody can stop it. We are selling at winter time nearly all our citrus from Spain. And we have to face the concurrence of other retailers selling from other production areas in the Mediterranean. As retailer, we (AH) apply strict policies in food safety and social compliance, so all growers from wherever origin can compete on equal conditions. We understand that reduction of pesticides has an implicit cost. Social Security, safety at work, working in legal and reasonable conditions have costs. But citrus is synonymous of

Health. We have to guarantee our consumers that what they buy in our shops is healthy. And furthermore, that those citrus have been grown packed and transported in legal and safe conditions.

Spain is a privileged area. Since centuries, weather and soil have provided the optimal environment for growing citrus. But the global context also counts. Therefore, we all are obliged to keep on working on the same axes: quality and efficiency. As grower, sometimes size can be a difficulty. There are several production areas, like in the Valencia Region, where orchards average surface is quite small. Maybe getting closer to a professional good organization with good positioning in the market, good knowledge of varieties and trends could help. On efficiency, maybe working together and sharing cultural practices costs could help small growers. I understand it is always better to do everything by yourself, not depending on anyone. But, unless you are big enough and efficient enough, world is pushing in the other direction: teamwork.

To keep a place in the market is always a challenge. And we all have to work hard to achieve it. But I invite you all to take profit of your unique conditions and keep on making consumers from all over the world, enjoying these magnificent fruits you are able to produce.

PC05

The experience of Huanglongbing control in Brazil

Antonio Juliano Ayres

Fundo de defesa da citricultura (FUNDECITRUS). Brazil

Symptoms of Huanglongbing (HLB) were first reported in São Paulo State (SPS) near Araraquara in 2004. However, the insect vector, the Asian citrus psyllid, Diaphorina citri, has been present in SPS since 1940 and is responsible for the spread of HLB to most regions of SPS.

Immediately after HLB was identified in March 2004, Fundecitrus recommended HLB-management in all the citrus farms of SPS as an attempt to control the disease and save the industry from destruction. The management involved three classic measures: (i) insecticide sprays of all trees, several times a year, to decrease the insect-vector population, (ii) identification and immediate removal of symptomatic trees, several times a year, to rid orchards of sources of inoculum, and (iii) replacement of removed trees by young, HLB-free trees from covered, insect-proof nurseries. HLB-management by this “three-pronged system” (TPS) was evaluated by determining the total number of symptomatic trees removed each year in the various blocks. Indeed, successful management would result in decrease of the total number of symptomatic trees removed per year, while unsuccessful management would lead to an increase of such trees.

Since 2004, crucial factors having a strong influence on HLB-management by the TPS have been identified. 1) HLB incidence in the region where the farm is located and 2) HLB incidence in the farm. For instance, when HLB is first detected in a region or country where the psyllid vector has been present for some time (Brazil in 2004, Florida in 2005, Texas and California in 2012) the incidence of the disease is generally low. HLB management by the TPS is easier when the HLB incidence at onset of the management is low (≤ 7%). 3) When a farm with good HLB management is close to a farm with no or poor management, HLB-control in the “good” farm becomes much more difficult because the highly infected psyllids from the “poor” farm invade and contaminate the “good” farm. In this case, more aggressive psyllid control has to be applied in the contaminated periphery of the good farm 4) HLB-control was found to be easier when applied to large orchard surfaces (≥1000ha) rather than to smaller ones. 5) Young orchards which have multiple flushes were more susceptible than mature ones with lesser flushes and required systemic insecticides to achieve better control of the psyllid-vectors. In contrast to the five above factors which are characteristic of the farm at onset of the management, the following factors are those that can be modified and through which management can change the HLB incidence in the farm: 1) Number of inspections for early detection and removal of symptomatic trees. The use of tractor-pulled platforms with two or four inspectors made surveys more efficient and less strenuous. 2) Number of psyllid control operations, nature of insecticides, application methods, prevention of insecticide resistance. 3) Acording to the HLB incidence, removed trees are replaced or not replaced by reset trees. 4) Old, lesser productive citrus blocks are removed to prevent their role as sources of inoculums (restructuring). 5) Last but not least, it was recommended that HLB-management be

regional, i.e. applied on an area-wide scale, including either many growers with small/middle size farms or only a few growers with large farms.

The surveys conducted by Fundecitrus in 2011 on the incidence of HLB in SPS have confirmed that the efforts of the Citrus Industry to control HLB since 2004, when HLB was first identified in SPS, have met with success. The results of these surveys show that more than 200,000ha (500,000acres) of citrus orchards, i.e. more than one third of the total SPS citrus acreage, have an HLB-incidence as low as 1%. This level might even become lower as many citrus orchards with poor management and high HLB-incidence had to be removed or turned into fields of sugar cane: they are no more a serious danger for the well-managed neighboring farms. These 200,000ha of well-managed citrus farms with low HLB incidence prove that HLB–management has been successful in many parts of SPS, including regions of high initial HLB incidence, and that a large part of the Paulista citrus industry has survived HLB. Furthermore, in the years to come, more farms with low HLB-incidence might be seen because HLB-management will probably become easier.

All these farms with low HLB incidence represent the "hard-core-orchards" on which the future of the citrus industry will be constructed. Research is under way in SPS to produce "new generation" citrus trees resistant to HLB or protected from infection by the psyllid-vectors. When these genetically modified trees (GMTs) will become available, in five to ten years, the hard-core-orchards with low-HLB-incidence, will offer the proper conditions for rapid growth and development of the young, new-generation GMTs. It is even well possible that the healthy, non-genetically modified trees from the successful TPS system can be kept in parallel with the GMTS, giving SPS two options for the future: orchards with GMTs in the regions of high HLB incidence and orchards with essentially healthy, non genetically modified trees.

At this moment, Brazil is the only country in the world where HLB control is effective on a large scale. In other parts of the world, HLB could not be met with proper management. Worse, methods have been recommended and/or adopted, which result in a dramatic increase of HLB-incidence. Such methods should not be used in SPS as a substitute for the successful HLB-management developed in the region.

PC06

New genetic and genomic tools for citrus breeding

Mikeal L. Roose

Department of Botany and Plant Sciences. University of California, Riverside, CA, USA

Citrus breeders face many challenges in their quest to develop scion and rootstock varieties that provide growers with productive, disease resistant trees and consumers with tasty, high-quality and nutrient-packed fruit. Three major approaches are used to develop new cultivars: mutation, hybridization, and gene introduction (transgenics). In citrus, mutation breeding has been used mainly to develop low seeded cultivars, but in annual crops it has also been used to improve many other traits because it is easier and less costly to screen very large numbers of plants. Breeders identify rare mutants that express useful traits and then transfer the mutant gene into other cultivars by hybridization methods. In citrus and other fruit crops with long generation times and in which the elite genotypes used as commercial cultivars are very heterozygous, such breeding is so difficult that it has rarely been attempted. Several new developments promise to facilitate this relatively conventional approach to breeding. Cultural practices to shorten the juvenile stage are being more widely used and may reduce the generation time of citrus to about 3 years. Transgenic varieties with reduced juvenility have also been developed and could facilitate transfer of novel mutations into other cultivar types. A shorter generation time makes multiple generation breeding much more feasible. Targeted mutation is another potential approach for citrus breeding. One approach, commonly called TILLING, screens specific genes in a large population of mutagenized plants to identify individuals with mutations in the targeted gene. This method requires knowledge of gene function to identify the target gene or genes, but knowledge of gene function is developing rapidly in citrus and many potential targets are already known. An alternative approach, sometimes called targeted gene modification, is to express genes for enzymes such as zinc-finger nucleases (ZFN) that induce chromosome breaks at specific target sites. Repair of these breaks is frequently imprecise, resulting in small deletions or insertions at the cleavage site. Many such mutations will affect the phenotype. At present, transgenes are introduced that specify these enzymes and they are removed from the genome by segregation in later generations.

In citrus, it would be difficult to recover the elite cultivar (such as a sweet orange) following a cross, so an alternative method to introduce these ZFN genes will be needed. Such genes could be carried on a virus that infects the initial cultivar and is later removed by thermotherapy or shoot-tip grafting before the new mutated cultivar is released. These approaches will allow breeders to reduce or eliminate function of one copy of a gene. To have a phenotypic effect, the mutation would have to have some level of dominance. In annual crops new mutations are commonly made homozygous by selfing or sib-mating, methods that are either not possible in citrus or which yield progeny very unlike the parental cultivar type. Possibly targeted gene modification can be efficient enough to produce mutations in both alleles. It is also important to recognize the limits of these methods – they produce mostly loss-of-function mutations, and not all desired phenotypes can be created by such mutations.

High quality reference genome sequences for citrus have been developed by the International Citrus Genomics Consortium (haploid from clementine), the University of Florida (diploid sweet orange) and Huazhong University (haploid of sweet orange). With these reference sequences available, advances in genome sequencing now make it simple and inexpensive to determine most of the genome sequence of interesting mutants and cultivars. Comparison of sequences from a few varieties indicates the ancestral species that contributed each chromosome segment to hybrids and may even indicate the precise genotype of each parent. Comparative genome sequence information will allow identification of candidate genes that may cause phenotypic differences among cultivars derived by mutation (navel oranges with different maturity dates, grapefruit differing in flesh color etc.). Once genes are known, they can be more easily transferred to other cultivar groups by hybridization-selection, transgenics, or targeted mutation approaches. Breeding by hybridization-selection methods is also increasing in efficiency through use of genome sequence information and methods. Several groups have now developed SNP arrays that can be used to rapidly determine the overall genotype of each individual progeny. Projects that required years of work using “one at a time” markers such as SSRs can now be completed in a few weeks. Genotyping-by-nextgen sequencing methods, in which a defined fraction of genome fragments is sequenced in multiplex, is another approach to this end. Development of these methods, particularly when combined with gene expression studies, will greatly facilitate identification of genes that confer disease resistance, fruit quality and other important traits. Efficient, high-density genotyping should also facilitate identification of progeny plants that resemble specific genotypes such as sweet orange or clementine and thereby allow breeders to develop hybrids that closely resemble these cultivars except for chromosome segments carrying desired genes. These technologies will reduce the “random” element in hybridization-selection breeding, but exploiting them will not be inexpensive because achieving these objectives will require molecular analysis of large populations.

New methods also promise to revolutionize development of citrus cultivars using transgenic approaches. In the past, nearly all transgenes were introduced using methods such as *Agrobacterium* or biolistics that result in integration at more or less random locations in the genome. The genomic context in which a transgene integrates can affect its expression and this contributes to the need to screen a large number of transgenics to find those with appropriate expression levels. Random integration also complicates regulatory review of transgenics. Targeted integration of transgenes is now possible by engineering a “founder line” having a construct with good gene expression and an integration site developed with a recombinase-mediated cassette exchange strategy. Such lines can then be transformed with additional genes that will be integrated at this site. Another significant opportunity is development of cis-genic varieties in which the added DNA originates from citrus rather than from an unrelated species. It is possible to envision systems in which all genes introduced, including selectable markers, originate from citrus sequences. Such varieties may encounter less public opposition than those that contain genes from a non-citrus species. Despite the potential power and promise of these new tools, real progress will also depend on accurate plant phenotyping and subsequent field trials to identify superior cultivars. Success will also be facilitated by sharing information widely in the citrus community because there are many challenges to be addressed and few citrus breeding groups to address them.

PC07

Biological control and citrus: a long time fruitful story

Jacques Brodeur

Plant Biology Research Institute. Montreal University, Montréal, Qc, Canada.

From the earliest days, agriculture has been plague by pests. Growers have to deal with outbreaks of insects, weeds, and plant pathogens. There has been a continual increase in arthropod pest species invading citrus over the past century. Citrus systems are replete with mite and insect pests, both in terms of their abundance and diversity. Growers rely on several approaches to pest control, including mechanical, cultural, chemical and biological methods. In this conference I outline the practice of biological control.

Biological control refers to the use of living organisms, or products derived from living organisms, such as toxins, for control of insect pests, weeds and plant diseases

Biological control is considered by many to be the best alternative to pesticides. Biological control is efficient, safe for humans and the environment, and in most cases cheaper than chemical control. Natural enemies exert a significant, continuous and naturally level of pest population regulation in natural or managed ecosystems. Biological control also refers to the deliberate introduction of specific natural enemies to control specific populations of weeds, plant diseases or insect pests. Such an approach may involve the discovery and release into a crop of natural enemies, and in some instances their production and commercialization as biological control agents. At least 7,000 introductions of natural enemies involving almost 2,700 species have been made worldwide. The most widely used species have been introduced into more than 50 countries. In augmentative biological control, more than 170 species of natural enemies are produced and sold, but some 30 species make up more than 90% of the market worldwide. Biological control has a long tradition of success in citrus. In 1868, the cottony-cushion scale was found on acacia in California. Ten years later the citrus industry was at the verge of collapse because of the scale. Natural enemies were sought in the native home of the pest, southern Australia. This search resulted in the introduction into California in 1888 of a coccinellid predator, Rodolia cardinalis. The voracious beetle rapidly became established and by late 1889 the cottony-cushion scale was no longer regarded as a threat to citrus. The beetle saved the American citrus industry and has since become an icon in biological control.

By creation of partnerships that brought together growers, researchers and biological control companies, the citrus industry has since undertaken a transition from total chemical control to a balance of biological control and integrated pest management. Because the arthropod community in citrus is very complex, it remains challenging to conduct biological control and to predict the impacts arising from interactions among biological control agents, landscape structure and pesticide use. Understanding these factors will help to develop sustainable ecosystem communities that can maintain pest densities below economically damaging thresholds. A current challenge relates to the introduction of exotic pests and the subsequent release of exotic natural enemies. Due to its overall ecological complexity, the citrus orchard is characterized by high level of both stability and capacity for self-regulation. However, this condition is repeatedly jeopardized by a significant increase of exotic citrus pests in all producing areas. There is therefore a growing requirement for improved quarantine services and classical biological control programs that remains the best tool to tackle existing and future alien pest problems. But the biological control sector is facing increasing problems with access to natural enemies since the international Convention of Biological Diversity and its Access and Benefit Sharing regime. This situation has added another level of regulation that may slow, and even stop in certain countries, the use and exchange of biological control agents around the world. Global climate change is another growing concern for pest management in citrus orchard. The increasing temperatures, elevated carbon dioxide and changes in rainfall patterns associated with climate change will affect pest management programs and the movement of exotic organisms. In the near future we will need to modify the carefully developed pest management programs to respond to climate changes. The citrus industry will soon have to deal with changes in pest and beneficial species assemblages, developmental ranges and degree of synchrony between interacting species.

Biological control creates and sustains public good – food security, food quality, reduced pesticide use, human (farmer and worker) health, invasive alien species control, protection of biodiversity, and maintenance of ecosystem services. However, the adoption of biological control worldwide has lagged behind our scientific understanding of its potential. Development of biological control for citrus is a dynamic, constantly-evolving challenge. Perseverance is critical.

12 INTERNATIONAL
CITRUS CONGRESS
VALENCIA / SPAIN

Workshops

W01. HLB Control
Convener: J. Bové

W02. New mandarin varieties
Convener: P. Aleza

W03. Procedures for the exploitation of protected and/or patented varieties
Convener: M. Iborra

W04. New perspectives in pest control
Convener: A. Urbaneja

W05. Dwarf citrus trees in high-density plantings
Convener: K.D. Bowman

W06. Molecular identification of varieties
Convener: T. Shimizu

W07. Mechanization of citrus harvest
Convener: E. Moltó

W08. Global citrus industry collaboration on MRL regulatory issues
Convener J.R. Cranney Jr.

W09. Global conservation strategy for citrus genetic resources
Convener: K. E. Hummer

W10. Research activities to support the potential biological effects of citrus fruits in human health
Convener: Francisco A. Tomás-Barberán

W11. Quarantine security for tephritid fruit fly pests in citrus
Convener: N.J. Liquido

W01

HLB control

Convener: J.M. Bové

Université de Bordeaux Ségalen. INRA Center, Bordeaux-Aquitaine (France). E-mail: joseph.bove@wanadoo.fr

The University of Florida estimates that in the State, HLB has resulted in the loss of more than 6,600 jobs, $1,3 billion in revenue to growers and $3,6 billion in economic activity. Can HLB be controlled, how and when? Answering these questions is the goal of the workshop! Two forms of HLB have to be considered: (i) high temperature-sensitive African-HLB, with *Candidatus* (Ca.) Liberibacter (L.) africanus and the African Citrus Psyllid, *Trioza erytreae*, and (ii) high temperature-tolerant Asian-HLB, with Ca. L. asiaticus and the Asian Citrus *Psyllid, Diaphorina* citri (2). African-HLB is presumed to be less aggressive than Asian-HLB.

There is little or no evidence of genetic resistance to HLB in citrus. Apparently, citrus has had only a recent association with Liberibacter species, an association too short to have built up resistance to the bacterium. Hence, according to a general consensus, resistance to HLB will probably be obtained by engineering, into citrus, genes with anti-Liberibacter and/or anti-psyllid activity (8). However, such HLB-resistant cultivars will probably not become available to the growers for several years and, in the meantime, solutions must be developed to control HLB and save the existing citrus industry from destruction (1, 3, 9). Thus, for HLB control, short-term systems for "today" and long-term systems for "tomorrow" must be discussed.

Short-term systems. Even though they are called "short-term", these systems, when efficient, will probably have to be used for several years. Various systems are known and listed. **(i)** The classic, Three-Pronged Package (TPP) has been recommended in China since the 1950s and is comprised by (a) insecticide treatments against psyllids, (b) detection and removal of symptomatic trees, and (c) resets with disease-free trees from insect-proof nurseries. TPP is a "preventive" control system; as it "prevents" trees from becoming infected. Application of the TPP in São Paulo State, Brazil, since 2004 when HLB was first identified, has resulted in two hundred thousand hectares of citrus with less than 1% of HLB-affected trees, even in areas where the initial HLB incidence was high. In Florida, growers were reluctant to remove symptomatic trees and the TPP system has not gained much acceptance, except in some large citrus farms (6). Factors having a significant influence on the efficiency of the TPP system, including restructuring, will be highlighted (1). For instance, HLB control was found to be easier in large farms than in smaller ones. "Regional" management overcomes this problem in Brazil. In Florida, "Area Wide Control of Psyllids" has been developed and "Citrus Health Management Areas" have been established (8). Ways to improve the detection of symptomatic trees (rapid field detection methods, hyperspectral imaging, biomarkers, etc) and the treatments against the Asian and African citrus psyllid (biological control, insecticides, application methods, prevention of insecticide resistance, flush control, overwintering sites, etc) should be discussed. **(ii)** Foliar sprays of micronutrients, with or without inducers of Systemic Acquired Resistance (SAR), are also believed to give immediate protection against HLB. However, this system in which no tree removal is practiced, rapidly results in a high percentage of HLB-infected trees, in spite of good psyllid control (5, 9). The effect of nutritional treatments on HLB-control, fruit-quality, bacteria-titer and disease-progress has been challenged recently and found inconsequential (5). **(iii)** In the Mekong delta of Vietnam, farmers have found that the presence of guava trees surrounding citrus trees (interplanting) prevents or at least retards HLB. Volatiles from guava repel the Asian citrus psyllid, thus explaining the guava effect. However, recent experiments in Vietnam have shown that efficacy of guava interplanting on HLB is limited, as the effectiveness of guava against disease invasion breaks down after one year, probably because citrus trees outgrow guava trees (7). **(iv)** Advanced Citrus Production System (ACPS). The aim of the ACPS is to shorten and enhance the citrus production cycle through high density plantings, as well as intensive computerized daily fertirrigation (Open Hydroponics System) for automated irrigation and intensive nutrient management. (v) Additional short-term systems might be brought up from the floor and discussed.

Long-term systems. Long-term systems are still at the research and/or the experimental level, even though some are already under evaluation in the greenhouse and/or the field. These systems involve the production of genetically modified citrus plants expressing anti-Liberibacter and/or anti-psyllid genes (8). In one system, these genes are inserted into the citrus genome through classic Agrobacterium-mediated transformation. Transformation of mature citrus tissue ensures that the resulting transgenic cultivar is true to type, but seems to be more difficult to achieve than transformation of juvenile tissue. In a second, more recent system,

meant for regions where citrus tristeza virus (CTV) is endemic, the anti-HLB gene is first incorporated into the genome of CTV, thus using the virus as a gene vector (4). Inoculation into citrus of the CTV strain carrying the ant-HLB gene results in viral replication and expression of the viral proteins as well as those of the anti-HLB protein from the anti-HLB gene.

Genes used for resistance to Liberibacter spp. have essentially been anti-bacterial peptide genes from various sources (including plants) and genes involved in systemic acquired resistance. Recently, single-chain-fragment-variable (SCFV) antibodies have been produced. For the first time, SCFV antibodies are now available against almost any Liberibacter protein. Candidate SCFV antibodies for resistance to HLB are those directed against proteins found to be essential for Liberibacter growth and replication. Combination of the CTV gene vector and the SCFV anti-HLB antibodies might become a most efficient anti-HLB system. Liberibacter phage for potential control of HLB has also been proposed.

Anti-psyllid transgenic cultivars are developed to express volatile chemicals repelling the psyllids. Double-stranded RNA corresponding to an essential psyllid gene can also be used to transform citrus cultivars, hopefully resulting in mortality and suppression of the feeding psyllids through RNA interference

However, all these genetically modified plants (GMP), would have to be accepted by the consumers (from Europe in particular) before products from such GMPs can be marketed.

References

(1) Belasque Jr. et al. 2010. Lessons from Huanglongbing management in São Paulo State, Brazil. Journal of Plant Protection 92: 285-302.
(2) Bové, J. M. 2009. www.ivia.es/iocv Citrus diseases, Huanglongbing, text and image gallery.
(3) Chamberlain, H.L. 2010. Importance of awareness to growers, nursery growers and residents for the control of HLB and its vector. 2nd international workshop on Huanglongbing and Citrus Asian Psyllid, Merida, Yuc., July 2010, <www.senasica.gob.mx/?doc=18380>.
(4) Folimonov, A.S. et al. 2007. A stable RNA virus-based vector for citrus trees. *Virology*, 368:.205-216.
(5) Gottwald, T. R. et al. 2012. Inconsequential effect of nutritional treatments on Huanglongbing control, fruit quality, bacterial titer and disease progress. Crop Protection 36:73-82.
(6) Irey, M.S. 2010. Experiences of one Florida grower with the management of Huanglongbing—Success over time. 2nd international workshop on Huanglongbing and citrus Asian psyllid, Merida, Yuc., July 2010, <www.senasica.gob.mx/?doc=18380>.
(7) Katsuya, Ichinose et al. 2012. Limited efficacy of guava interplanting on citrus greening disease: Effectiveness of protection against disease invasion breaks down after one year. Crop Protection 34:119-126.
(8) National Research Council. 2010. Strategic Planning for the Florida Citrus Industry: Addressing Citrus Greening Disease. Washington, DC: The National Academies Press.
(9) Timmer, L. W. et al. 2011. It's not too late – yet. Citrus Industry, January 2011: 6, 7.

W02

New mandarin varieties

Convener: Pablo Aleza

Centro de Protección Vegetal y Biotecnología. Instituto Valenciano de Investigaciones Agrarias (IVIA). Moncada, Valencia (Spain)
E-mail: aleza@ivia.es

One of the major issues occurring during the last three decades was the increase of the fresh fruit market of mandarins. For clementines and satsumas, new cultivars have only been selected from spontaneous or induced mutations while both mutant selection and breeding programs have allowed varietal progress in other type of mandarins.

In relation with specific market demands and environmental conditions (biotic and abiotic constraints), the main goal of breeding may vary strongly between the production areas. Expanding the harvesting period with high quality seedless fruits is currently the main objective of new mandarin varieties for the fresh fruit market. This market promotes organoleptic qualities (aroma, taste, acidity, sugar) and pomological qualities (easy to peel, seedlessness, external appearance). The definition of organoleptic quality can vary according to the consumers. Citrus breeders must therefore endeavour to develop a wide range of varieties likely to meet these diverse needs. Nutritional quality based on vitamin C, carotenoid and polyphenol contents are now considered as breeding criteria in some projects. Some diseases cause considerable damages in orchards. Huanglongbing in Asia, South Africa and recently in Brazil and Florida, citrus canker in most tropical and subtropical areas, cercosporiosis in Africa, citrus variegated chlorosis and Sudden death in Brazil. Alternaria also becomes a problem for some

mandarin cultivars such as `Fortune´ in Spain. Ranges of varietal susceptibility have been established for most of these diseases and tolerant parents are selected in some breeding programs.

Conventional breeding in citrus has important limitations due to the complex reproductive biology of these species. Most genotypes are apomictic and adventitious embryos develop directly from nucellar cells limiting or precluding the development of zygotic embryos. This limits the recovery of large sexual populations and in practice apomictic genotypes are avoided as female parents in many programs. Several high quality genotypes have pollen and /or ovule sterility and thus cannot be used as parents in breeding programs. Self and cross-incompatibility are relatively common among many genotypes, also limiting the possibilities to select parents for specific crosses. They have a very long juvenile phase and in most species at least five years are required to star flowering and many more to completely loose the undesirable characters associated with juvenility. All this aspects, together with large progeny size hamper setting up breeding schemes over several generations and are the main reason for the relatively low success of conventional breeding programs carried out so far.

Citrus breeders, taking advantage of vegetative propagation, put their main effort in the research or induction of polymorphism on one cycle from which they made clonal selection. Selection relates thus, either to spontaneous mutation identified in the orchards, or on genotypes obtained by hybridizations, induced mutagenesis or after recourse to biotechnologies that have been strongly developed in citrus in part to solve the problems found in conventional breeding. The main methodologies used are:

- **Mutants' induction**

  Selection of spontaneous mutations is the oldest citrus breeding method and most of the varieties cultivated worldwide arose from this process. In Spain, Morocco and Corsica, it has provided good results for clementine, by extending the production period and enhancing fruit size and color. On a much longer time scale, similar results have been obtained for Satsuma mandarins in Japan.
  A number of experiments of induced mutagenesis were carried out since 1935 for cultivar improvement. Gamma irradiation has been the most common method of mutagenesis. Examples of mandarin cultivars obtained by irradiation programs are the low-seeded selection of `Murcott`, called `Mor´, low-seeded selection of `Clemenules´ clementine named `Nulessin´, and the seedless mandarin `Tango´ produced by irradiation of `Afourer´ (Nadorcott) budwood.

- **Sexual breeding at diploid level**

  Sexual breeding is mainly used for diversification in mandarins. The main limitation of this strategy is that most of the diploid hybrids are fertile and thus seedy. The selection of seedless cultivars displaying high quality and good yield requires the evaluation of very large progenies. Moreover, if seedlessness of these new hybrids is based on self-incompatibility or male sterility, important problems should be encountered in areas where self-incompatible varieties such as the clementine are the predominant production.

- **Seedlessness and ploidy manipulation for triploid creation**

  The selection of triploid lines is a very interesting way to develop seedless cultivars. Indeed triploidy is generally associated with both male and female sterility. Thus, most of the trees of a triploid progeny under field evaluation present these characters and an efficient selection can be carried out in other traits.
  Several methods have been developed for triploid citrus creation. One of them exploit natural events of polyploidization such as 2n gametes, using embryo rescue and flow cytometry to select triploids in 2x X 2x crosses. The most classical strategy is to cross diploid non-apomictic females with tetraploid males. Such tetraploid plants can be found in apomictic seedlings (natural doubling of the chromosome stock of nucellar cells) or are created by somatic hybridisation. Tetraploid non-apomictic lines have been obtained by colchicine treatment of shoot tips grafted in vitro and, these tetraploids open the avenue to 4x X 2x crosses with tetraploid female parent. As an example, in the Spanish mandarin triploid breeding program more that 15,500 triploids have been recovered using the three crossing strategies and using routinely in vitro embryo rescue and flow cytometry to determine the ploidy level of regenerated plants. In the last 10 years several new triploid cultivars resulting mostly from the oldest breeding strategy (diploid x autotetraploid sexual crosses) have been released in Italy, USA, and Japan, and some of these cultivars are now being produced for the market. Recently, four new triploid mandarin cultivars recovered after 2x x 2x crosses have been released in Spain.

- **Somatic hybridization**

  Plant somatic hybridization via protoplast fusion has become an important tool for ploidy manipulation in plant improvement programs, allowing researchers to combine somatic cells from different cultivars, species, or genera, resulting in novel allotetraploid and autotetraploid genetic combinations. This technique can facilitate conventional breeding, gene transfer, and cultivar development by bypassing some problems associated with conventional sexual hybridization including sexual incompatibility, nucellar embryogenesis, and male or female sterility. The most important application of somatic hybridization in plant breeding programs is the building of novel germplasm as a source of elite breeding parents for various types of conventional crosses. In Citrus, somatic hybridization is being used to generate key allotetraploid breeding parents for use in interploid crosses to generate seedless triploids and for creation of new cybrids and asymmetric hybrids. Countries supporting programs based in this technology include USA, Brazil, Japan, China, France, Italy, Brazil, New Zealand and Spain.

- **Genomics**

  Due to the generally important heterozygosity of genitors and long juvenile period, the development of markers for oriented introgression and early selection is a key point to improve the efficiency of citrus sexual breeding. Clementine haploid sequence is available (www.phytozome.net) and the clementine genetic map has been recently publicised. With these new tools citrus breeders will be able to survey and comprehend the citrus genetic diversity with the objective to assist and enhance breeding programs.

- **Genetic transformation**

  Genetic transformation allows the introduction of specific traits into known elite genotypes without altering their genetic background. In the mandarin groups like clementines and satsumas, and due to their highly heterozygous and complex genetic structure, genetic transformation should be considered as a very promising tool for improvement despite of the negative social acceptance of these plants.

Advantages and disadvantages of the different procedures for different breeding objectives will be discussed in the workshop, and also their relationship with the legislation related to essentially derived varieties, that in some cases may preclude the protection of breeder´s rights of new varieties.

W03

Procedures for the exploitation of protected and/or patented varieties

Convener: Mercedes Iborra

Norma Agrícola. Rafelbuñol, Valencia (Spain). E-mail: miborra@normagricola.com

Since the early 19th century, the introduction of new varieties is an essential component to maintain and sustain good and high crop productivity and quality. New varieties are constantly being bred for higher yields, for better agronomic and quality traits like taste, and for resistance to abiotic and biotic stresses.

Plant Breeders' Rights (PBR), also called Plant Variety Protection (PVP), is an exclusive right granted to breeders of new varieties to exploit their varieties and has features that are in common with patents for industrial inventions. Both forms of protection grant to their holders a form of exclusive right to pursue innovative activity. With the PVP legislation, growers are in a better position to have access to new and improved varieties for commercial growing.

Protection of a new variety is granted via a registration process based on validation of plant characteristics that distinguishes it from other varieties. The condition for registration of new plant varieties and the granting of breeder's right is that the plant variety is new, distinct, uniform and stable. The scope of a breeder's right extends to acts carried out on a commercial basis including producing or reproducing, conditioning for the purpose of propagation, offering for sale, marketing, exporting, importing and stocking the material for the earlier activities. Hence, unauthorized conduct of such acts will constitute an infringement under the law.

Today practically all new citrus varieties are being protected, and their exploitation is being done following different models:

- **Open:** All growers have free access after paying royalties (per plant or per surface, ie. 'Pri23', 'Sunnyridge').
- **Restricted:** There is partial access to rights of exploitation by limiting the supply (by surface or by production, ie. ASF club) or by restricted markets (only a few destinations or on certain dates, ie. 'Garbí' or 'Safor' mandarins, so far restricted to the Spanish market).
- **Closed:** No one has free access to the operating rights, except the company that has the rights in the country. (ie. 'Orri', 'Unifrutti' with 'Regal' seedless) or closed to producers who have made custom production (ie. 'Zespri gold').
- **Variety Club**: There is no free access, as the right to development and commercialization has been delivered by the breeder to a closed group of farmers or propagating and exporting companies (ie. 'M7').

In all cases it is very important to take additional precautions, control the varieties information (where they are grown), by producing it only within a given organization or granting licenses or contracts to trusted seed/plants producers, enforce protection, stay alert for unauthorized sales, notify any individuals, companies, or other organizations that sell or distribute your variety.

If the breeders, both public or private, have a good system to protect their PVP, it will be possible to get returns on their investments, which can then be used to develop the next round of improved varieties from their breeding programs. This implies that the use of new varieties is subject to the payment of royalties and involves the protection of the breeder's rights and the variety value. Many breeders and master Licenses of the new varieties are not aware about control mechanisms, so have the risk of uncontrolled propagation of the variety and the loss of incomeIn all exploitation models, it is important to avoid irregular commercial exploitation in order to effectively protect plant breeders' rights. The utilization of systems for planting geolocation has become a critical activity to identify the location and origin of the plantations that have cultivation rights (exploitation licenses), in order to minimize the incidence of illegal activity. It is also of significant importance to know the behavior of varieties in different geographical areas, with the aim of improving their knowledge.

Some producers start to share once they have a protected variety, and a failure to control the spread has generated a mess. The main issue of piracy has a lot to do with auditing or genetic identification techniques, but also with ethical education in respecting IP. Major industry players are very concerned about the issue, and what would happen to their image if some competitors do not play by the rules. A solution to the problem could be the creation of clubs where businesses across the supply chain are identified to minimize risks, but not all varieties are suited to this form. Having the mapping of where the orchards are a profitable information.

Visual NACert will be presented as an example of geolocation systems. It is an innovative, global information system designed initially for protected varieties management that operates through Internet. It easily identifies plantations in the earth's surface and integrates modern technologies of visualization and data management into a single system.

During the workshop the different exploitation models for protected varieties and control systems based in geolocation will be discussed.

W04

New perspectives in pest control

Convener: Alberto Urbaneja

Centro de Protección Vegetal y Biotecnología. Instituto Valenciano de Investigaciones Agrarias (IVIA). Moncada, Valencia (Spain)

Co-conveners: J.A. Jacas[1] and P.A. Stansly[2]

[1]Universitat Jaume I (Spain); jacas@uji.es. [2]University of Florida (USA); pstansly@ufl.edu. E-mail: aurbaneja@ivia.es

Citrus is an international crop, accessible to exotic pests, but also with options to access solutions from around the world. The introduction to California of cottony cushion scale followed by the vedalia beetle in 1888, is just one early example of how citrus entomologists have worked together to develop ways to safeguard this important crop. Recent years have continued to see the spread of serious invasive pests and vectored diseases that challenge our scientific and grower communities. Climate change may also serve to exacerbate impacts from new invaders as well as resident pests. Current options to deal with these challenges are many, from

-omic approaches to food-web engineering. The overall objective is to increase the sustainability of citrus while providing the public with a safe wholesome product that can be justifiably promoted for enhancement of human health. Strategies to be discussed for achieving this goal include:

- **Augmentative and conservation biological control**

  Evergreen agro-ecosystems like citrus support complex food webs with diverse natural enemies that can be conserved and manipulated to maintain most pests below economic injury levels. Studies focused on the role of indigenous natural enemies in the citrus agrosystem and the conservation of both native and exotic natural enemies are being emphasized in recent years. Such studies and the management systems they lead to will probably continue to play more important role in the citrus production worldwide.

- **Selective chemistry**

  While an important goal is decreased dependence on pesticides, not all pests can always be adequately controlled biologically. Selective "smart" chemistry that can selectively suppress difficult pests with minimal collateral damage to non-target organisms will continue to be important tools in the near term as the industry moves toward more sustainable management systems.

- **Semiochemical-based pest management**

  Semiochemicals are the most selective of all chemicals and therefore the most compatible with biologically based management systems. New sex pheromones for monitoring, mass trapping and mating disruption are continually being made available and finding their place in citrus pest management.

- **Pest management based on citrus molecular biology**

  The rapid development of molecular tools and techniques has led to major breakthroughs in entomology that impact agricultural production. These include use of molecular markers for the correct taxonomic identification of citrus invasive pests, the molecular gut-content analysis to identify food web interactions between predators and pests, and detection of resistance to insecticides in different citrus key pests. We can anticipate many new molecular advances, especially in the fields of comparative genomic and metabolomic analyses used for identifying and manipulating candidate pathways involved in the response of citrus to pests and vectored diseases.

These and other new biorational approaches must be combined into functional systems adapted to diverse environmental conditions if current Integrated Pest Management (IPM) programs are to progress in the future. Researchers and IPM practitioners must remain current in order to better serve their clientele and also help inform the general public to better accept new and more sustainable technologies. Experts and practitioners of these and other pest management strategies are invited to present recent advances and join the discussion on ways to maintain citrus profitable and sustainable in the 21st century.

W05

Dwarf citrus trees in high density plantings

Convener: Kim D. Bowman

U. S. Horticultural Research Laboratory. Fort Pierce, Florida (USA). Email: Kim.Bowman@ars.usda.gov

Dwarf citrus trees have the potential to be used in high density plantings that will have greater yield during the first few years after tree establishment, and be easier to care for and harvest as the trees mature. Dwarf citrus may be obtained by management practices that restrict growth of standard cultivars or by genetic dwarfing traits of the scion, interstock, or rootstock. In this workshop, detailed information will be presented on the characteristics of two new USDA-ARS size-controlling rootstocks, considerations in the design and management of high density plantings with dwarfing rootstocks, and the trends in commercial use in Florida. The workshop will also include an overview of rootstock effects on tree size, the potential for producing dwarf citrus trees through the use of a dwarf scion, and management techniques that could be used to restrict tree growth and thus produce dwarf trees for use in high density plantings. Additional information on dwarf citrus and high density plantings will be presented by workshop attendees. The challenges and benefits associated with dwarf citrus trees in high density plantings will be discussed.

Genetic restriction of fruit tree size is most commonly obtained by using a size-controlling rootstock. The most widely recognized citrus size-controlling rootstock is 'Flying Dragon' trifoliate orange (*Poncirus trifoliata*), and this rootstock is currently used to a limited extent in many citrus growing areas to establish high density plantings. Genetic transmission of the dwarfing trait to progeny of 'Flying Dragon' has been reported, and many hybrids have been produced and tested. Two hybrids of mandarin with 'Flying Dragon' have been released by USDA-ARS for commercial use in Florida over the last five years. US-897, a hybrid of *Citrus reticulata* 'Cleopatra' × 'Flying Dragon', was released by USDA in 2007. This rootstock combines the induction of dwarfing on the grafted scion, along with the induction of good fruit production and fruit quality, and good resistance to several disease problems, including citrus tristeza virus (CTV) and tolerance to the Phytophthora-Diaprepes weevil complex (PDC). Tree size obtained with 'US-897' rootstock varies by scion and tree age, but in one typical trial with 'Hamlin' sweet orange (*Citrus sinensis*) scion, 16-year-old trees on 'US-897' were 3 meters tall, while those on standard size rootstocks were 5-7 meters tall. 'US-942' is a hybrid of *Citrus reticulata* 'Sunki' × 'Flying Dragon' and was released by USDA-ARS for commercial use in 2010. 'US-942' also produces a grafted tree that is highly productive of good quality fruit and resistant to CTV and PDC. Trees with sweet orange scion on 'US-942' vary in size by cultivar, but tree volumes on 'US-942' are typically 65-85% that of trees on standard-size rootstocks, like 'Carrizo' citrange. In the year ending summer of 2012, official propagations in Florida were listed as 73,220 trees on 'US-897' rootstock and 38,489 trees on 'US-942'.

This workshop will provide researchers the opportunity to present information about size-controlling rootstocks and other approaches to developing dwarf trees, as well as other studies related to development and management of high density plantings. The challenges and benefits associated with dwarf citrus trees in high density plantings will be discussed.

W06

Molecular identification of varieties

Convener: Tokurou Shimizu

NARO Institute of Fruit Tree Science (NIFTS), Citrus Research, Shizuoka (Japan). E-mail: tshimizu@affrc.go.jp

Varieties of genus *Citrus* and its relatives show large diversity on their appearance and physiological characteristics, but some of them often look similar and are difficult to distinguish from others. Molecular identification of citrus varieties is beneficial to distinguish from similar ones by eliminating to depend on unstable morphological dissimilarity or empiricism. Genotyping analysis by DNA marker has been used for its accuracy, reliability, and reproducibility by detecting differences of nucleotide sequence among varieties that is called nucleotide polymorphism. Type and frequency of nucleotide polymorphism at different loci observed at a set of varieties correlates to genetic distance of them. Scoring genotypes by multiple DNA markers is therefore useful to reveal identity of individual variety, and phylogenetic relationships among citrus varieties.

Initial attempts for DNA-based markers analysis used random markers like RAPD or ISSR. These DNA markers were advantageous by revealing sufficient number of polymorphism with less cost and required no prior nucleotide sequence data. But those kinds of markers are not suited for genetic study since they are unstable and less informative dominant markers. Nowadays co-dominant type DNA markers that improved stability, reproducibility and transferability including simple sequence repeat (SSR), insertion/deletion (indels) or single nucleotide polymorphism (SNP) are used in studies of citrus. These DNA markers are sufficiently informative for detailed genetic analysis but require nucleotide sequences of target organisms to design them. EST sequences or BAC end sequences were initially applied to design these markers. Recent citrus genome sequencing analysis revealed many polymorphic nucleotide sequence loci, and it is facilitating DNA marker development. Further efforts for nucleotide sequencing of various citrus varieties in the future will increase genome-wide DNA markers and improve their availability.

With the rapid advance of DNA marker development, molecular identification analysis becomes a common technique. It has been applied for phylogenetic study to understand origin and development of modern citrus varieties, and also used in citrus breeding program to discard hopeless seedlings. Meanwhile, strong demand to protect breeders' right of patented variety has developed. DNA marker analysis contributes to determine violation in accordance with scientific evidence by authorities. Applications of DNA marker analysis now cover

processed products of fruit for juice, canned fruit, dried fruit and succade, but special care must be taken to evaluate these samples.

Although current DNA marker analysis can identify natural varieties or crossbred varieties, technical limitation still remains for assured identification of mutant-origin varieties that covers most of major citrus varieties including sweet orange, Satsuma, grapefruit, Clementine, and lemon. On the other hand, rapid development of high-throughput genotyping analysis utilizing accumulated genome sequence information will allow distinguishing varieties of mutant origin in future. Besides potential of DNA marker analysis, DNA sample preparation method, cost, equipment or data analysis as well as number or type of DNA marker are still practically important points to be considered for each purpose.

In this workshop, current status of DNA marker analysis and typical applications will be introduced. Feasibility of the technology and possible constraint for future challenge are the issues to be discussed. Setting up 'standard' protocol of genotyping analysis and putting genotypes of patented varieties to public database is another valuable issue to discuss. Sharing these points among scientists, administrative agency and commercial growers would contribute for concerted development of scientific study and citrus industry.

W07

Mechanization of citrus harvest

Convener: Enrique Moltó

Centro de Agroingenieria. nstituto Valenciano de Investigaciones Agrarias (IVIA). Moncada, Valencia (Spain). E-mail: molto_enr@gva.es

Co-convener: Reza Ehsani

UF, Florida, US

In most parts of the world, citrus are currently manually harvested, loaded in boxes and transported to packing houses or to processing facilities where fruit needs some kind of sorting. Although there are two clearly differentiated ways of cultivating citrus: for the fresh and for the processed market, in both cases manual harvesting accounts for the most part of the total production costs, with a high net share in the final price of the product. This situation has lead to major producing countries to invest in developing methods for mechanical mass harvesting. Moreover, the use of abscission agents for improving the performance of harvesting machines has been under research for more than a decade.

This workshop is aimed at sharing with the audience the state of the art in mechanical harvest of citrus around the World and discussing about the efficiency, adaptability and socio-economic implications of the proposed solutions, including:

- A historical review of engineering approaches to mechanical harvesting of citrus in Florida, where the most relevant efforts for mechanization of citrus for juice have been made since the 50's.
- Trunk shakers and fruit catchers experiences in Spain for harvesting fruits for the fresh market.
- Historical attempts for robotic harvesting and current solutions for in-field sorting of fruit during harvesting in order to reduce packing costs. Limited energy and space availability are always critical issues for the electronic and mechanical designs of these machines.
- The effect of mechanical harvesting on tree stresses and production in the subsequent years.
- The advantages and disadvantages of the use of chemical abscission agents for facilitating the mechanical operation.
- Needs and solutions for debris removal after mechanical harvesting.

After a series of presentations by key researchers in these topics [(J. Syvertsen (UF), T. Spann (UF), J. Burns (UF), A. Torregrosa (UPV, Spain)], the participants in the workshop will have the opportunity to participate in an open discussion about these and any other matters related to de development of harvesting machines for the citrus industry.

W08

Global citrus industry collaboration on MRL regulatory issues

Convener: James R. Cranney, Jr.

California Citrus Quality Council. Auburn, CA (USA). Email: jcranney@CalCitrusQuality.org

A significant number of citrus producers depend on exports as a critical part of their export marketing programs. Conversely, citrus fruits are not domestically produced in countries that represent a significant portion of the global population, so in those countries, citrus fruits must be imported. As a result, global trade of citrus fruits has become a high priority for citrus producers and consumers.

While the demand for citrus fruits may be great, no fruit can be exported unless it meets the pesticide residue regulatory standards of the importing country. As the global citrus trade has become more important, there has been a steady increase in the regulation of pesticide residues on traded citrus commodities. With increasing global demand for citrus fruits and greater regulation, the need for more pesticide maximum residue levels (MRLs) for citrus has never been greater.

This workshop will explore the possibility that citrus producers around the globe could work collaboratively to advocate for more citrus MRLs and for global regulatory policies that benefit citrus producers and trade. The goal of the workshop is to generate coordinated activity among citrus industry leaders to facilitate more global citrus trade.

An overview of technical and regulatory issues under consideration within the Codex Committee on Pesticide Residues and report on the outcome of the Global Minor Use Summit will be presented.

Within the discussions in this workshop we hope to identify common pesticide-related problems of citrus exporters and country-specific leaders willing to advocate for change in regulatory policies. Problems and solutions could be regional or global in nature. Citrus growers, trade association leaders, exporters and packers are encouraged to attend this organizational meeting.

W09

Global conservation strategy for citrus genetic resources

Convener: Kim Hummer

USDA ARS National Clonal Germplasm Repository. Corvallis, Oregon (USA). Kim.Hummer@ARS.USDA.GOV

Co-conveners: Mikeal Roose[1], Fred Gmitter[2] and Richard Lee[3]

[1] Department of Botany and Plant Sciences, University of California. Riverside, CA (USA); [2]University of Florida, Citrus Research and Education Center. Lake Alfred, FL (USA); [3]USDA-ARS National Clonal Germplasm Repository for Citrus and Dates. Riverside, CA (USA). mikeal.roose@ucr.edu; fgg@crec.ifas.ufl.edu; Richard.Lee@ARS.USDA.GOV

Citrus is an important world tree fruit crop with production in more than 146 countries. The center of origin for citrus is considered to be Southeastern Asia including southern China, northeastern India, and Malaysia, with secondary centers in surrounding areas. Novel and commercially significant scion and rootstock cultivars originating by natural mutation or directed hybridization were introduced during the past century. Significant genetic resource collections exist in many countries. A global citrus germplasm network was developed in 1997. Now that the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGR) has recognized Citrus as an Annex 1 crop, a global conservation strategy needs to be established. The objective of this workshop is to plan the development of this strategy. A statement reviewing germplasm exploration and collection gaps needs to be updated. A survey of Citrus ex situ genebanks will define specific protocols. Alternative conservation strategies such as cryogenic approaches will be included. Global disease and pest pressure need to be summarized. A statement detailing protocols for safe movement of germplasm needs to be updated. The success of this strategy will provide a granting opportunity where ex situ genebanks could be supported to improve conservation and health of critical Citrus germplasm of global significance. Participation of all interested parties in this workshop is encouraged.

W10

Citrus and health

Convener: Francisco A. Tomás-Barberán

CEBAS-CSIC. Murcia (Spain). Email: fatomas@cebas.csic.es

The effect of citrus fruits in human health has been an active field of research particularly during the last twenty years. This activity has been associated with benefits for cardiovascular and bone health, and with the decrease of cancer risk. Many of these studies have used in vitro models that do not reflect the complex situation in vivo. Clinical studies, however, have shown large inter-individual variability, leading to non-significant effects in many cases. The recent advances in the knowledge of citrus bioactives metabolism and bioavailability, and in human nutrigenetics, and gut microbiome open great expectations to understand the effects of citrus phytochemicals on health. In this workshop the research strategies, trends and perspectives, to support the role of citrus fruits in human health will be discussed.

Are the in vitro studies valid to demonstrate citrus health effects?

Many mechanistic studies use human cell cultures to evaluate the biological effect of citrus extracts, or isolated bioactives from citrus, and these studies are often extrapolated to the potential effects *in vivo*. Most of these studies, however, have some mayor flaws, although they can be published in very good scientific journals, although they usually do not correlate with the effects observed *in vivo*. The main problems found are associated with the dose, the chemical nature of the citrus bioactive tested and the human tissue used in the assay. The dose used is often much higher than the amounts found *in vivo*, and these high doses are necessary to see a relevant effect. The long term approach, using low metabolite concentration or mixtures of them, which is the situation found *in vivo*, is difficult to evaluate with the classical models. The type of metabolite used in the assays is often that found in the food products, although these compounds are metabolized but the gut microbiota and the human Phase II enzymes to produce metabolites that differ from those found in citrus fruits. In addition, the tissue model used is often inappropriate for the metabolites tested as many of these metabolites do not reach the tissues that are assayed for the effects. Therefore, a new scientific approach is needed to evaluate the mechanisms of action using the appropriate cell lines, with the appropriate metabolites and at the concentrations that reach the specific tissues, and providing systems that mimic the long term effects that are found *in vivo*.

Can we explain the large inter-individual variability observed in clinical studies?

In clinical studies with citrus fruits and extracts, a large inter-individual variability is generally observed with individuals that respond to the intervention with relevant biological effects, while other individuals do not respond to the intervention in the same way. This behavior could be explained by differences in the genetic background of the individuals (nutrigenetics, transporters, metabolic enzymes isoforms, etc.), by differences in the metabolism of the bioactive compounds by the colon microbiota, or by a combination of both factors. The relevance of these two factors in the evaluation of the health effects of citrus products will be discussed, and the need of stratification of the population used in the studies to explain the results obtained will be presented.

W11

Quarantine security for Tephritid fruit flies pests in citrus

Convener: N. J. Liquido

USDA-APHIS-PPQ, Center for Plant Health, Science and Technology, Plant Epidemiology and Risk Analysis Laboratory. Honolulu, Hawaii (USA). Email: Nicanor.J.Liquido@aphis.usda.gov

Co-convener: R.L. Griffin

USDA-APHIS-PPQ, Center for Plant Health, Science and Technology, Plant Epidemiology and Risk Analysis Laboratory. Raleigh, North Carolina (USA). Email: Robert.L.Griffin@aphis.usda.gov

Tephritid fruit flies are serious pests of citrus. They cause direct fruit damage through oviposition and larval feeding, and warrant regulatory restriction on the movement of citrus fruits across national and international boundaries. Current quarantine mitigations for fruit flies in citrus include fumigation, high-temperature

forced-air, vapor heat, cold, and irradiation treatments. Cold treatment appears to be a treatment of choice by several citrus producing countries, with a multitude of schedules and combinations of low temperature and treatment duration, depending on species of citrus and the guild of associated fruit fly pests.

Participants are encouraged to join the discussion on evaluating current quarantine treatment schedules and exploring measures of achieving quarantine security other than Probit 9, including possibilities of integrating less than probit 9 treatments in developing systems approaches to mitigate risk of fruit fly pests.

Probit analysis is a statistical method for determining a dose-response relationship and calculating the appropriate dose for a specific response. The probit 9 mortality response estimates 32 survivors, or less, from 1,000,000 treated pest individuals. At the 95% level of confidence, the probit 9 statistics predicts no survivors in a minimum of 93,613 test insects.

The probit 9 efficacy (99.9968% desired response, e.g., mortality) is the unofficial obligatory de facto standard of treatment efficacy for high risk pests in highly susceptible hosts adapted by the United States and many countries. For naturally poor hosts, the probit 9 standard may be too rigid and impractical, and treatment efficacy lower than probit 9 may be sufficient to achieve the desired quarantine security. In the alternative treatment efficacy approach, risk may be defined either as the number of survivors or as the probability of a mating pair being present in a shipment, rather than as the effective mortality of the treated infesting individuals.

The less than probit 9 approach has regulatory recognition and acceptance. Japan, Australia, and New Zealand accept quarantine treatment efficacy at 99.99%. Furthermore, 99.99% mortality, or survivorship of 0.0001, is the experimental benchmark for verifying the conditional non-host status of a fruit or vegetable commodity to a fruit fly pest. The 99.99% mortality at the 95% confidence level is approximately probit 8.72.

Probit 9 treatments are mandated in many situations without regard to rates of natural infestation; gregariousness (single versus multiple infestation); survival and reproductive capacity of the pest; inherent hardiness of the pest to environmental stress during packaging and shipment; seasonality of shipment; distribution of the commodity in the importing country; and other biological and nonbiological parameters. However, risk managers are familiar with them; a number of precedent-setting probit 9 treatments have been established based on accords during bilateral agreement to facilitate trade, with scientific justification possibly from liberal extrapolation of meager technical data.

Thus, a discussion of these approaches is needed to effectively mitigate the risk of fruit fly pests for a specific host commodity and the infesting pest of interest.

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Session 01

CITRUS GERMPLASM AND PHYLOGENETICS

S01O01

Citron germplasm in Yunnan, China

Karp D.[1], Krueger R.R.[2], Kahn T.[1], and Hu X.[1]

[1]University of California, Riverside (UCR), Department of Botany and Plant Sciences, United States; and [2]United States Department of Agriculture, Agricultural Research Service (USDA-ARS), National Clonal Germplasm Repository for Citrus and Dates, United States.
dkarp@ucr.edu

The center of origin and diversity for citron (*Citrus medica*) is Southwestern China, Northeastern India, and adjacent regions of Southeast Asia, but very little is known about citron germplasm resources in this area. Citron germplasm resources are rapidly being eroded as forests are cleared for development and farmers abandon citron cultivation for more profitable crops. Starting in 2008, researchers for the Chinese Citron Germplasm Project travelled around Yunnan and other provinces of China, observing both cultivated and wild citron. We collected information about germplasm, horticultural practices, and economic uses, as well as cuttings and seeds, which were used to establish a germplasm collection with 30 accessions in Jianshui, Yunnan. Wild citrons typically have smaller fruits and thinner rinds than commercial cultivars; it is likely that over the centuries farmers have selected for a thick rind, as the most economically valuable part, for preserving and eating fresh. The majority of common (non-fingered) citrons grown in Yunnan have thick, sweet albedos, but no juice vesicles. Some grow as large as 15 kg, making them the largest known citrus fruits. Common citron cultivars include 'Large Wrinkled', 'Small Wrinkled', 'Pillow', 'Sour', 'Water', 'Dog Head', 'Bullet', 'Yunnanensis', 'Oblate', and 'Stigma Persistens'. Fingered (Buddha's Hand) citrons are grown primarily as ornamental plants and to be dried for use in Chinese Traditional Medicine, but also for candying. Many distinct cultivars exist, which vary in flower color, size, thickness of fingers, and the point at which the fingers branch out from the body of the fruit. Fruit types of fingered citron include the common, 'Octopus', 'Fist', and 'Half-and-Half'. There is also a cultivar native to southern Sichuan, 'Muli' citron, which is intermediate between fingered and common citron.

S01O02

Cryopreservation of citrus for long-term conservation

Volk G.M., Bonnart R., Shepherd A., Krueger R.R., and Lee R.F.

United States Department of Agriculture, Agricultural Research Service (USDA-ARS), National Clonal Germplasm Repository for Citrus and Dates, United States. gvolk@lamar.colostate.edu

More than 850 varieties of *Citrus*, *Fortunella*, and *Citrus*-related species are maintained within the USDA-ARS National Plant Germplasm System and the University of California Citrus Variety Collection. These genetic resources are held within duplicated field, screenhouse, and greenhouse collections. Plant materials are at risk of disease infestations, abiotic stresses, and natural disasters. We have developed a cryopreservation method by which we can back-up citrus genetic resources for the long term at liquid nitrogen temperatures. We excise shoot tips directly from greenhouse or screenhouse source plants, surface sterilize, and then treat with cryoprotectants. These solutions dehydrate and allow the shoot tips to survive liquid nitrogen exposure. Plants are recovered by micrografting thawed shoot tips onto 'Carrizo' seedling rootstocks. Plants representing diverse species exhibit a high regrowth (more than 50%), and can be transferred to greenhouse conditions within months. Cryopreserved plants did not revert to a juvenile state. Accessions of *Citrus aurantifolia*, *Citrus clementina*, *Citrus celebica*, and *Citrus paradisi* all flowered within 13 to 15 months after cryoexposure. These methods are also applicable for disease eradication using cryotherapy techniques.

S01O03

Genetic diversity and population structure of the mandarin germplasm revealed by nuclear and mitochondrial markers analysis

Garcia-Lor A.[1], Luro F.[2], Ancillo G.[1], Ollitrault P.[3], and Navarro L.[1]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Proteccion Vegetal Y Biotecnología, Spain; [2]Institut National de la Recherche Agronomique (INRA), Unité de recherche GEQA, France; and [3]Centre de coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), BIOS, France. angarcia@ivia.es

Mandarins (*C. reticulata*) are considered as one of the four main species involved in the origin of cultivated citrus. However, the classification of the mandarin germplasm is still controversial and numerous cases of introgression from other species are known or suspected in this germplasm. The main objective of this work was to analyze the genetic diversity structure of mandarin germplasm and its relationship with the other citrus species. Fifty

microsatellite (SSR) markers, 25 Insertion-Deletion (InDel) nuclear markers and four mitochondrial InDel markers were genotyped for 223 accessions. 'Structure' software was applied on nuclear data to check and quantify potential interspecific introgressions in the mandarin germplasm, mainly the pummelo and papeda genomes. Within the mandarin germplasm without identified introgression, seven clusters were revealed by 'Structure' analysis. Five of them should be true basic mandarin groups and the other two include genotypes of known or supposed hybrid origin. The contributions of these seven groups to the mandarin genotypes were estimated. The mitochondrial InDel analysis revealed eight mitotypes, in which the mandarin germplasm was represented in four of them. In this work, new insights in the organization of mandarin germplasm and its structure have been found, and different mandarin core collections were determined. This will allow a better management and use of citrus germplasm collections and to perform genetic association studies.

S01O04

Study on genetic diversity of 39 Citron germplasm resources with SCoT and ISSR markers

Shu-wei Z., Gui-Xiang H., Xin-Hua H., Jie-Chun P., and Feng D.

Agricultural College of Guangxi University, P.R.China. honest66222@163.com

The genetic relationships and diversity of 39 citron (*Citrus medica*) germplasm resources were examined by SCoT and ISSR markers in this study. Of the 80 SCoT primers screened, 9 primers gave reproducible, polymorphic DNA amplification patterns with a total 69 amplified bands. The polymorphic percentage was 76.8%. Of the 100 ISSR primers screened, 9 primers gave reproducible, polymorphic DNA amplification patterns with a total 84 of amplified bands. The percentage of polymorphic band was 77.4%. An UPGMA relationship tree was established based on the similarity coefficients. At the coefficient level of 0. 85 for SCoT and 0.77 for ISSR, the 39 germplasm resources were divided into six groups and five groups, respectively. The application of SCoT and ISSR markers is discussed in this paper.

S01O05

Origin of dwarf, thornless-type trifoliate orange varieties deduced from genome-wide genotyping analysis

Shimizu T., Yoshioka T., Kita M., and Ohta S.

NARO Institute of Fruit Tree Science (NIFTS), Citrus Research, Japan. tshimizu@affrc.go.jp

Trifoliate orange (*Poncirus trifoliata*) is commonly used as a rootstock in Japan. Several varieties that are different for tree height or tree morphology are recognized. However, origin and phylogenetic relationship among them are not evaluated. We compared genotypes of three varieties, a thornless type trifoliate orange that lacks thorns at the leaf axil and two dwarf types, 'Hiryu' ('Flying dragon') and 'Unryu' ('Cloudy dragon'), to a reference accession of trifoliate orange in our citrus collection. Genotyping analysis with 289 genome wide SSR markers gave 265 valid genotypes. Among them, 'Hiryu' lost five loci but 96.2% of genotypes were matched to those of the reference. In contrast, the thornless-type lost 4 loci and 78.8% of genotypes were matched to those of the reference, and 'Unryu' lost 6 loci and 81.4% of genotypes were matched to those of wild type. Assignment of SSR marker genotypes to a citrus linkage map revealed that genotypes of 'Hiryu' were well conserved among all linkage groups. However, loss of heterozygosity (LOH) was observed at various regions of linkage groups for the thornless-type and 'Unryu'. Ratios of homozygous loci of the reference, the thornless-type, 'Hiryu' and 'Unryu' were 70.3%, 88.5%, 69.1%, and 84.9%, respectively. These observations suggested that 'Hiryu' is a sport of trifoliate orange but the thornless-type and 'Unryu' resulted from trifoliate orange by self-pollination and mutation at particular loci.

S01O06

Multilocus haplotyping by parallel sequencing to decipher the interspecific mosaic genome structure of cultivated citrus

Curk F.[1], Ancillo G.[2], Garcia-Lor A.[2], Luro F.[1], Navarro L.[2], and Ollitrault P.[3]

[1]INRA, UR1103 Génétique et Ecophysiologie de la Qualité des Agrumes (INRA UR GEQA), France; [2]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain; and [3]Centre de Coopération International en Recherche Agronomique pour le Développement (CIRAD), BIOS, France. curk@corse.inra.fr

Recent studies support the theory that four basic taxa (*Citrus medica, Citrus maxima, Citrus reticulata* and *Citrus micrantha*) have generated all cultivated *Citrus* species. It is supposed that the genomes of most of the actual

citrus cultivars are interspecific mosaics of large DNA fragments issued from a limited number of interspecific meiotic events. In the present work, we analyzed how haplotypic multilocus study of closely linked SNPs allows phylogenetic assignment of DNA fragments for the main cultivated species. We have developed a new method based on universal primers to prepare the amplicons to be analyzed by 454 technology (Roche). It was applied for direct multilocus haplotyping of 12 gene fragments of 48 *Citrus* genotypes. Moreover, Sanger sequencing was performed on a subset of these amplicons (seven gene fragments of 24 citrus genotypes) to validate the 454 results. Consensus haplotype sequences were successfully identified from 454 sequencing. Sanger and 454 results were mostly identical. *C. reticulata* was the most polymorphic basic taxa. The average differentiation between the basic taxa was about 20 SNPs/kb. These polymorphisms were enough for unambiguous multilocus differentiation of the basic species and assignment of phylogenetic origin for each haplotype of the secondary species. Multilocus haplotyping by parallel sequencing will be a powerful tool to decipher the interspecific mosaic genome structure of cultivated citrus.

S01P01

Citrus ichangensis in Sichuan and Chongqing

Zhang Y., and Zhou X.

Citrus Experiment Station of the Middle Reaches of the Yangtze River, Chongqing Academy of Agricultural Sciences, P. R. China. zhangyg66@gmail.com

Citrus ichangensis from Sichuan and Chongqing Provinces occurs in a wild state in the hills of 42 counties, growing at altitudes of 800 to 2400m with soil pH 5 to 6.5. The dominant vegetation around this species consists of pine, *Chimonobambusa utilis*, plum, kiwifruit, wild cherry, pear, waxberry, fern, rhododendron, couch grass, et cetera. The typical *C. ichangensis* is a shrub or tree, usually 1 to 8 meters high; the corolla of the flowers is purple, light purple, or white; the fruits are ellipsoid, obloid, spheroid or pyriform, 36 to 170 g in weight; the seeds are very large, very numerous (from 20 to 67 in a single fruit), and monoembryonic.

S01P02

Diverse genetic resources of citrus in North-East region of India

Huidrom S.D.[1], Singh S.R.[1], and Handique P.J.[2]

[1]Institute of Bioresources and Sustainable Development (IBSD), Dept. of Biotechnology, Govt. of India., India; and [2]Gauhati University (GU), Department of Biotechnology, India. huidrom_sunitibala@rediffmail.com

The diverse gene pool of citrus in the North-East India is represented by 23 species, a subspecies, and 68 varieties. Collection, conservation and characterization are necessary for incorporating the useful agronomically important traits present in this large gene pool for breeding of improved citrus rootstocks and cultivars and exploring bioactive compounds present in them. Twenty eight accessions of citrus belonging to different species have been collected and morphologically characterized. Variability observed among the citrus accessions collected is reflected by five different types of fruit shape, five different types of fruit base shape, four different types of fruit apex shape, four types of leaf lamina shape, three different types of petiole wing shape, three types of leaf apex, four different types of leaf margin, two types of leaf lamina attachment and five different types of seed shape. Citrus accessions collected have fruit color ranging from light yellow to yellow and orange to orange red color. Various forms of citrus types such as pummelos, citron, acid lime and other hybrids are available in cultivated, semi-cultivated and wild forms which required immediate intervention of a scientific way of conservation to prevent the continuing threat of genetic erosion.

S01P03

Observations of graft compatibility between *Citrus* spp and related Aurantioideae taxa

Siebert T.[1], Kahn T.[1], and Krueger R.R.[2]

[1]University of California, Riverside (UCR), Botany and Plant Science, United States; and [2]USDA-ARS National Clonal Germplasm Repository, United States. tsiebert@ucr.edu

Although *Citrus* has long been known to be graft compatible with the genus *Poncirus*, compatibility between *Citrus* and other genera of the Aurantioideae is less well characterized. Graft compatibility between

Aurantioideae genera has historically been associated with the use of the related taxa as rootstocks for *Citrus* spp. However, the use of *Citrus* spp as rootstocks for related taxa can be useful in maintaining germplasm accessions, in propagating specimen trees or specific commercial taxa, and biological indexing in a sanitation program. One of the largest collections of taxa related to *Citrus* is maintained by the University of California and the United States Department of Agriculture in Riverside, California. This paper reviews historical observations made in Riverside of graft compatibility between Aurantioideae genera and updates them with current observations. Specific combinations have been observed to be incompatible whereas others have shown either short- or long-term compatibility and survival.

S01P04

Fertility relationships among *Citrus* and its relatives in the subfamily Aurantioideae

Siebert T., Ellstrand N., and Kahn T.

University of California, Riverside, Botany and Plant Science, United States. tsiebert@ucr.edu

Data on fertility relationships between crop species and their relatives is of immediate practical value because they can be used to predict the ease of introgression of valuable traits into cultivars. Likewise, those data are of evolutionary significance as evidence of phylogenetic relationships. Here, we review what is known regarding the relative cross-compatibility of various *Citrus* taxa with each other as well as with members of other genera in the Rutaceae subfamily Aurantioideae. For the most part these data were collected as collateral information from breeding studies and therefore are uneven within the group. We compare how the extant fertility relationship data support the various molecular phylogenies of the Aurantioideae. We conclude with the identification of significant gaps in the data that could be filled with a series of strategic and feasible crosses for the purposes of better defining the systematic relationships of the group as well as guiding future citrus improvement by breeding.

S01P05

Characterization of seed and embryo abortion during fruit development in several citrus cultivars pollinated by 'Nishiuchi Konatsu' (*Citrus tamurana*) and preliminary trial of embryo rescue of aborting embryos

Honsho C.[1], Tsuruta K.[1], Ryuto K.[1], Sakata A.[1], Kuroki S.[2], Nishiwaki A.[2], and Tetsumura T.[1]

[1]University of Miyazaki, Faculty of Agriculture, Japan; and [2]University of Miyazaki, Field Science Center, Japan.
chitose@cc.miyazaki-u.ac.jp

'Hyuganatsu' (HY; *Citrus tamurana*) is a late-season citrus grown in several specific prefectures in Japan. Although it is self-incompatible, its bud mutation, 'Nishiuchi Konatsu' (NK), is self-compatible, and interestingly most of the seeds in harvested fruits are aborted. In our previous study we revealed that NK produces a proportion of unreduced 2N pollen grains, resulting in unusual seed development. In this study, we have focused on seed and embryo development when pollen from NK was used to pollinate HY, NK, and 'Hassaku' (HS; *Citrus hassaku*), which is compatible with both HY and NK. When NK pollen was used to pollinate HY or NK, most of the seeds aborted, whereas all seeds developed successfully from crossing using HS pollen. The seeds from NK-pollinated fruits were found to be significantly smaller than those from HS-pollinated fruits 10-12 weeks after pollination during periodic samplings of fruits, suggesting that the seed abortion phenotype is expressed during this time period. Embryo development was observed using differential interference contrast or stereo microscopy. Embryos that had formed from NK self-pollination had aborted at 10 weeks after pollination, whereas control embryos had reached the globular or heart-shaped stage. In the cross HS × NK, both normal and aborted seeds were present in the resulting fruit. Embryo development was found to cease at 12 weeks after pollination, and at 14 weeks after pollination, normal and aborted seeds were easily distinguishable. In addition to the characterization of seed development, preliminary attempts at embryo rescue have been made to recover triploid seedlings. After making the crosses HY × NK or NK × NK, aborting embryos were placed on MT media supplemented with 500 mg/L malt extract, 50 g/L sucrose, and 5 mM GA3. As a result, several triploid plantlets confirmed by flow cytometry were recovered from both pollination combinations, although the success rate was not high.

S01P06

Diversity analysis of citrus fruit pulp acidity and sweetness: toward to understand the genetic control of the fruit quality parameters

Luro F., Gatto J., Costantino G., and Pailly O.

Unité de Recherche 03 Génétique et Ecophysiologie de la Qualité des Agrumes (GEQA), Station de Recherche INRA, France. costantino@corse.inra.fr

Acidity level is the main criteria of quality and physiological maturity evaluation for citrus fruit, especially for mandarins and oranges. The acidity level varies during fruit development but also among species and varieties, and also depends upon rootstock and environmental factors such as temperature and rainfall. Acid metabolism is linked to sugar metabolism. To better control fruit maturity and harvest time, it is necessary to understand the genetic control and the parameters influencing the variation of acidity and sweetness. *Citrus* diversity was investigated by selecting 87 varieties belonging to the 8 major *Citrus* species grown under the same environmental and cultivation conditions. The juice acidity and sweetness were analysed by assessing pH Na-neutralization and by refraction index, respectively, and their components by HPLC. The sequence polymorphisms of 9 candidate genes encoding for key enzymes of sugars and organic acids metabolic pathways were investigated by SSCP (Single Strand Conformation Polymorphism). Whatever the biochemical or molecular analysis, the observed structure of the *Citrus* diversity was organized around the ancestral species (mandarin, pummelo and citron). As expected, the secondary species were closely related to their putative species progenitors. The biochemical diversity was strongly correlated to molecular SSCP diversity without having any certainty on the cause and effect relationship.

S01P07

Screening a core collection of citrus genetic resources for resistance to *Fusarium solani*

Krueger R.R.[1], and Bender G.S.[2]

[1]USDA-ARS National Clonal Germplasm Repository for Citrus & Dates (USDA-ARS-NCGRCD), USA; and [2]University of California Cooperative Extension, San Diego County (UCCE-SD), USA. robert.krueger@ars.usda.gov

A causal agent for Dry Root Rot (DRR) of citrus has not been definitively identified, but the organism most consistently associated with DRR is *Fusarium solani*. To efficiently screen a citrus germplasm collection for resistance to *F. solani*, a core subset of the collection was evaluated. Seedlings of forty five accessions were wounded by girdling. Treatment consisted of inoculation with *F. solani*, with uninoculated controls. Three parameters were evaluated: recovery from girdling (0 - 3); amount of DRR (0 - 4); and percentage of healthy feeder roots. Thirty three accessions showed no significant difference in recovery from girdling between inoculated and uninoculated seedlings and 11 of these accessions showed good recovery from girdling (> 2.7). Twenty four accessions showed no significant difference in DRR between inoculated and uninoculated seedlings, and 12 of these accessions showed low development of DRR (< 1). Twenty four accessions showed no significant difference in % healthy feeder roots between inoculated and uninoculated seedlings and 8 of these showed a high percentage (> 80%) of healthy feeder roots. Five accessions were superior in all thee parameters: 'Fremont' mandarin (PI 539507), 'Lamas' lemon (PI 539226), 'Mato Buntan' pummelo (PI 529398), 'Olivelands' sour orange (PI 539164), and 'South Coast Field Station' citron (PI 539435). These accessions would probably represent the best candidates for further study of *F. solani* resistance.

S01P08

Nuclear phylogeny of *Citrus* and four related genera

Garcia-Lor A.[1], Curk F.[2], Snoussi H.[3], Morillon R.[4], Ancillo G.[1], Luro F.[2], Navarro L.[1], and Ollitrault P.[4]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal Y Biotecnología, Spain; [2] INRA, UR1103 Génétique et Ecophysiologie de la Qualité des Agrumes (INRA UR GEQA), DGAP, France; [3]Institut National de la Recherche Agronomique de Tunisie (INRAT), Laboratoire d'Horticulture, Tunisie; and [4]Centre de coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), BIOS, France. angarcia@ivia.es

Despite considerable differences in morphology, the genera representing "true citrus fruit trees" are sexually compatible, but their phylogenetic relationships remain unclear. Most of the important commercial species

of *Citrus* are believed to be of interspecific origin. By studying SNP and InDel polymorphisms of 27 nuclear genes on 45 genotypes of *Citrus* and related taxa, the average molecular differentiation between species was estimated, and the phylogenetic relationship between "true citrus fruit trees" was clarified. A total of 16238 bp of DNA was sequenced for each genotype, and 1097 SNPs and 50 InDels were identified. Nuclear phylogenetic analysis revealed that *Citrus reticulata* and *Fortunella* form a clade clearly differentiated from the other two basic taxa of cultivated citrus (*Citrus maxima*, *Citrus medica*). A few genes displayed positive selection patterns within or between species, but most of them displayed neutral patterns. The phylogenetic inheritance patterns of the analysed genes were inferred for commercial *Citrus* species. The SNPs and InDels identified are potentially very useful for the analysis of interspecific genetic structures. The nuclear phylogeny of *Citrus* and its sexually compatible relatives was consistent with their geographic origin. The positive selection observed for a few genes will orient further work to analyze the molecular basis of the variability of the associated traits. This study presents new insights into the origin of *Citrus sinensis*.

S01P09

New insights on limes and lemons origin from targeted nuclear gene sequencing and cytoplasmic markers genotyping

Curk F.[1], Garcia-Lor A.[2], Snoussi H.[3], Froelicher Y.[4], Ancillo G.[2], Navarro L.[2], and Ollitrault P.[4]

[1]INRA, UR1103 Génétique et Ecophysiologie de la Qualité des Agrumes (INRA UR GEQA), DGAP, France; [2]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain; [3]INRAT (Institut National de la Recherche Agronomique de Tunisie), Tunisia; and [4]Centre de Coopération International en Recherche Agronomique pour le Développement (CIRAD), BIOS, France. curk@corse.inra.fr

It is believed that *Citrus medica, Citrus maxima, Citrus reticulata* and *Citrus micrantha* have generated all cultivated *Citrus* species. Depending on the classification, lemons and limes are classified either into two species, *Citrus limon* and *Citrus aurantifolia* (Swingle and Reece) or into more than 30 (Tanaka). In order to study the molecular phylogeny of this *Citrus* group, we analyzed 20 targeted sequenced nuclear genes and used 3 mitochondrial and 3 chloroplastic markers for 21 lemons and limes compared with representatives of the 4 basic taxa. We observed 3 main groups, each one derived from direct interspecific hybridizations: (1) the Mexican lime group (*C. aurantifolia*), including *Citrus macrophylla*, arising from hybridization between papeda (*C. micrantha*) and citron (*C. medica*); (2) the yellow lemon group (*C. limon*) that are hybrids between sour orange (*Citrus aurantium*, which is believed to be a hybrid between *C. maxima* and *C. reticulata*) and citron; and (3) a rootstock lemon/lime group (Rough lemon and Rangpur lime) that are hybrids between the acid small mandarin group and citron. We also identified different probable backcrosses and genotypes with more complex origins. None of the analyzed limes and lemons shared the *C. medica* cytoplasm, while this taxon is the common nuclear contributor of all limes and lemons. Limes and lemons appear to be a very complex citrus varietal group with the contribution of the 4 basic taxa. Neither the Swingle and Reece nor the Tanaka classifications fit with the genetic evidence.

S01P10

Diversity of citron (*Citrus medica*) and phylogenetic analysis of related citron hybrids using molecular markers and essential oil compositions

Luro F.[1], Venturini N.[2], Costantino G.[1], Tur I.[1], Paolini J.[2], Ollitrault P.[3], and Costa J.[2]

[1]Institut National de Recherche Agronomique (INRA), Génétique et Écophysiologie de la Qualité des Agrumes, France; [2]CNRS UMR 6134 (SPE), Laboratoire de Chimie des Produits Naturels, France; and [3]Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), AGAP, France. luro@corse.inra.fr

It is commonly accepted that citron (*Citrus medica*) was the first citrus fruit to reach the Mediterranean area about 3 centuries B.C. The fragrance due to the essential oils is probably the main characteristic that has contributed to the reputation of this fruit in all Mediterranean countries for multiple uses including cosmetics, food, medicinal, ornamental and also as religious symbol. The Citron group is supposed genetically low diversified compared to mandarins and pummelos. To assess the diversity of the citron group we analyzed the molecular polymorphism of nuclear and cytoplasmic genetic markers and the variation in composition of essential oils from leaves and peels of 24 varieties of citron or apparent hybrids of citron.

Our results demonstrate that the citron group has a relatively high allelic diversity, probably due to multiple introductions of several varieties. Self-fertilization was the mode of reproduction, which most frequently led to the development of commercial varieties with specific aromas and flavors such as the cultivar 'Corsican', a putative 'Common Poncire' self-crossed hybrid. However, some varieties appeared to be interspecific hybrids related to citron but only by male parent. This varietal diversity was probably favored by spreading seeds to extend the cultivation of citron in Mediterranean countries. The chemical diversity does not always correspond to genetic diversity but nevertheless reveals specific profiles for some genotypes.

S01P11

Analysis of genetic diversity in Tunisian citrus rootstocks

Snoussi H.[1], Duval M.F.[2], Garcia-Lor A.[3], Perrier X.[2], Jacquemoud-Collet J.C.[2], Navarro L.[3], and Ollitrault P.[2]

[1]Tunisian National Agronomic Research Institute (INRAT), Horticultural Laboratory, Tunisia; [2]International Center for of Agricultural Research for Development (CIRAD), Department BIOS. TGU. AGAP, France; and [3]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain. hagersnoussi@gmail.com

Breeding and selection of new citrus rootstocks are nowadays of the utmost importance in the Mediterranean Basin because the citrus industry faces increasing biotic and abiotic constraints. In Tunisia, citrus contributes significantly to the national economy, and its extension is favored by natural conditions and economic considerations. Sour orange, the most widespread traditional rootstock of the Mediterranean area, is also the main one in Tunisia. In addition to sour orange, other citrus rootstocks well adapted to local environmental conditions are traditionally used and should be important genetic resources for breeding. Prior to initiation of any breeding program, the exploration of Tunisian citrus rootstock diversity was a priority. Two hundred and one local accessions belonging to four facultative apomictic species (*Citrus aurantium*, sour orange; *Citrus sinensis*, sweet orange; *Citrus limon*, lemon; and *Citrus aurantifolia*, lime) were collected and genotyped using 20 nuclear SSR markers and four InDel mitochondrial markers. Sixteen distinct Multi-locus genotypes (MLGs) were identified and compared to references from French and Spanish collections. The differentiation of the four varietal groups was well-marked. Each group displayed a relatively high allelic diversity, primarily due to very high heterozygosity. The Tunisian citrus rootstock genetic diversity is predominantly due to high heterozygosity and differentiation between the four varietal groups. The phenotypic diversity within the varietal groups has resulted from multiple introductions, somatic mutations and rare sexual recombination events. This diversity study enabled the identification of a core sample of accessions for further physiological and agronomic evaluations. These core accessions will be integrated into citrus rootstock breeding programs for the Mediterranean Basin.

S01P12

Characteristics of 'Pompia' a natural citrus hybrid cultivated in Sardinia

Mignani I.[1], Mulas M.[2], Mantegazza R.[1], Lovigu N.[1], Spada A.[1], Nicolosi E.[3], and Bassi D.[1]

[1]University of Milan (DAES), Department of Agricultural and Environmental Science, Italy; [2]University of Sassari (DIPNET), Department of the Nature and Land Sciences, Italy; and [3]University of Catania (DAFS), Department of Agricultural and Food Science, Italy. mmulas@uniss.it

The 'Pompia' is a probable citrus natural hybrid sporadically growing in the "Baronia" area (East Sardinia, Italy). The origin and taxonomic classification of this *Citrus* taxon is unknown. The fruit shows a yellow-amber flavedo at maturity and large size (weight from 200 to 700 g), irregular shape, light-yellow pulp, and low juice content. The traditional use as food is limited to the thick albedo after removal of the flavedo tissue and of the acid, non-edible pulp, and candying. This research had as its objective a morphological study of nine accessions selected from four orchards of 'Pompia' in order to sample the observed variability of trees, shoots, leaves, and fruits. The results of morphological determinations showed a high similarity of 'Pompia' samples to lemon and citron species for thorns, fruit size and colour. The presence of small petiole winglets was observed only on one accession. AFLP, RAPD, and SCAR analysis allowed the comparison of 'Pompia' with seven *Citrus* species: citron, lemon, pummelo, grapefruit, bitter orange, and mandarin. The analysis with molecular markers demonstrated a close genetic relationship among 'Pompia', lemon, and citron. Moreover, the elaboration of the phenotypic and molecular analysis allowed evidencing a high level of similarity of the nine 'Pompia' accessions. Seven of them probably genetically correspond to the same clone.

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CITRUS GENETICS AND BREEDING

S02O01

The triploid mandarin breeding program in Spain

Navarro L., Aleza P., Cuenca J., Juárez J., Pina J.A., Ortega C., Navarro A., and Ortega V.

Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain. lnavarro@ivia.es

Production of seedless citrus fruits is required for the fresh market because consumers do not accept seedy fruits. Development of new seedless mandarin cultivars has a high priority for many citrus industries worldwide. The recovery of triploid hybrids is the most promising approach to achieve this goal, since triploids have a very low pollen and ovule fertility and usually are seedless or produce very low number of seeds, and do not induce the formation of seeds in other cultivars by cross pollination. In Spain we are carrying a triploid breeding program since 1996 based on 2x X 2x, 2x X 4x and 4x X 2x pollinations followed by embryo rescue and flow cytometry, and the use of different approaches to produce new tetraploid parents. The objective is to produce new high quality easy peeling and seedless mandarin cultivars. More than thirty spontaneous autotetraploid apomictic genotypes to be used as male parents have been selected from seedbeds by flow cytometry. Also nine autotetraploid non apomictic genotypes mainly to be used as female parents have been obtained by chromosome duplication with treatments of micrografted shoot tips with colchicine. Symmetric protoplast fusion is also being used to produce allotetraploid genotypes. So far, we have obtained more than 5,500 triploid hybrids from 130 parental combinations by 2x X 2x pollinations, more than 4,300 triploid hybrids from 100 parental combinations by 2x X 4x pollinations, and more than 5,600 triploid hybrids from 103 parental combinations by 4x X 2x pollinations. Recently we have released the first cultivars originated in the program, the seedless late maturing mandarins 'Garbí' and 'Safor', obtained from 2x X 2x crosses. More than 400,000 trees of these varieties have been planted by growers during the last three years.

S02O02

Triploid seedless mandarin breeding in France

Froelicher Y., Bouffin J., Dambier D., and Ollitrault P.

Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), UMR AGAP, France. froelicher@cirad.fr

Small citrus is an increasing component of the world citrus industry. New high quality, parthenocarpic, sterile mandarin varieties will play a pivotal role for its sustainable development. To produce seedless varieties, the CIRAD breeding program is focused on triploid hybrid selection. The first method to develop triploid progenies exploits 2n gametes that are naturally produced by diploid cultivars to obtain triploids in 2x X 2x crosses. The second method is based on interploid crossings (2x X 4x and 4x X 2x). For the last strategy the parental tetraploid gene pool has been diversified by selecting spontaneous tetraploids in apomictic cultivars, generating doubled diploids by colchicine treatments and production of allotetraploids by somatic hybridization. Several thousand triploid hybrids have been created by CIRAD using these strategies with the support of embryo rescue and ploidy evaluation by flow cytometry. This breeding program is supported by basic and methodological research performed in collaboration with IVIA (Spain), DAK (Morocco) and INRA (France) in the following topics: (1) citrus germplasm management and characterization, (2) studies of polyploidization mechanisms, tetraploid meiosis and its implications on the genetic and phenotypic structure of triploid progenies, and (3) studies of the implication of polyploidy on genomic and phenotypic expression.

S02O03

Mechanism of 2n gametes formation and centromere mapping in citrus

Aleza P.[1], Cuenca J.[1], Juárez J.[1], Navarro L.[1], and Ollitrault P.[2]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain; and [2]Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), BIOS, France. aleza@ivia.es

Citrus triploid hybrids can be recovered by 2x X 2x hybridisations as a consequence of 2n gamete formation. Two main meiotic processes are at the origin of such gametes, the first division restitution (FDR) and the second division restitution (SDR). These two mechanisms lead to very different gamete genetic structures.

Particularly, the opposite feature of parental heterozygosity restitution (PHR) is observed in relation with the distance to the centromere. Without previous knowledge on centromere location, the determination of the restitution mechanism requires an analysis of the frequency of PHR within a large population with numerous codominant markers. With this method we determined that SDR is the mechanism involved in clementines and 'Fortune' mandarin. With SDR, PHR is a direct function of the distance of the considered locus to the centromere. Therefore the analysis of the evolution of PHR, within a linkage group (LG) allows mapping of the centromere position. Centromere has been located in the 9 LGs of the clementine genetic map by genotyping triploid hybrids with SSR and SNP markers. Analysis of the 2n gamete allelic configuration with loci close to the centromere can shed light on the restitution mechanism at the individual level and open the way for simplified study of this mechanism in a large range of genotypes. We are applying this approach to analyse the 2n gamete origin in several genotypes of mandarin and one genotype of sweet orange.

S02O04

Experiences in the development, release and commercialization of new irradiated citrus varieties from the citrus breeding program at the University of California Riverside

Williams T.E.

University of California Riverside (UCR), Botany and Plant Sciences, United States. timwill@ucr.edu

The use of irradiation in the mutation breeding scheme of citrus breeding programs is now quite widespread with most programs in the major citrus producing countries actively pursuing new selections. Irradiation, used principally to reduce the seed content of normally seedy varieties, represents a more rapid method of achieving these goals than does hybridization. Some programs, notably in Israel and California, have a longer history with these techniques, not only in the development of new varieties but especially as relates to the release and commercialization of the newly developed selections. The experiences in the Citrus Breeding Program at the University of California Riverside have included the development, release and commercialization of several mandarin selections including 'Tango', 'DaisySL', 'KinnowLS', and 'FairchildLS' along with others in the 'pipeline'. The presentation will discuss the specific approaches used by the UCR Citrus Breeding Program during the irradiation process, the criteria and standards used in the selection of promising new candidates and the general experiences and problems encountered during the release and commercialization phases.

S02O05

Citrus breeding program in Chile

Martiz J., and Montañola M.J.

Pontificia Universidad Católica de Chile (PUC), Facultad de Agronomía e ingeniería forestal, departamento de Fruticultura, Chile. mmontano@uc.cl

Mandarin production and exports from Chile have shown a huge growth in the last ten years. During the 90's the most important cultivar was 'Clemenules' and since 2005 mandarin hybrids are being planted to supply late season markets. This has resulted in the production of fruit with seeds due to cross pollination causing big losses to Chilean producers. In 2007 citrus breeding program was initiated in the Pontificia Universidad Católica de Chile with the support of CIRAD (France) and University of California Riverside. The aim of this program was to obtain seedless mandarin and lemon cultivars. Strategies used are induced mutation by gamma irradiation, *in vitro* triploid rescue obtained from diploid crosses, production of auto-tetraploid plants and the establishment of a protoplast fusion protocol. Irradiation technique has resulted in the establishment of 5700 mandarins and 2500 lemons in a field trial located in Pomaire, Chile (33°39'S, 71°10'W). Field evaluation resulted in the selection of 164 lemon and 14 mandarin seedless selections, 4 lemon thornless selection, and 2 ornamental types. Our methodology allowed an early selection of seedless genotypes and quickly reduced the number of plants under evaluation. 118 triploids were obtained through *in vitro* rescue from diploid mandarins cross pollinations. These genotypes are currently under agricultural practices to induce flowering and are prone to be planted in commercial trials. 9 tetraploid of reduced juvenility were obtained with the use of colchicine in axillary buds. They will be used in cross pollinations in 2012 spring. Finally a protoplast fusion protocol has been developed under our conditions. The results of this program have a high impact for the Chilean citrus industry and the academy, since they have allowed creating varieties adapted to local conditions, a new line of research and training for new professionals.

S02O06

Citrus breeding in South Africa: the latest developments in the programme run by the ARC-Institute for tropical and subtropical crops.

Sippel A.D., Bijzet Z., Froneman I.J., Combrink N.K., Maritz J.G.J., Hannweg K.F., Severn-Ellis A.A., and Manicom B.Q.

Agricultural Research Council - Institute for Tropical and Subtropical Crops (ARC - ITSC), Plant Improvement, South Africa. arthur@arc.agric.za

The South African Agricultural Research Council's Citrus Plant Improvement Programme run by the Institute for Tropical and Subtropical Crops consists of a number of separate projects. These involve conventional scion breeding to improve productivity and quality, rootstock breeding for soil adaption, disease resistance and improved yields, mutation breeding, and evaluation of both scions and rootstocks in different climatic zones. These projects are also supported through the Citrus Improvement Programme, a vast germplasm collection, various biotechnology techniques such as embryo rescue and ploidy manipulation, and the use of molecular markers to distinguish between, at this stage citrus mandarin selections. This paper will give a short overview of the research activities within each of the projects, as well as the most important results achieved by each.

S02O07

Highlights of the University of Florida, Citrus Research and Education Center's comprehensive citrus breeding and genetics program

Grosser J.W., Gmitter Jr. F.G., Ling P., and Castle W.S.

University of Florida, Institute of Food and Agricultural Sciences (IFAS), Citrus Research and Education Center (CREC), USA. jgrosser@ufl.edu

A comprehensive citrus breeding and genetics program was established at the UF/CREC during the mid-1980's combining conventional breeding with tools provided by tissue culture, emerging biotechnologies, molecular genetics and genomics. Scion Improvement: The Florida citrus industry is 90% processing sweet oranges. A major goal has been to develop new cultivars for Not From Concentrate juice production with emphasis on increasing juice quality and expanding seasonal availability. Improved clones from seedling introductions, budwood irradiation and somaclonal variation will be discussed. The fresh fruit industry in Florida is dominated by grapefruit and seedy mandarins. Primary objectives for fresh fruit improvement include expanding seasonal availability, development of Canker tolerant and low furanocoumarin grapefruit, and development of seedless, easy-peeling mandarins. The focus of our program has been on parent development, and seedless triploid production via interploid crosses; strategies and successes will be discussed. Rootstock Improvement: Initial focus was on improving soil adaptation, tree-size control to facilitate harvesting and cold-protection, and disease resistance, especially to blight, CTV induced quick decline and Phytophthora. Breeding objectives have since been expanded to include tolerance of the *Diaprepes*/*Phytophthora* complex and HLB, and development of rootstocks that facilitate advanced citrus production systems (ACPS). Progress from conventional breeding (both 2x and 4x) and somatic hybridization will be discussed. Genetic Engineering and Genomics: Due to the HLB and Canker epidemics, focus has been on disease resistance gene discovery, transformation, and evaluation of transgenic plants; progress will be discussed.

S02O08

Advance in *S* allele determination, *S* allele frequencies and *S*-genotyping in citrus

Wakana A., Handayani E., Kim J.H., Miyazaki R., Mori T., Sato M., and Sakai K.

Kyushu Univesity, Horticultural Science, Japan. wakana@agr.kyushu-u.ac.jp

Gametophytic self-incompatibility (SI) is known in pummelo, mandarin and cultivars with pummelo in their pedigrees. Despite the importance of SI for citrus cultivation and breeding, little is known about the SI genotypes of citrus cultivars. In this study, homozygous S_1 seedlings for the *S* gene were produced through bud self-pollination of five monoembryonic SI cultivars, i.e., 'Banpeiyu' and 'Hirado Buntan' pummelos (*Citrus grandis*), clementine (*Citrus clementina*) and 'Ellendale' mandarins and 'Hassaku' (*Citrus hassaku*). The homozygosity of S_1 seedlings for *S* gene was determined by pollen tube penetration in the stylar base 8

days after pollination with the parent, and homozygous genotypes were determined similarly by pollinations between the homozygous S_1 seedlings. The determined homozygous S_1 seedlings, whose *S* genotypes were S_1S_1, S_2S_2, S_3S_3 and so on, were pollinated to about 100 SI ($S_nS_{n'}$) or SC (S_fS_f or S_fS_n) cultivars and accessions. The cultivars or accessions arresting pollen tubes in their stylar base were determined to have the same allele as that of the pollinated homozygous seedling. Cultivars whose *S* genotypes were determined were 'Banpeiyu' (S_1S_2), 'Natsudaidai' (S_fS_2), 'Tosa Buntan' (S_1S_3), clementine (S_3S_{11}), 'Hirado Buntan' (S_9S_{10}), and so on. Frequency of S_n alleles, except for S_f allele, was 16.4% for S_1, 11.6% for S_2, 11.8% for S_3, 10.6% for S_4, 5.1% for S_5, 5.7% for S_9, 2.9% for S_{10} and 13.7% for S_{11}. Among Japanese cultivars and accessions with the determined *S* alleles, those with S_4 allele were considered to originate from 'Kunenbo' tangor (*Citrus nobilis*) or hybrids with 'Kunenbo' in their pedigrees. The Japanese cultivars or accessions with the S_4 allele were satsuma mandarin (*Citrus unshiu*), 'Hassaku' (S_4S_5), 'Ogonkan' (*Citrus flaviculpus*), 'Keraji' (*Citrus keraji*), 'Kabuchi' (*C. keraji*) and so on. Introgression of the S_4 allele into citrus cultivars is discussed.

S02O09

Integrated profiling of furanocoumarins (FCs) in grapefruit and derived hybrids toward selection of low FCs varieties

Chen C.[1], Gmitter Jr. F.G.[1], Cancalon P.[2], and Greenblatt D.J.[3]

[1]University of Florida, Citrus Research and Education Center (UF-CREC), Department of Horticultural Sciences, USA; [2]Florida Department of Citrus, Scientific Division (FDOC), USA; and [3]Tufts University, School of Medicine (TUSM), Department of Molecular Physiology and Pharmacology, USA. cxchen@ufl.edu

Furanocoumarins (FCs) are a class of organic chemical components in grapefruits and other food plants. Consumption of grapefruit juice can potentially increase the bioavailability of a number of clinically prescribed drugs, induce grapefruit-drug interactions, and therefore, some physicians recommend that grapefruit juice should be avoided by patients taking these drugs. To develop low FC grapefruit cultivars, integrated approaches to profile FCs were used to facilitate achieving the goal, including quantifying different FCs in grapefruit and pummelo, characterizing the inheritance using hybrid populations, identifying some key controlling genes in the furanocoumarin biosynthesis pathway, and determining the inhibitory potency of different hybrids and FCs. We monitored seven FCs, 6',7'-dihydroxybergamottin (6,7-DHB), bergamottin, bergaptol, isoimperatorin, epoxybergamottin (EBM), 5',8'-dimethylallyloxypsoralen (5,8-DMP), and Paradisin C, in randomly selected hybrids, and found 6,7-DHB, bergamottin, paradisin C, and bergaptol inherited in a 1:1 co-segregating manner, with strong correlations (R^2 up to 0.909) among them, as well as with *in vitro* inhibitory potency versus human cytochrome P450-3A activity (R^2=0.96). At least one gene in the pathway was identified to show differential expression between high and low FC varieties. The phytochemical, genetic, genomic, and pharmaceutical profiles of the FCs are leading to a genomic and breeding solution to the grapefruit FC-drug interaction issue.

S02O10

Breeding new grapefruit-like varieties with low furanocoumarin contents

Weissberg M., Yaniv Y., Sobolev I., Fidle L., and Carmi N.

The Volcani Center (ARO), Fruit Tree Sciences, Israel. nircarmi@agri.gov.il

Furanocoumarins are compounds present in pummelo and grapefruit (grapefruit is a probable hybrid of pummelo and sweet-orange) and absent in mandarin and orange fruit. People that consume different types of drugs, such as statins, antihistamines, and certain antibiotics, are forbidden to consume grapefruits and pummelo because of the inhibitory affect of furanocoumarins on cytochrome P450 3A4 activity, a key enzyme involved in drug metabolism, and its inhibition may lead to severe health risks. Since it is anticipated that 50% of the population above the age of 40 will need to take statins, we predict a drastic drop in grapefruit juice consumption in the near future. Therefore, to address this problem, we aimed to develop a new grapefruit-like variety without furanocoumarins. For that purpose, we screened using LC-MS various grapefruit-like hybrids for presence of furanocoumarins, and found that most of the genotypes tested, such as 'Marsh' grapefruit, 'Chandler' and 'Flamingo' pummelos, and various grapefruit-like hybrids, such as 'Einat' ('Hudson' grapefruit X 'acidless pummelo') and 'Michal' mandarin X 'Duncan' grapefruit, contained high amounts of

furanocoumarins. However, a particular hybrid between 'Chandler' pummelo X 'Orah' mandarin, named 'Aliza', had low furanocoumarin content. At the same time, the observed level of naringin, which provides typical grapefruit bitter flavor, in 'Aliza' fruit was the same as that detected in 'Marsh' grapefruit.

S02O11

Genotypic variation of rind colour in citrus tangor 'Kiyomi' progenies

Combrink N.K.[1], Bijzet Z.[1], Sippel A.D.[1], Booyse M.[2], and Labuschagne M.T.[3]

[1]Agricultural Research Council - Insitute for tropical and Subtripical Crops (ARC-ITSC), Plant Improvement, South Africa; [2]Agricultural Research Council - Biometry (ARC-Biometry), Biometry, South Africa; and [3]University of the Free State (UFS), Plant Breeding, South Africa. combrinkn@arc.agric.za

Rind colour is the most important characteristic contributing to a fruit's appearance and therefore the improvement of rind colour has been a longstanding aim of citrus breeding programmes. However, due to the lack of information on the inheritance of rind colour in citrus, the breeder faces a difficult task when planning crosses for breeding new cultivars. By quantifying the variation in a population the breeder can study the relationships between the hybrids and parents and gain an understanding of how certain characteristics are inherited. Therefore a study was undertaken to investigate the variation in rind colour, as chroma coordinates L*, a* and b*, in six mandarin families, where female parent 'Kiyomi' tangor (*Citrus unshiu* x *Citrus sinensis*) was crossed with male parents 'Dancy', 'Hansen', 'Rishon', 'Roma', 'Shani' and 'Sunburst' mandarins (*Citrus reticulata*). A significant level of variation was found between both the parents and the families for all the colour coordinates. A greater variation was found within the families than between the families, indicating a high level of genetic variation within the families, while the within tree variation was lower than the within family variation. All the families showed an improvement in the population for rind colour over the female parent 'Kiyomi' for L* and b*, while all the families except for the 'Kiyomi' x 'Dancy' family showed an improvement for a*. The 'Kiyomi' x 'Dancy' and 'Kiyomi' x 'Roma' families were found to have a population with a lighter, more yellow-orange rind colour, while the 'Kiyomi' x 'Hansen', 'Kiyomi' x 'Rishon', 'Kiyomi' x 'Shani' and 'Kiyomi' x 'Sunburst' families had a population with a deeper, more orange-red rind colour. The intraclass correlation coefficient relevant to selection within the families was fairly low, indicating the variation was only partly genetic and the environment contributed to the phenotypic variation. Therefore a mean performance of multiple year's data should be used for effective selection.

S02O12

Rootstock effects on mandarin fruit traits under control and salinity conditions

Raga V.[1], Bernet G.P.[1], Carbonell E.A.[2], and Asíns M.J.[1]

[1]Instituto Valenciano de Investigaciones Agrarias (I.V.I.A.), Centro de Protección Vegetal y Biotecnología, Spain; and [2]Instituto Valenciano de Investigaciones Agrarias (I.V.I.A.), Biometría, Spain. raga_ver@gva.es

The objective of this study is to explore rootstock genetic effects on salt tolerance in terms of fruit yield. A seedling population from hybrids between *Citrus volkameriana* (V) and *Poncirus trifoliata* (P), and its grandparents (V and P) were grafted with 'Hashimoto' mandarin. Trees were maintained in a screenhouse and salt treatment (25 mM NaCl) was applied from June to September for 5 years (2004-2008) to 32 genotypes and 18 controls. Rootstocks were genotyped for 5 salt tolerance candidate genes. Alleles of 96 and 73 markers segregating at the V and P genomes, respectively, in the VxP nucellar seedlings were studied for marker-trait associations. Significant effects for rootstock genotype (G) and treatment (E) were found for most traits. Salinity decreased yield and juice volume, but improved total soluble solids and rind thickness. Year effects were highly significant in most cases. GxE interaction was found for fruit weight, total fruit weight, leaf water content and leaf [Na]. Trait heritabilities of rootstock effects ranged from low to very low and no fruit yield trait had consistent, significant correlations through years with any non-fruit trait. Two salt tolerant candidates were associated with fruit yield traits, but only under control conditions. Segregations at 8 and 9 loci on the V and P genomes, respectively, were highly associated with fruit yield under the salinity treatment. Therefore, phenotypic selection assisted by markers linked to these QTL is more efficient than just phenotypic selection to obtain rootstocks that confer salt tolerance to the grafted mandarin.

S02O13

Extension of a citrus genetic linkage map for QTL mapping of freeze tolerance

Hong Q.-B., Ma X.J., Gong G.-Z., and Peng Z.C.

Citrus Research Institute, Chinese Academy of Agricultural Sciences and Southwest University, National Citrus Engineering Research Center, Chongqing, China. hongqb@sina.com

Freeze limits the cultivated area of citrus. Periodically freeze events in some producing areas caused severe damages to citrus harvest and low temperature during extreme climate change often affected the citrus global production in recent years. To develop and cultivate freeze tolerant cultivars is the most effective way to avoid damages caused by freeze. Cold tolerance is considered a quantitative trait in plant. In this research, QTL mapping was used to find loci and molecular markers putatively related with freeze tolerance in citrus. A new linkage map was established by combining the segregating data of markers in a published map and SSR markers newly developed from citrus EST and clementine BAC-end sequences. New map integrated 452 SSR markers with 9 linkage groups and covered 923.3 cM of citrus genome with an average genetic distance at 2.04 cM between adjacent markers. Freeze tolerance of the mapping population of parents and 68 progenies was tested by the method of electrolyte leakage in the end of December after more than one month low temperature hardening and semi-lethal low temperature (LT50) was estimated with logistic equation. The distribution of LT50 in the mapping population was not deviated from a normal distribution. QTL analysis was carried out with Map QTL5.0 software. Four QTLs were indentified at LOD≥3.0 using Multiple QTL Mapping (MQM) procedure, which could explain 29.3%, 23.1%, 44.7% and 21.0% of the phenotypic variation. These QTLs were mapped on the linkage group 2, 4, 3 and 1 respectively. The identified QTL areas and nearby markers should be useful for citrus freeze tolerance research and breeding in the future.

S02O14

Location of a chromosome region linked to Alternaria Brown Spot resistance from the evaluation of triploid mandarin populations

Cuenca J.[1], Aleza P.[1], Iborra E.[1], Vicent A.[1], Ollitrault P.[2], and Navarro L.[1]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain; and [2]Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), BIOS, France. jcuenca@ivia.es

Alternaria Brown Spot resistance in citrus has been described in diploid progenies as controlled by a single recessive allele (r). The objectives of this study were (1) to map chromosome regions that could be associated to *Alternaria alternata* resistance and (2) to analyse the inheritance of the resistance in triploid families resulting from 2*n* gametes and interploid crosses. Bulk segregant analysis coupled with genome scan (650 Single Nucleotide Polymorphism –SNP– markers genotyped with GoldenGate array) was used to identify genomic regions linked to the *A. alternata* resistance gene using a triploid progeny from a SrXrr cross. The results confirmed the monolocus inheritance of *A. alternata* resistance and revealed 44 SNPs differentiating the resistant and susceptible bulks within a 17 cM region near the centromere on chromosome III. De novo mapping from individual genotyping with 9 markers revealed that the *A. alternata* resistance gene could be located at 9.7 cM from the centromere. Two flanking markers were selected to perform marker assisted selection. Three populations from 2xX2x crosses sharing the female genitor (SrXrr, SrXSr and SrXSS) and one population from a 2xX4x cross (rrXSSrr) were evaluated for *A. alternata* resistance. Segregations indicate that 2xX2x crosses are better to obtain more resistant genotypes for a centromeric gene controlled by a recessive allele than 2xX4x crosses, since less heterozygosity is transmitted by 2*n* gametes than by gametes of doubled-diploids.

S02P01

Investigating the parentage of 'Orri' and 'Fortune' mandarin hybrids

Barry G.H.[1], Gmitter F.G.[2], Chunxian C.[2], Roose M.L.[3], and McCollum G.[4]

[1]XLnT Citrus company (XLnT Citrus), South Africa; [2]University of Florida-Institute of Food and Agricultural Science, Citrus Research and Education Center (UF-IFAS, CREC), USA; [3]University of California (UCR), Department of Botany and Plant Sciences, USA; and [4]United States Horticultural Research Laboratory, Agricultural Research Service, United States Department of Agriculture (USHRL-ARS, USDA), USA. ghbarry@gmail.com

Germplasm characterization is now possible with improved molecular analysis tools with a greater ability to detect polymorphisms. Previously citrus cultivars were primarily described according to their morphological

or horticultural traits, but trueness-to-type can now be confirmed using molecular markers, thereby limiting inadvertent cultivar misidentification. However, following routine testing the reported parentage of two mandarin hybrids is now questioned. 'Orri' mandarin is derived from 'Orah' mandarin hybrid by induced mutation, and was bred by Spiegel-Roy and Vardi (The Volcani Center, Israel) with 'Temple' tangor and 'Dancy' mandarin reported as the parents. 'Orri' is prized in many markets for its excellent organoleptic qualities, and other favorable traits. With 'Dancy' as the pollen parent, at least some degree of susceptibility of 'Orri' to Alternaria Brown Spot (ABS) might be expected, but more likely to have inherited a high degree of susceptibility; yet it appears to be immune to ABS. SSR analysis provided evidence that excludes 'Dancy' as the pollen parent of 'Orah'. Further testing has revealed the likely candidate as the pollen parent. 'Fortune' mandarin was a popular late-maturing mandarin cultivar until the high incidence of ABS rendered the cultivar uneconomical to produce. This exceptionally late, high quality, attractive cultivar is reported to have originated from a clementine mandarin x 'Dancy' cross made by Furr (USDA, Indio, California). 'Fortune' is used as a female parent in breeding programs. However, two independent molecular marker studies revealed that the pollen parent of 'Fortune' is actually 'Orlando' tangelo and not 'Dancy'. In both cases, the pollen parent of 'Orri' and 'Fortune' mandarin hybrids appears to not be 'Dancy' as published. This information will assist geneticists and breeders to re-interpret heritability studies on ABS susceptibility of mandarins and their hybrids.

S02P02

Simple PCR-based tolls provide molecular identification of the main rootstock used in Sao Paulo State, Brazil.

Coletta-Filho H.D., Pompeu-Jr. J., Francisco C.S., and Machado M.A.

Centro APTA Citros Sylvio Moreira (IAC), Laboratório Biotecnologia, Brazil. carolinaf@centrodecitricultura.br

Distinguishing between 'Sunki' and 'Cleopatra' mandarins as well as between 'Carrizo' or 'Troyer' citranges and 'Swingle' citrumelo very early in the nursery based only based on their respective leaf morphology could result in erroneous interpretation. Horticultural advantages of 'Swingle' rootstock are incomparable to 'Carrizo' under our conditions (Sao Paulo State, Brazil). The crescent diversification of citrus rootstock varieties in SP state has demanded, in particular situations, for identification of rootstock varieties very early in the nursery, sometimes as a consequence of accidental mixture of seeds. To help growers and nurseryman accurately identify rootstock varieties, we developed PCR-based tools that identify most of rootstocks used in SP state. Information from three-selected RAPD 10mer primers (M04-ggcggttgtc, M15-ctggcgtgtc, and AB18-gacctaccac) can be successfully used to identify and genetically separate citrange, citrumelo, and *Poncirus trifoliata.* Another 10mer primer (R04-cccgtagcac) identifies 'Rangpur' lime (*Citrus limonia*) and separates it from 'Volkameriano', and 'Siciliano' lemons. From the fingerprints generated by those 10-mer primers, repeatable and consistently well-amplified DNA fragments are used as variety-specific markers. Also, 'Sunki' and 'Cleopatra' mandarins are identified by the microsatellites CCSM-6R (cctctattaatgtgcctg) and CCSM-6F (atctgtgtgaggactgaa) primers. These simple PCR based protocols have been used to avoid significant economic losses by the early and correct identification of rootstocks in SP state nurseries.

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S02P03

Assignment of SNP allelic configuration in polyploids using Competitive Allele-Specific PCR: application to triploid citrus progenies

Cuenca J.[1], Aleza P.[1], Navarro L.[1], and Ollitrault P.[2]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain; and [2]Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), BIOS, France. jcuenca@ivia.es

Estimation of DNA allele doses in polyploid genotypes is essential to assign the allelic configuration for the different types of heterozygosity for parents and segregating progenies. It is of special interest in citrus with the implementation of extensive triploid breeding programs to develop new seedless varieties. Single nucleotide polymorphisms (SNPs) are becoming the most important class of molecular markers and we have tested the potential of KASPar technology (based on competitive allele-specific PCR) to infer allele doses of SNP markers in

polyploid citrus. DNAs from haploids *Citrus clementina* and *Citrus maxima* were mixed at different proportions to test the accuracy of the technique for quantitative analysis. All analyzed 7 SNPs provided clear clustering related with allele doses. DNA mixes at intermediary proportions revealed high correlation coefficients between observed and expected data (mean = 0.9796; sd = 0.0094). For all SNPs, separated cluster analyses and ANOVA from mixed DNA data formed all expected homogeneous groups, with correct assignation for practically all samples. Moreover, two triploid populations were easily genotyped and results were in agreement with expected segregations. KASPar technology is a routine and a cost-effective technique to assess the allele doses at the DNA level, which is especially interesting in citrus triploid breeding programs. Moreover, it could be also used to correlate genomic and transcriptomic doses in allele specific expression analyses.

S02P04

The parentage analysis contributes to the validation of high throughput SNP genotype calls of citrus

Fujii H.[1], Nonaka K.[1], Kita M.[1], Shimada T.[1], Endo T.[1], Kuniga T.[2], Ikoma Y.[1], and Omura M.[3]

[1]NARO Institute of Fruit Tree Science (NIFTS), Japan; [2]NARO Western Region Agricultural Research Center (NARO/WARC), Japan; and [3]Shizuoka University, Faculty of Agriculture, Japan. hfujii@affrc.go.jp

High-throughput genotyping is an important breeding tool to provide genome wide genotyping of germplasm for breeding and the selection of markers through linkage mapping. However, the validation system for genotyping calls from the automatic analysis has not been adequately developed to apply the high-throughput genotyping system in a practical fashion, because the marker data sets derived from DNA markers often contain missing or questionable genotype calls. Therefore, the objective of this study was to develop validation procedures using SNP data sets from a previously developed 384 multiplexed SNP array, named *CitSGA-1*, for the genotyping of *Citrus* cultivars. For this purpose, we initially used the manufacture's criterion that included: (1) the call frequency scores (over 0.9) for SNPs and (2) the GC10 and GC50 scores of samples. Thereafter, the following validation procedures were investigated: (3) removal of monomorphic SNPs genotype calls, (4) removal of No Call SNPs, and (5) removal of SNPs with discrepancies in the parentage analysis. In these procedures, the parentage analysis could detect the genetic discrepancy between parents and progeny in the reliable criteria. The obtained reliable SNP types were also tested for the reproducibility of calls by the replicated genotyping of accessions on the same SNPs. The results confirm these the validation procedures, which include the design of samples for parentage assays and the replicates necessary to obtain the reliability of large genotype calls.

S02P05

Comparative values of SSRs, SNPs and InDels for citrus genetic diversity analysis

Ollitrault P.[1], Garcia-Lor A.[2], Terol J.[3], Curk F.[4], Ollitrault F.[2], Talon M.[3], and Navarro L.[2]

[1]Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), BIOS, France; [2]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain; [3]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Genómica, Spain; and [4]Institut National de la Recherche Agronomique (INRA), GAP, France.
patrick.ollitrault @cirad.fr

SSRs have long been considered as almost ideal markers for genetic diversity analysis. With the increasing availability of sequencing data, SNPs and InDels become major classes of codominant markers with genome wide coverage. We have analyzed the respective values of SSRs, InDels, and SNPs for intra and interspecific *Citrus* genetic diversity analysis. Moreover, we have compared the diversity structure revealed by markers mined in a single heterozygous genotype (the clementine) and markers mined in a large interspecific survey. A random set of 25 markers was selected for each marker class to genotype 48 citrus accessions. SSRs were the most polymorphic markers at the intraspecific level allowing complete varietal differentiation within basic taxa (*Citrus reticulata*, *Citrus maxima*, *Citrus medica*). However, SSRs gave the lowest values for interspecific differentiation, followed by SNPs and InDels, that displayed low intraspecific variability but high interspecific differentiation. A clear effect of the discovery panel was observed for SNPs and InDels. The ascertainment biases associated with the clementine heterozygosity mining resulted mainly in an over estimation of within *C. reticulata* diversity and an underestimation of the interspecific differentiation. Therefore SSRs are very useful for intraspecific structure analysis while SNPs and InDels mined in large discovery panel will be more powerful to decipher the interspecific mosaic structure of secondary cultivated species.

S02P06

Preliminary research on genes and proteins related to a spontaneous mutant of *Citrus reticulata*

Zeng J.W.[1], Yi G.J.[2], Jiang B.[1], Zhong Y.[1], and Zhong G.Y.[1]

[1]Institute of Fruit Tree Research, Guangdong Academy of Agricultural Science, China; and [2]Guangdong Academy of Agricultural Science, China. zhongyun99cn@163.com

Bud mutations of citrus happen frequently and are the main natural resource for new cultivars. 'Mingliutianju' (*Citrus reticulata* 'Mingliutianju', MP) is a novel late ripening, very productive mandarin cultivar selected from 'Chuntianju' (*C. reticulata* 'Chuntianju', CP) through spontaneous bud mutation. In addition, the cultivar is morphologically contrast to the smooth fruit of its parent 'Chuntianju' by having prominent vertical narrow strips on its fruit surface. Large-scale analysis of gene expression differences between peels of MP and CP was performed at the 12th week and the 23rd week post flowering using Affymetrix GeneChip® citrus genome array. Altogether, 395 differentially expressed genes, including 132 up-regulated and 263 down-regulated genes, were identified in the mutant. Singular Enrichment Analysis (SEA) and Parametric Analysis of Gene Set Enrichment (PAGE) were performed and 7 statistically significantly differential-expressed genes were selected for further studies. Results from semiquantitative RT-PCR performed on these 7 genes were generally correlated with those observed with the microarray. These seven genes include three UDP-glucosyltransferase genes, two disease resistance-responsive protein-related genes, one chitinase gene and one protein kinase gene. Thirty three differentially expressed proteins were identified by two-dimensional gel electrophoresis using the same material. The differential proteins included 17 up-regulated and 16 down-regulated in the mutant. Pathway identification was conducted on these 33 differential expressed proteins through the web-based platform KOBAS. When these KEGG pathways were arranged according to their P values, flavonol biosynthetic process pathway ranked first.

S02P07

Allelic diversity of *Ferritin III* and *PMT IV* genes related to iron chlorosis in some citrus rootstocks

Aka Kacar Y.[1], Simsek O.[1], Donmez D.[1], Boncuk M.[1], Yesiloglu T.[1], and Ollitrault P.[2]

[1]Cukurova University (Cu), Horticulture Department, Turkey; and [2]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain. ykacar@cu.edu.tr

Iron (Fe) is one of the most important elements in plant mineral nutrition. Fe deficiency is critical abiotic stress factor for Mediterranean citriculture and the development of marker assisted selection (MAS) for this trait would be a great aid for rootstock breeding. In this study DNA sequencing and SSCP (Single Stranded Conformation Polymorphism) analysis were performed to discover allelic diversity of genes related to iron chlorosis tolerance in citrus. Two iron chlorosis tolerance candidate genes were selected from existing Citrus ESTs databases and *Arabidopsis thaliana* genome databases. *Ferritin-3* chloroplast precursor and putative membrane transporter candidate gene sequences were used to define primers in conserved regions. Six citrus genotypes from the basic taxon of *Citrus* where used to identify polymorphic areas in the genes. Direct sequencing of amplified DNA fragments of candidate genes was performed and SNPs (single-nucleotide polymorphisms) and InDels (Insertions/Deletions) where searched after sequence alignment. A total of 6840 bp DNA fragments were sequenced and 263 SNPs and 15 InDels were determined. New primers were defined, in conserved areas flanking polymorphic ones, for Single Strand Conformation Polymorphism (SSCP) analysis. SSCP-PCR analysis was performed with twenty-five *Citrus* genotypes. The neighbor joining method was used for cluster analysis. Phylogenetic origin of the alleles and genetic relationships of genotypes were discussed.

S02P08

Genetic mapping of QTLs associated with drought tolerance in citrus

Dutra-Souza J.[1], Cristofani-Yaly M.[2], Machado M.A.[2], and Oliveira A.C.[1]

[1]Universidade Estadual do Sudoeste da Bahia (UESB), Brazil; and [2]Centro de Citricultura Sylvio Moreira, Instituto Agronômico (IAC), Laboratório de Biotecnologia, Brazil. mariangela@centrodecitricultura.br

A population of 73 hybrids of 'Rangpur' lime (*Citrus limonia*) and 'Swingle' citrumelo (*Citrus paradisi* x *Poncirus trifoliata*), kept in plastic bags of 3.8 liters in a greenhouse, had water removed for 15 days. The gradient

of drought stress symptoms, after irrigation suspension, was measured by scores on a scale [absence of symptoms (0), wilting of leaves (1), leaf rolling (2), complete drying of the leaf, characterized by the change from green to brown (3) and drop of leaves (4)]. The symptoms were evaluated in four periods, the scores were pooled and the 'Index of Intensity of Symptoms of Drought' (IISD, from 0 to 100) was created, adapted from Czemainski (1999). Results of IISD were subsequently used to find QTLs to drought tolerance in an integrated genetic map of 'Rangpur' lime and 'Swingle' citrumelo, previously constructed, allowing the identification of seven QTLs in two linkage groups (LG) of the map.

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S02P09

Evaluation of resistance of 'Pera' (*Citrus sinensis*) genotypes to Citrus Canker in field and greenhouse conditions

Goncalves-Zuliani A.[1], Nunes W.M.C.[1], Zanutto C.[1], Croce Filho J.[2], and Nocchi P.T.M.[1]

[1]Universidade Estadual de Maringa (UEM), Nucleo de Pesquisa em Biotecnologia Aplicada-NBA, Brazil; and [2]Secretaria de Estado da Agricultura e Abastecimento (SEAB), Departamento de Fiscalizacao - DEFIS, Brazil. william.nunes@pq.cnpq.br

The use of resistant genotypes is an important alternative in the control of Citrus Canker caused to *Xanthomonas citri*. The objective of this study was to evaluate genotypes of the 'Pera' sweet orange for resistance to Citrus Canker in the field and under greenhouse conditions. A total of 25 genotypes of 'Pera' sweet orange were evaluated in orchards and in a greenhouse in the state of Parana, Brazil. To determine the incidence and severity of disease in the field 10 plants were evaluated per genotype, with samples consisting of four branches per plant. Data collected included counts of the total number of leaves, the number of diseased leaves, number of lesions and an inferred average of severity using a diagrammatic scale. In greenhouse treatment, leaves were inoculated by piercing with a needle (0.55 x 0.20 mm), containing *X. citri* inoculum adjusted to 10^8 CFU / ml at 600 nm and evaluated with the aid of a micrometer. The results were submitted to ANOVA and means were compared by Scott Knott test at 5% probability. In field conditions 'Pera EEL', 'Pera Coroada' and 'Pera Ovale Siracusa' genotypes showed no symptoms of disease in the three regions evaluated. In the greenhouse test, 'Pera EEL' (1.74 mm) and 'IAC 2000/1' (1.73 mm) genotypes showed smaller diameters lesions. An increase in diameter of the lesions was observed over time, requiring 61 days after inoculation (DAI) for the lesion size to be stabilized.

S02P10

Susceptibility of mandarins and tangors to Citrus Leprosis under greenhouse conditions

Nunes M.A.[1], Pereira J.A.[1], Freitas-Astua J.[2], Novelli V.M.[1], and Bastianel M.[1]

[1]Instituto Agronômico (CCSM-IAC), Centro de Citricultura Sylvio Moreira, Brazil; and [2]Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Cassava and Fruits, Cruz das Almas, Brazil. valdenice@centrodecitricultura.br

Citrus Leprosis is an important disease affecting sweet oranges in Brazil, and almost all of the studies performed to date have included only sweet oranges. The disease is caused by *Citrus leprosis virus* C (CiLV-C), which infects several citrus and non-citrus host plants, and is transmitted by *Brevipalpus phoenicis*, a cosmopolitan and polyphagous mite. Few studies are available on the response of other citrus genotypes, such as mandarins and their hybrids, to Leprosis. In this work, 56 mandarin (*Citrus reticulata*) and five tangor (*C. reticulata* x *C. sinensis*) genotypes from the Citrus Active Germplasm Collection of Centro Apta Citros Sylvio Moreira (BAG Citros IAC) were assessed for their response to Leprosis using a score scale. Five reps of each genotype were infested with viruliferous mites maintained on infected sweet orange fruits. Among the genotypes tested, only 'Shikai' mandarin (accession CN 551) and 'Dieberger 5' (accession CV 456) did not present any symptoms. 'Small-fruited 627' mandarin (accession CN561) was the most susceptible genotype evaluated, with the highest average score (3.2). The average general score to all genotypes was 1.25. The results demonstrated that some mandarins and tangors are resistant to Leprosis, as widely reported in the literature. However, most genotypes exhibit differential levels of susceptibility to the virus. Financial support: Fapesp, CNPq and Embrapa.

S02P11

Development CTV resistant citrus rootstocks using hybridization

Pinar H.[1], Seday U.[1], Unlu M.[1], and Uzun A.[2]

[1]Alata Horticultural Research Station, Genetic and Breeding, Turkey; and [2]Erciyes University, Department of Horticulture, Turkey. usd75@hotmail.com

Citrus is widely grown throughout tropical and subtropical regions of the world with 117 million tons of production. There are some serious problems affecting citrus production. One of them is *Citrus tristeza virus* (CTV) that is the most significant viral pathogen of citrus. This disease causes quick decline of trees grafted on sour orange (*Citrus aurantium*) rootstock. The development of CTV resistant rootstocks has become a priority objective of citrus breeding programs. Genetic studies revealed that CTV resistance is controlled by a single dominant gene in *Poncirus trifoliata*. Regarding resistance to CTV some molecular markers had been identified. Seven of them were cloned and were converted as SCARs. Three of them are SCB 11, SC007, SCTO8. In this study some citrus rootstocks ('Volkameriana', 'Rough lemon', 'Cleopatra', Citrumelo, 'Troyer' and 'Carrizo' citranges, 'Taiwanica', sour orange, Yuzu, 'Rangpur' lime, pummelo and *Fortunella* spp.) and hybrids of *P. trifoliata* X sour orange were screened with the three markers at Alata Horticultural Research Station. SCT08 marker yielded two bands for 'Troyer' and 'Carrizo' and one band for *P. trifoliata* and Citrumelo. On the other hand other citrus rootstocks had sensitive band at 400 bp. Five of *P. trifoliata* X sour orange hybrids have only one resistance band (homozygous resistant) and 30 of them have two bands (heterozygous resistant). SCB 11, SC007 SCAR markers were tested at this study. This study showed that although SCB 11, SC007 SCAR markers could be use for CTV resistance. In the next step all materials will be evaluated by biological inoculation of plants for CTV resistance.

S02P12

Lime Bush (*Citrus glauca*) hybrids resistant to CTV

Smith M.W., Gultzow D.L., Newman T.K., and Parfitt S.C.

Bundaberg Research Station, Queensland Department of Agriculture, Fisheries and Forestry, Australia. malcolmsmith1966@hotmail.com

The potential contribution of endemic Australian species to conventional citriculture has long been recognised, but nothing of commercial value has ever emerged. Rootstock experiments in Queensland, Australia have demonstrated that severe CTV reaction is their major limitation, totally masking the expression of useful traits these species are known to possess. In the case of *Citrus glauca*, the extremely slow growth of seedlings and cuttings during the nursery phase further prevents commercial application. A breeding program commenced in 2002 to address these limitations and has already developed 2nd generation hybrids of *C. glauca*. Obstacles encountered by previous breeders have been overcome by using *Citrus wakonai* as a bridging species, resulting in F1s that flower within two years, and readily hybridise with *Poncirus trifoliata*. F2 populations (85 hybrids) have been free of delayed lethality, segregate ~50:50 for CTV resistance, readily propagate from cuttings, and display vigour comparable with conventional citrus rootstocks. Cuttings from CTV resistant F2 hybrids will be deployed as rootstocks in field experiments in 2013.

S02P13

Determination of self-incompatibility status of some clementine (*Citrus clementina*) genotypes by histological analysis

Aka Kacar Y.[1], Aslan F.[1], Eti S.[1], Boncuk M.[1], Simsek O.[1], Yildirim B.[1], Yalcin Mendi Y.[1], Yesiloglu T.[1], Distefano G.[2], and La Malfa S.[2]

[1]Cukurova University (Cu), Horticulture Department, Turkey; and [2]University of Catania, Dipartimento di OrtoFloroArboricoltura e Tecnologie Agroalimentari, Italy. ykacar@cu.edu.tr

Most of clementine mandarins belonging to *Citrus* that is extremely important fruit group in the world show self-incompatible features as sexual. Several cultivars, characterized for high compatibility level with other varieties, often produce several seeds in fruits. Although seeds in mandarins-like represent a huge problem for citrus industry, the mechanisms related to citrus incompatibility have not been deeply investigated. In this study, self-compatible/incompatible status of 40 clementine mandarins located at the 'University of Cukurova, Faculty of Agriculture, Tuzcu Citrus Collection' were determined by histological analysis. For this purpose, self-

pollination was performed and following the self-pollination, pistil samples until the fifteenth day from the third day were taken at intervals of two days (3, 5, 7, 9, 11, 13, 15, 20th days after pollination). All samples fixed in FPA70 solution and stored at 4 °C. Pistil samples were stained with aniline blue for microscope observation. The preparations were observed by fluorescence microscope after staining and squashing. All pollen tubes of the genotypes arrested in the upper or middle part of style and none reached the base of the style. As a result of investigations, 40 varieties of clementines have been identified certainly as a self-incompatible.

S02P14

Genotyping for male sterility (MS) and MS gene mapping with RAPD markers in citrus, especially with precocious flowering seedlings from a cross of HY16 x grapefruit

Dewi P.S.[1], Wakana A.[2], Tanimoto Y.[1], Fujiwara Y.[1], and Sakai K.[2]

[1]Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University (Grad. School of Bioresource and Bioenvironmental Sciences Kyushu Uni.), Agriculture, Japan; and [2]Horticultural Laboratory, Kyushu University (Horticultural Lab., Kyushu University), Agriculture, Japan. p_saridewi@yahoo.com

Seedlings from *Citrus* species and cultivars show precocious flowering after autumn seed germination. HY16 is a monoembryonic and male sterile hybrid-derived from a cross of 'Hanayu' (*Citrus hanaju*) × 'Yuzu' (*Citrus junos*). 'Kiyomi' tangor (satsuma × 'Trovita'), a male sterile and monoembryonic plant, was also used to determine the male sterile genotype of grapefruit. Both seed parents generated seedlings with precocious flowering ability. Seeds were collected in November of 2006-2011 in Fukuoka, Japan, and allowed to germinate in a greenhouse (>10°C). Male sterile anthers were observed in hybrid seedlings from several crosses, i.e., HY16 × grapefruit, (HY16 × grapefruit) × grapefruit, HY16 × 'Ruby Blood' orange, (HY16 × grapefruit) × 'Ruby Blood' orange, HY16 × 'Trovita' orange, and 'Kiyomi' × grapefruit. On the basis of segregation analysis for MS in these progenies, and on the fact that HY16 and 'Kiyomi' did not have cytoplasmic restoration factor R for MS, male sterile genotype was estimated to be $ms_1ms_1Ms_2ms_2ms_3ms_3$ (R^-) for HY16, $Ms_1ms_1ms_2ms_2ms_3ms_3$ (R^-) for 'Kiyomi' and $ms_1ms_1Ms_2Ms_2Ms_3ms_3$ or $Ms_1ms_1Ms_2Ms_2ms_3ms_3$ ($R^)$ for grapefruit with male fertile anthers. Here, MS is a recessive character; ms_1 and ms_2 are complementary genes with upper stream expression of ms_2, and ms_3 is an epistatic gene to ms_1 and ms_2 genes. Male sterile seedlings appear when ms_3 is recessive homozygous (ms_3ms_3) and either ms_2 or ms_1 is recessive homozygous (ms_2ms_2 or ms_1ms_1). A population of 101 individuals from the cross of HY16 × grapefruit was chosen for mapping of *Ms* genes. Using bulked segregant analysis with 260 12-mer oligonucleotide random primers, seven RAPD markers linked to Ms_1 or Ms_3 allele of grapefruit were detected and mapped. Five markers flanked at the closest 9.9cM with the dominant of Ms_1 or Ms_3 alleles and two markers flanked at the closest 18.6cM with the recessive of ms_1 or ms_3 alleles.

S02P15

Embryological studies on 'URS Campestre', a new seedless orange cultivar

Guerra D.[1], dos Santos R.P.[2], Montero C.R.S[3], Schwarz S.F.[1], and Bender R.J.[1]

[1]UFRGS, Horticultural Sciences Department, Brazil; [2]UFRGS, Department of Botany, Brazil; and [3]Fepagro, Research Station Caxias do Sul, Brazil. rjbe@ufrgs.br

In citrus, seedlessness is a sought-after trait. Absence of seeds is advantageous for the juice industry as well as is meeting consumer demands. Close to the city of São Sebastião do Caí, 70 km north of Porto Alegre, the capital city of the southernmost state in Brazil, a new orange cultivar was identified and selected as a seedless cultivar. However, the cause for the nonexistence of seeds has yet to be determined. The objective of the present work was to analyze reproductive flower structures such as anthers and seminal rudiments to determine the grounds for the absence of seeds. ´URS Campestre´ flowers of different developmental stages were collected, fixed and examined. The flowers have anthers and ovaries of typical dimensions. Pollen grains characteristics analysis point to a standard developmental pattern, normal meiosis and high viability (84%) and elevated *in vitro* pollen tube germination rates (63%). However, so far, characteristics of generative cell and sperm cells have not been evaluated. Preliminary studies of seminal rudiments allowed detect abnormalities at the nucellus and embryo sac development, which seems to be one of the limiting causes of the fecundation process hindering seed production.

S02P16

Mechanism of seedlessness in a new lemon cultivar 'Xiangshui' (*Citrus limon*)

Zhang S.-W., Huang G.H., Ding F., He X.-H., and Pan J.C.

Agricultural College of Guangxi University, P.R.China. honest66222@163.com

Seedlessness is an important economic trait of lemon. Pollen and embryo sac fertility, embryo development, compatibility of self-pollinated 'Xiangshui' lemon and cross-pollinated 'Xiangshui' lemon were studied for investigating mechanism of seedlessness in 'Xiangshui' lemon. The results showed that the fertility of pollen and mature embryo sac was normal. The development of embryo sacs belonged to polygonum type. The mature embryo sac had one egg, two synergids, three antipodal cells and one big central cell containing two polar nuclei. Pollen tube in self- or cross-pollination grew well in stigma. Pollen tube of cross-pollinated 'Xiangshui' lemon could grow normally in style and ovary, and then entered into the embryo sac, double fertilization was accompanied. However, the growth of pollen tube in self-pollination was finally stopped in the bottom of stigma. Embryonic development in cross-pollination was normal. Zygote began to divide 2 weeks after cross-pollination (WACP). Early globular embryos were observed 3 WACP. Globular embryos and heart-shaped embryos were observed 4 WACP. Torpedo-shaped embryos were observed 5 WACP. Cotyledonary embryos were observed 6 WACP and developed into complete seeds. While in self-pollination, ovules began to abort at 2 weeks after self-pollination (WASP), disappeared 5 WASP later and produced seedless fruits. The emasculated flowers without any pollination finally developed into seedless fruits also. Results indicated that parthenocarpy is contributed to seedlessness. However, gametophytic self-incompatibility has a main role in seedlessness of 'Xiangshui' lemon by blocking fertilization in the bottom of stigma.

S02P17

Differences in the genetic structure of citrus triploid hybrids recovered from 2x X 2x and 4x X 2x sexual hybridisations

Aleza P.[1], Cuenca J.[1], Juárez J.[1], Ollitrault P.[2], and Navarro L.[1]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain; and [2]Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), BIOS, France. aleza@ivia.es

Citrus triploid hybrids can be recovered by 2x X 2x hybridisations as a consequence of the formation of 2n gametes or by interploid hybridisation. Most of the 4x parents used in our program are doubled-diploid (DD), also known as autotetraploid. The genetic structure of diploid gametes and particularly the rate of parental heterozygosity restitution (PHR) depend on the meiotic process by which they were originated. Second-division restitution (SDR) is the 2n gamete formation mechanism involved in 'Fortune' and clementines. With SDR, PHR is positively linked with the distance of each locus to the centromere. With DD parents, PHR depends on the rate of preferential pairing and thus the proportion of disomic versus tetrasomic segregations. We have compared the genetic structure of two populations of diploid gametes of clementine (one population of 2n gametes and one of 2x gametes produced by DD) with SNP and SSR markers covering the 9 citrus chromosomes. The DD displays mostly tetrasomic segregation; however three linkage groups present intermediate segregation and one displays a tendency for disomy. The PHR in the 2n gametes is 2/3 of that obtained in 2x gametes produced by DD. The two methods of triploid production appear complementary in terms of genotypic variability. 4x X 2x hybridizations are more efficient than 2x X 2x for developing new cultivars phenotypically closer to the diploid parent of the DD. Conversely, 2x X 2x hybridisations provides a greater opportunity to select innovative products.

S02P18

Efficient haploid production on 'Wilking' mandarin by induced gynogenesis

Jedidi E.[1], Kamiri M.[2], Poullet T.[3], Ollitrault P.[3], and Froelicher Y.[3]

[1]Tunisian National Agronomic Research Institute (INRAT), Horticultural Laboratory, Tunisia; [2]Groupe Kabbage (DAK), Agadir, Morocco; and [3]International Center for of Agricultural Research for Development (CIRAD), UMR AGAP, France. jedidi.emy@gmail.com

Seedlessness is a major criterion for the citrus fresh fruit market. Therefore, triploid breeding appears very promising for the selection of new citrus cultivars. CIRAD has developed a method to produce triploid hybrids

by somatic hybridization between diploid and haploid lines, and demonstrates the potential for haploid citrus production by induced gynogenesis in clementine. In this work we analysed the efficiency of induced gynogenesis by irradiated pollen in 'Wilking' mandarin (*Citrus reticulata*). Five doses of gamma irradiation of 'Meyer' lemon pollen were tested (0, 150, 200, 250, 300 Grays). Embryos were extracted from small seeds, obtained in immature fruits, and cultivated *in vitro.* The ploidy level of plantlets was determined by flow cytometry analysis. Irradiated pollen appeared to be efficient for gynogenesis induction in 'Wilking'. A relatively high number of haploids (7 plantlets) were obtained, essentially after hybridisation with 'Meyer' lemon pollen irradiated at 250 Grays. The ploidy level of all haploid plantlets was confirmed by chromosome counts, and molecular markers demonstrated their gynogenetic origin. These haploid lines should be useful for triploid breeding by somatic hybridisation, and also constitute interesting resources for genomic studies.

S02P19

Differences in ploidy levels of interploidal crosses progenies between diploids and tetraploid somatic hybrids in citrus

Kamiri M.[1], Srairi I.[1], Poullet T.[2], Ollitrault P.[2], and Froelicher Y.[2]

[1]Domaines Abbes Kabbage (DAK), Morocco; and [2]Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), UMR AGAP, France. kamirimourad@hotmail.com

One of the main objectives of citrus breeders is to obtain sterile mandarins that produce seedless fruits. Triploids are a response to these criteria. A way for triploid creation is sexual crosses between diploids and tetraploids. However, the scarcity of the natural tetraploid gene pool has been a restriction for using this method. Citrus somatic hybridisation via protoplast fusion allowed the creation of allotetraploid somatic hybrids that can be used as parents to generate triploid cultivars. Several crosses using diploids (female) and allotetraploid somatic hybrids (male) were conducted by CIRAD: a) 'Fortune' mandarin x ('Willow leaf' mandarin 'Star Ruby' grapefruit tetraploid somatic hybrid); b) 'Eureka' lemon x ('Star Ruby' grapefruit 'Corsican' citron tetraploid somatic hybrid); and c) 'Eureka' lemon x ('Mexican lime' 'Shamouti' orange tetraploid somatic hybrid). The majority of progenies were triploid from the 3 crosses (61-76%). However diploid and tetraploid progenies were also obtained in all of them. Molecular analysis of progenies with SSR markers revealed that tetraploids were issued from a diploid male gamete and an unreduced female gamete, while diploids could be issued from a haploid ovule and a haploid male gamete. This study reveals that ploidy variations were owed to meiotic dysfunction of either the tetraploid somatic hybrids or the diploid female parent.

S02P20

Gametic configuration and inheritance of SSR markers in tetraploid interspecific and intergeneric citrus somatic hybrids

Kamiri M.[1], Stift M.[2], Srairi I.[1], Chahidi B.[1], Poullet T.[3], Costantino G.[4], Dambier D.[3], Ollitrault P.[3], and Froelicher Y.[3]

[1]Domaines Abbes Kabbage (DAK), Morocco; [2]University of Konstanz, Ecology, Department of Biology, Germany; [3]Centre de coopération internationale en recherche agronomique pour le développement (CIRAD), UMR AGAP, France; and [4]Institut national de recherche agronomique (INRA), UR1103 Génétique et Ecophysiologie de la Qualité des Agrumes, France. kamirimourad@hotmail.com

Citrus somatic hybridization has become an integral part of citrus improvement programs worldwide. More than 100 somatic hybrids have been created by CIRAD. Intra and interspecific somatic hybrids will be used mostly for the triploid scion breeding. Intergeneric somatic hybrids can have a direct application as rootstocks or be used as parents for sexual 'tetrazyg' breeding. To optimize the design of such breeding schemes, it is essential to have knowledge of the inheritance mode in such allotetraploid hybrids. The aim of the present study was to investigate the inheritance (disomic, tetrasomic or intermediate) in a citrus interspecific hybrid (*Citrus reticulata x Citrus limon*) and an intergeneric somatic hybrid (*C. reticulata x Poncirus trifoliata*). Two triploid populations were generated using the somatic hybrids as pollinators in crosses with the diploid 'Chandler' pummelo. The triploid progenies were genotyped with SSRs to infer the allelic constitution of the somatic hybrid gametes. A likelihood-based approach was used to estimate for each locus the proportion of disomic versus tetrasomic segregations. For the two somatic hybrids, intermediate inheritance was observed with a major tendency for tetrasomic segregation in the interspecific hybrid, but a tendency for disomic inheritance in the intergeneric hybrid. The implications of the observed inheritance mode for citrus breeding are discussed with special focus on heterozygosity restitution.

S02P21

Haploid and polyploid hybrids obtained from cross of diploid citrus

Liu J.J., Chen K.L., He J., and Guan B.

Horticulture Research Institute, Sichuan Academy of Agricultural Sciences (HRI,SAAS), Centre of Fruit Reseach, China.
sc.liujianjun@163.com

Ploidy breeding is an effective and economical method in citrus to create seedless cultivars. Haploid, triploid and tetraploid hybrids were obtained and evaluated in our cross-breeding program. 'Tangor 15-1', a selection of local natural hybrid of *Citrus reticulata* × *Citrus sinensis*, was used as a female parent to cross with 'Red Tangerine' (*C. reticulata*) in 2000. Both parents are diploid. The obtained seeds were sowed in 2001 and seedlings began to flower and fruit successively since 2005. Traits of the seedlings were evaluated. During the evaluation process four seedlings were suspected to be variant for ploidy level. Further identification was performed by chromosome count and flow cytometry. Identification confirmed that one seedling was haploid, which was weak in growth with very small and long-shaped leaf; one was tetraploid, which was strong in growth with thick and dark-green leaf; the other two were triploid, which were strong in growth with seedless fruit. Bud sticks of these plants were taken for propagating conservation in the breeding nursery, and the biological/botanical characteristics of these haploid and polyploid plants were recorded and their fruit quality were analyzed. The tetraploid hybrid has been used as a new male parent in our subsequent breeding program.

S02P22

Chromosome redundancy and phenotypic variation in autotetraploid trifoliate orange seedlings

Oh E.U.[1], Chae C.W.[2], Kim S.B.[1], Park J.H.[1], Yun S.H.[2], Koh S.W.[2], and Song K.W.[1]

[1]Jeju National University (JNU), Faculty of Bioscience and Industry, Korea; and [2]National Institute of Horticultural and Herbal Science (NIHHS), Citrus Research Station, Korea. kwansong@jejunu.ac.kr

The study was conducted to evaluate the phenotypic variation in autotetraploid seedlings occurred spontaneously from diploid trifoliate orange (*Poncirus trifoliata*). Tetraploid nucellar seedlings were selected by flow cytometry and then ploidy level without aneuploid was confirmed by chromosome analysis. These young tetraploid seedlings were transplanted in open field and phenotypic variation such as tree vigor, growth habit, and photosynthesis activity was investigated. Tree vigor such as tree height, trunk diameter, internode length, and thorn length varied at juvenile stage. Specially, distinctively morphological difference was observed in growth habit including branch number and branching angle. Also, there was variation in photosynthesis activity such as leaf size, stomata density, photosynthesis rate, and chlorophyll index (SPAD value). These phenotypic variations had no correlation among phenotypic traits. The effect of chromosome redundancy on phenotypic variation was analyzed by karyotyping chromosomes with chromomycin A3 (CMA) staining and analyzing DNA methylation level. The results indicated that phenotypic variations of autotetraploid seedlings might be related to epigenetic effect.

S02P23

New cybrids resulting from asexual pathway: a promise of the cybridization for creating new rootstocks and varieties

Dambier D., Petit F., Barantin P., and Ollitrault P.

Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), France.
dominique.dambier@cirad.fr

Different breeding strategies have been developed by CIRAD to overcome agronomic and economical issues of the citrus industry in intertropical, subtropical and Mediterranean conditions. The present work is focused on the selection of new cybrids through different approaches of protoplast fusion. Two main objectives were targeted: (i) the manipulation of the nucleocytoplasmic male sterility in the framework of our seedless mandarin breeding project and (ii) the development of 'Rangpur' lime and 'Rough' lemon lines tolerant of *Alternaria*. 22 diploid cybrids have been obtained by targeted cybridization or as by-products of symmetric somatic hybridization, from interspecific and intergeneric combinations. For targeted cybridization, 4 genotypes 'Eureka' lemon, 'Volkamer' lemon, 'Rough' lemon and 'Boukhobza' orange, were combined via the cytoplasts method with 'Star

Ruby' grapefruit callus-derived cytoplasts. These combinations regenerated some diploid and tetraploid cybrids. The nuclear origin was confirmed by SSR markers to have arisen from the leaf-derived parent, while universal chloroplastic and InDel PCR markers exhibited respectively random chloroplast segregations but systematic inheritance of grapefruit mitochondria. The analysis of nucleocytoplasmic interaction should open in the near future a new pathway for rootstock improvement and sterility management for new seedless cultivar breeding.

S02P24

Several seedless citrus hybrids selected from tangor x 'Ponkan' cross

Liu J.J., Chen K.L., He J., and Guan B.

Horticulture Research Institute, Sichuan Academy of Agricultural Sciences (HRI,SAAS), Centre of Fruit Reseach, China. sc.liujianjun@163.com

Seedlessness is important for the consumption and marketing of fresh citrus fruit. 'Ponkan' (*Citrus reticulata*) is easy to peel and has excellent inner quality, but many 'Ponkan' cultivars in China are seedy (13～14 seeds per fruit). The goal of this breeding program is to breed new cultivars with the advantages of 'Ponkan' and less seeds. In 2000, 'Ponkan' was used as male parent to cross with 'tangor 15-1', a selection of local natural hybrid (*C. reticulata* × *C. sinensis*). 'Tangor 15-1' produces big fruit without seeds, deep orange-red rind, reddish albedo without cracks, and 'Ponkan' produces fruit with light orange rind, white albedo with cracks. The obtained seeds were sowed in 2001 and seedlings began to flower and fruit successively since 2005. Traits of the seedlings were evaluated. Six seedlings were found to be seedless or low seed. All of these six plants produced fruits of big size, but the color of rind and albedo as well as albedo crack, looked more like 'Ponkan'. The average seed numbers per fruit of six hybrids were 2.45, 2.86, 3.33, 4.33, 1.4 and 3.0. The biological/ botanical characteristics of these hybrid plants were recorded and their fruit quality was analyzed for 3 years. Preliminary observations show that some of them are promising for the citrus industry.

S02P25

New citrus hybrids: selection and genetic studies

Novelli V.M., Bastianel M., Fernandes K.A., Misságlia J., Latado R.R., Cristofani-Yaly M., and Machado M.A.

Instituto Agronomico - Centro de Citricultura Sylvio Moreira (IAC-CCSM), Brazil. valdenice@centrodecitricultura.br

The tools of biotechnology have enabled the improvement of citrus with significant reduction in cycle identification and selection of new materials, considering that the genetic studies and breeding in *Citrus* sp. sometimes has been hampered because of characteristics related to the reproductive biology of these species, i.e. high interspecific fertility, apomictic reproduction, polyembryony, a long juvenile phase and others. The Centro de Citricultura Sylvio Moreira-IAC has a breeding program for generating populations of scions and rootstocks hybrids. Phenotypic evaluations and genetic studies are underway to select promising materials, mainly for resistance to major diseases of citrus. The objectives of this study were to identify triploid plants and to construct genetic map with molecular markers using a backcrossing population of TMxLP163 ('Murcott' tangor x 'Pêra sweet' orange), a hybrid pre-selected in the breeding program, crossed with 'Pêra April' orange. Of the approximately 700 plants evaluated, 66 triploid (9.1%) were identified by flow cytometry . We evaluated 239 microsatellite loci, developed from EST library and 34 (14,2%) loci were selected with potential for genetic mapping. With this work, we expect to obtain new combinations resistant to Citrus Variegated Chlorosis (CVC) and with excellent fruit characteristics for the juice industry. Financial support: FAPESP, CNPq, INCT-Citros.

S02P26

Characterization of fruits of hybrids between 'Sunki' mandarin (*Citrus sunki*) and Sour orange (*Citrus aurantium*)

Schinor E.H., Michielin T.H.V., Simonetti L.M., Cristofani-Yaly M., Pompeu Jr. J., And Bastianel M.

Centro Apta Citrus Sylvio Moreira/IAC, Brazil. evandro@centrodecitricultura.br

The Centro de Citricultura Sylvio Moreira, Instituto Agronômico, since 1990, has been developing a breeding program of rootstocks, via hybridization, aiming to increase the number of varieties used in Brazilian citriculture and to improve the genetic variability as a way to overcome biotic and abiotic problems. In this work, we have

agronomically characterized 79 hybrids from reciprocal crosses between 'Sunki' (*Citrus sunki*) (TS) and sour orange (*Citrus aurantium*) (LA). Fifteen fruit of each hybrid were evaluated for the following characteristics: mass (M), height (H), diameter (D) and number of viable (SV) and aborted seeds (SA) per fruit, and the number of embryos of the seeds. The experimental design was a randomized complete block with fifteen repetitions. The averages of the variables were subjected to analysis of variance by F test and the comparison of the means made by the Scott Knott parametric test. There were differences among hybrids for all traits. The (M) of the fruits ranged from 28.2 to 116.7 g and (H) and (D) ranged from 3.03 to 5.92 cm and 1.21 to 7.57 cm, respectively. The fruits presented up to 22 (SV) and 1-18 (SA). The fruits of the hybrid TSxLA 7010 did not show (SV). The viable seeds showed 1-6 embryos. The results obtained for the characteristics evaluated showed that there was great variability among the hybrids.

S02P27

Evaluation of clementine x 'Kara' mandarin hybrids

Seday U.[1], Kafa G.[1], Uysal O.[1], Polatoz S.[1], Uzun A.[2], and Gulsen O.[2]

[1]Alata Horticultural Research Station, Mersin (AHRS), Turkey; and [2]Erciyes University Department of Horticulture, Kayseri (EUDH), Turkey. usd75@hotmail.com

Mandarin is one of the most important citrus species and has been subject of breeding research to obtain new cultivars by hybridization. Mandarin breeding program has been continued for a long time in Alata Horticultural Research Station, Mersin, Turkey. In the program, a lot of cultivars have been used as parents. We report here the results obtained by crossing mono embryonic clementine and late maturing 'Kara' mandarin. One hundred -twenty hybrids were evaluated for their fruit characteristics and 25 fruits per tree for four years were examined. We found a high level of variation in fruit parameters between hybrids. Fruit weight of hybrid plants ranged between 44-231 g. Seed number of hybrids varied from 3.85 to 45.80 whereas juice content ranged between 13.6% and 61.0%. According to four years of observation the harvesting period of hybrids extended from mid-October to end-February. Some promising hybrids were selected due to their good fruit quality. '9-16' hybrid was selected because of its large fruit size (168 g), early maturity and easy peeling features. On the other hand, '10-26' hybrid was superior with large fruits size (178 g), higher juice content (61%) and late maturity. Results of this study showed that increasing of fruit quality and extending maturity time of mandarin are possible using conventional hybridization methods.

S02P28

Distribution of leaf features in citrus hybrids obtained from clementine

Uysal O., Kafa G., Seday U., and Polatöz S.

Alata Horticultural Research Station, Turkey. onuruysal33@gmail.com

In this study, leaf length, leaf width and petiole wings dispersion were investigated in 41 hybrids obtained from clementine and 'Minneola' tangelo. Measurements were carried out using 40 leaves derived from all sides of four years plants. Clementine leaf length and width were 108.7 mm and 37.1 mm respectively whereas they were 107.8 mm and 51.3 mm respectively for 'Minneola' tangelo. The leaf length of hybrid plants varied between 72.5 mm and 144.0 mm. For forty-one hybrid plants the distribution of leaf length was as follows: 72.5-85.0 mm (14.63%), 85.0-100.0 mm (51.22%), 100.0-115.0 mm (19.52%), 115.0-145.0 mm (14.63%). Most of hybrids had lower leaf length than clementine and 'Minneola'. Leaf width varied between 24.0 mm and 50.1 mm among the hybrids with the following distribution: 2.4-3.0 mm (7.32%), 3.0-4.0 mm (60.97%), 4.0-5.1 mm (31.71%). Therefore leaf width, in hybrids mostly came from clementine mandarin. Petiole wings were observed in 36 of 41 plants.

S02P29

Optimal dose of gamma irradiation for mutation induction in mandarins

Handaji N.[1], Benyahia H.[1], Arsalane N.[1], Mouhib M.[2], Ibriz M.[3], Srairi I.[4], Chahidi B.[4], and Bourachde Y.[4]

[1]Institut National de la Recherche Agronomique Kenitra (INRA), Morocco; [2]Institut National de la Recherche Agronomique Tanger (INRA), Morocco; [3]Universite Bn Toufail Faculte de Sciences Kentra, Morocco; and [4]Domaine Kabbage Agadir, Morocco. citrusinra@yahoo.fr

A new program of irradiation was carried out by INRA Morocco to develop seedless fruits of existing citrus varieties. A first experiment was established to determine the optimal dose range of gamma source radiation

for mutagenic induction on the mandarins 'Anana', 'Vietnam' and 'Kara'. Seeds, budwood and seedlings were irradiated with a ^{60}Co gamma source with five doses (25Gy, 30Gy, 50Gy, 70Gy, 90Gy). No significant difference in radio sensitivity was observed between varieties depending on the dose of treatment. Single dose 90Gy caused the decrease of buds viability. Peeled seed showed no differences in radio sensitivity. Some stimulation of germination rate and early growth was observed with low doses. The LD_{50} for seedlings was between 70Gy and 90Gy. Field evaluation of the obtained mutants is currently performed in Kabbage domain.

S02P30

Effect of budwood irradiation on seed number of clementine, 'Nova' and 'Robinson' mandarins

Kafa G., Seday U., Uysal O., and Polatöz S.
Alata Horticultural Research Station Mersin (AHRS), Turkey. gucerkafa@gmail.com

We have investigated the effect of budwood irradiation on seed number of clementine, 'Nova' and 'Robinson' mandarin derived mutant populations. A total 675 plants issued from budwood irradiation and 10 non-irradiated plants of each parental variety as controls were planted in the field. For two years, seed number of 135 clementine mutants, 110 'Nova' mutants, 165 'Robinson' mutants and control plants were determined. Clementine mutant plants average seed number was 15.22 for 30 gray, 18.43 for 50 gray and 18.28 for non-irradiated in 2010. 'Nova' mutant plants average seed number was 8.25 for 30 gray, 7.36 for 50 gray and 18.38 for non-irradiated in 2010. 'Robinson' mutant plants average seed number was 10.75 for 30 gray, 11.76 for 50 gray and 23.66 for non-irradiated in 2010. Clementine mutant plants average seed number was 17.10 for 30 gray, 17.19 for 50 gray and 17.54 for non-irradiated in 2011. 'Nova' mutant plants average seed number was 11.01 for 30 gray, 15.53 for 50 gray and 18.61 for non-irradiated in 2011. 'Robinson' mutant plants average seed number was 11.32 for 30 gray, 12.11 for 50 gray and 22.38 for non-irradiated in 2011. As a result budwood irradiation decreases seed number in resulting mutant plants, especially for 'Nova' and 'Robinson' mandarins.

S02P31

Three variegated clementines

Kafa G., Seday U., Uysal O., and Polatöz S.
Alata Horticultural Research Station Mersin (AHRS), Turkey. gucerkafa@gmail.com

Three variegated clementine mutants among the M1V3 clementine plants were selected at the Alata Horticultural Research Station of Mersin. Three variegated clementine mutants obtained from budwood irradiation of 30 gray Gamma ray. Young fruit rind color attractively variegated but fruit rind color variegation fades with maturity and mature fruit rind color could easily masquerade as an ordinary clementine. The young fruit rind is striped green and orange, but mature fruit rind has ridges. Seed number is 7.5 for variegated clementine No.1, 13.07 for variegated clementine No.2, 11.94 for variegated clementine No.3 and 17.54 for control clementine plants. Fruit weight are 72.73 g for variegated clementine No.1, 69.27 g for variegated clementine No.2, 80.94 g for variegated clementine No.3 and 110.10 g for control clementine plants. Other pomological characteristics are as same variegated and control plants.

S02P32

New low seeded mandarins (*Citrus reticulata*) and lemons (*Citrus lemon*) selections obtained by gamma irradiation in Chile

Montañola M.J., and Martiz J.
Pontificia Universidad Católica de Chile (PUC), Facultad de Agronomíe e Ingeniería Forestal, Departamento de Fruticultura, Chile. mmontano@uc.cl

Gamma irradiation of buds is a technique that has been successfully used in citrus breeding programs that seek to remove seeds from fruits. In 2007, Pontificia Universidad Católica de Chile CITRUS lab started a new breeding program of mandarins and lemons using this technique. Irradiated buds were grafted and plants were grown in a commercial nursery. In 2009, a field trial was established with 4500 hybrid mandarins, 1200 clementines and 2500 lemons, in Pomaire, Chile (33°39'S, 71°10'W). First evaluation of fruit was made in

2011. Early production was achieved by the use of high density planting, minimal pruning managements, fertirrigation and enhancement of cross pollination using 10 to 12 hives/ha. After 2 harvest seasons, most phenotypical variations have been observed in lemons, obtaining 164 seedless selections, 4 thornless selections and a compact selection interesting for ornamental production. In the case of mandarins 316 plants have been described as low seeded and 14 were completely seedless. Techniques used in this program allow shortening in 5 years the time spent obtaining new citrus varieties. The stability of mutations detected is being evaluated and new commercial field trials will be established with the selected materials.

S02P33

Radiosensitivity of seeds and nodal segments of citrus rootstocks irradiated in vitro with γ-rays from ^{60}Co

Tallón C.I., Porras I., and Perez-Tornero O.

Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA), Citricultura, Spain. olalla.perez@carm.es

The first step for a breeding program through physical mutagenesis by radiation is the evaluation of the sensitivity in the plant material. Seeds of *Citrus aurantium* and *Citrus macrophylla* and nodal segments, where the buds were completely removed, from micropropagated explants of *C. macrophylla*, were used to determine their radiosensitivity to gamma radiation. A ^{60}Co source of γ-rays was used to deliver doses of 0, 10, 20, 30, 40 y 50 Gy for nodal segments, or between 0 and 250 Gy for seeds. After irradiation, the loss of germination percentage or organogenesis efficiency was measured over two consecutive culture periods and the dose resulting in a 50% loss in germination or regeneration frequency was computed as the LD50 value. For nodal segments of *C. macrophylla*, the LD50 was determined as 29.2 Gy. Seeds of sour orange were the least sensitive to radiation, its LD50 was determined at a dose of 155.6 Gy while the LD50 in seeds of *C. macrophylla* was of 129 Gy.

S02P34

Studies on mutation breeding for seedless and low-seeded cultivars of citrus

Tang X.L., and Ma P.Q.

Institute of Fruit Tree Research of Guangdong Academy of Agricultural Sciences, Key Laboratory of South Subtropical Fruit Biology and Genetic Resource Utilization, Ministry of Agriculture, China. tangxlgaas@21cn.com

Mature autumn shoots were taken from the adult seedy 'Nianju' (*Citrus reticulata*) and 'Luogangtiancheng' (*Citrus sinensis*) trees and then the leaves of the shoots were stripped. The shoots were irradiated with gamma rays from ^{60}Co at the doses of 0, 4, 6 and 8 Kr with the dose rate of 230 r/min in January of 2005. All the treated buds were single-bud-grafted on 'Hongningmeng' (*Citrus limonia*) rootstocks after irradiation treatment. The survival rates were 93.2%, 68.4%, 41.3% and 16.5% for the 0, 4, 6 and 8 Kr treatment of 'Nianju', and 98.9%,84%,73% and 57.1% of 'Luogangtiancheng', respectively. The plantlets were grown in the orchard in May of 2006. Through fruit investigation for 2 years, 4 low-seeded mutants (4-10, 6-11, 6-6, 3-11) with good characters of 'Nianju' were screened and their seed contents were 3.3-6.6 seeds/fruit, which were remarkably less than 17.6 seeds/fruit of the control. In the meanwhile, 4 commercial seedless mutants (5-2-1. 7-1-5, 7-3-1, 7-3-8) with good characters of 'Luogangtiancheng' were screened and their seed contents were 1.1-2.7 seeds/fruit, which were obviously less than 13.5 seeds/fruit of the control. The pollen alive rates and the pollen germination rates of the seedless and low-seeded mutants were obviously lower than their control respectively. One of the main causes producing seedless and low-seeded fruits was higher rate of pollen abortion.

S02P35

Agronomic performance of twenty six clones of 'Pera' sweet orange in São Paulo state, Brazil

Carvalho S.A., Latado R.R., Silva L.F.C., and Müller G.W.

Instituto Agronômico (IAC), Centro de Citricultura Sylvio Moreira, Brazil. sergio@centrodecitricultura.br

'Pera' sweet orange, one of the most important Brazilian citrus varieties, is highly susceptible to *Citrus tristeza virus* (CTV), causing reduced yield and fruit quality, even on tolerant rootstocks. The 'Pera' IAC

clone, preimunized with "PIAC" mild protective CTV isolate, is the most widespread, but several other clones derived from spontaneous mutations have been selected. In order to define the best 'Pera' sweet orange clones for each environmental condition, the agronomic performance of 26 clones was evaluated in 9 experiments carried out in three regions of the São Paulo state: Northern, Central and Southern, among which there is a temperature gradient decrease. The experiments were established as randomized blocks with one plant per plot and ten repetitions. Results from 10 to 17 years of evaluation show that genetic differences between clones reflect on growth, production and interaction with the climate and CTV. Natural breakdown in protection by severe CTV isolates in clones preimmunized with "PIAC" occurred in colder climates, even in vigorous clones such as IAC 2000, indicating the need of further studies for effective cross protection. The best clones for the Southern region are IAC 2000, Bianchi, IAC and EEL, but Olimpia and Ipiguá are not recommended. For the Central region, recommended clones include IAC 2000, Bianchi, IAC, EEL and Olimpia. These clones are also recommended to the Northern and warmer regions, with the exception of IAC 2000 that exhibits excessive vegetative growth, delaying the first commercial productions.

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S02P36

Distribution of fruit characteristics in the group of citrus Setoka nucellar seedlings

Park J.H., Yun S.H., Koh S.W., and Chae C.W.

Citrus Research Station (CRS), National Institute of Horticultural & Herbal Science RDA, Korea. jeju4444@korea.kr

'Setoka' tangor was developed at Fruit Research Institute in Japan. This variety has top fruit quality and also shape is graceful oblate. No seed is in it. The strategies of the breeding for nucellar progenies might be targeted for the early mature type, vigor, and improvement of fruit quality. We worked for the new nucellar breeds with the excellent fragrance better than the 'Setoka' tangor. In 2004, we obtained seeds from the hybridization by seed parent tangor and pollen parent 'Orlando' or *Citrus Natsudaidai*. In 2011, we analyzed and classified them using the data of main fruit trait in 150 flowered progenies. The average fruit fresh weight was 236g and peel thickness was approximately 2.8mm. Sugar content per fruit indicated 14.3°Brix and acid content was 1.49%. Sugar/acid ratio was 9.92. The highest frequency was 31.3% with the 14.1 ~ 15.0 °Brix of seedlings. Especially, excess of 16 °Brix progenies occupied the 9.4 percentage. Seedlings with 1.31 to 1.50% of acid content took up 28 percentage, following an 1.11 to 1.30%, 1.51 to 1.70% range, respectively. On the correlation between fruit weight and both °Brix and acidity, the greater fruit fresh weight tended to be the lower sugar and acid content in fruit. In the relation between °Brix and acidity, the sugar content in fruit tended to increase the acid content of the fruit. On the other hand, this tendency was not affect by the pollen genitors. In fruit quality of certain citrus, the desirable sugar/acid ratio is 13.0 referred to more than 13 °Brix and 1% or so. From the above results to develop new varieties of late matured citrus, the higher °Brix of nucellar seedlings might be selected carefully, we proved that the higher sugar content tended to increase the higher acid content of fruit in case of nucellar seedlings. We suggested that the selection of promising nucellar variety may be considered the profitable sugar/acid ratio by the seasonable analysis.

S02P37

'Qing-ougan': The green-peel mandarin mutation of 'Ougan' in Zhejiang province, China

Xu J.G.[1], Ke F.Z.[2], and Huang J.Z.[3]

[1]Zhejiang Citrus Research Institute (ZCRI), Research, China; [2]Zhejiang Branch for National Center for Citrus Variety Improvement (ZBNCCVI), Research, China; and [3]Ouhai Agriculture, forestry and fisheries Bureau of Wenzhou city (OAFFB), Extension, China.
xujg@mail.zaas.ac.cn

'Ougan' (*Citrus suavissima*) is a very special variety of mandarin in Wenzhou city, Zhejiang province, China. 'Qing-ougan' mandarin is a mutation of 'Ougan' that maintains green peel even after fruit reach maturity. The first trees discovered were found in Ouhai District of Wenzhou city in 2001. 'Qing-ougan' mandarin has the same or very similar characters of tree, leaves and flowers with other 'Ougan' selections, which

are vigorous with nearly round-shaped fruit, high yields and good storage quality. The length of shoots, thorns and petals is longer than with other clones. The average fruit weight is about 130 g, much smaller than 'Ougan', rind thickness is 3.8 mm, thinner than 'Ougan', and the edible rate is 69.9%, significantly higher than common and seedless 'Ougan'. The total soluble solids of fruit juice is 9.5%, lower than other clones. Both of the content of total acidity and Vitamin C do not differ significantly with other clones of 'Ougan'. The fruit shape of 'Qing-ougan' is depressed at the stalk end, but the common 'Ougan' is usually slightly rounded. 'Qing-ougan' can maintain green peel up to the following March under common storage conditions, but color can change under ethylene 'de-greening' treatment. This indicates some chlorophyll degradation. For these reasons, 'Qing-ougan' could be an important germplasm material for breeding and genetic studies.

S02P38

Hybridization-based citrus breeding program in Turkey

Uzun A.[1], Gulsen O.[1], Seday S.[2], and Kafa G.[2]

[1]Erciyes University, Department of Horticulture, Turkey; and [2]Alata Horticultural Research Station, Genetics and Breeding, Turkey. aydinuzun@erciyes.edu.tr

Breeding studies to develop new cultivars in citrus have been carried out using traditional methods, such as selection, mutation and hybridization. We have carried out in Turkey a mandarin breeding program based on hybridization for the last ten years. Clementine mandarin was used as maternal parent and many cultivars from several species were used as male parents. So far, over 4000 hybrids have been already evaluated. Up to now several promising hybrids with different characteristics have been selected. The genotype '33-6' from a clementine X 'Orlando' tangelo cross is the most promising one, with large fruit (175 g) and dark orange rind color and it exhibits early maturity (October). The hybrid genotype '20-2' from a clementine X 'Valencia' orange cross was found to be late maturing (April) and it has large fruits and good appearance. A hybrid from a clementine x 'Cocktail' grapefruit, '39-9' cross has very large fruit (244 g) with attractive shape and it is harvested in November. 'CO-44', from a clementine X 'Orlando' tangelo cross has large (167 g) and early maturing fruit (October). In addition to these hybrids, many genotypes with good characteristics are also being selected. In the near future the new superior cultivars will be released.

S02P39

'BATEM Fatihi': A New Orange Selection from cv. 'Washington' navel

Eryılmaz Z.[1], Dal B.[1], Tuncay M.[2], Balkılıç R.[3], and Gübbük H.[3]

[1]Batı Akdeniz Agricultural Research Institute (BATEM), Fruit Research, Turkey; [2]Retired agricultural engineer; and [3]University of Akdeniz, Faculty of Agriculture, Department of Horticulture, Turkey. zeyneper07@gmail.com

Citrus variety improvement programme has been continuing in Turkey during last 30 years. During the period 1979 to 1983, a total 42 promising genotypes grown in different citrus locations and that have better earliness, yield and quality characteristics from main cultivars 'Washington' navel has been selected. An adaptation parcel was established with these selected types in 1991 in Antalya. After a long term evaluation, 4 promising types were determined and 2 of them has been registered in 2011 as new orange cultivars. One of the registered cultivars named as 'BATEM Fatihi' has been compared with cv. 'Washington' navel in terms of maturation time, yield and quality characteristics. The results of the study showed that cv. 'BATEM Fatihi' has better fruit and tree characteristics such as earlier harvest, compact growth habit, higher yield capacity and bigger fruit, thinner peel, higher juice yield and soluble solid content than cultivar 'Washington' navel. The fruit maturation time was second week of November for cv. 'BATEM Fatihi' and the first week od December for cv. 'Washington' navel. The yield per tree was recorded as 120 kg for cv. 'BATEM Fatihi' and 80 kg for cv. 'Washington' navel. The peel thickness, soluble solid content and fruit juice yield were 5.49 mm, 10.62% and 49.57% for cv. 'Washington' navel and these values were 5.07 mm, 10.57% and 49.57% in cv. 'BATEM Fatihi', respectively.

S02P40

A new orange cultivar 'BATEM Bahari' selected from cv. 'Valencia Late'

Eryılmaz Z.[1], Ercişli S.[2], Gübbük H.[3], and Tuncay M.[4]

[1]Batı Akdeniz Agricultural Research Institute (BATEM), Department of Fruit Research, Turkey; [2]Ataturk University, Faculty of Agriculture, Department of Horticulture, Turkey; [3]Akdeniz University, Faculty of Agriculture, Department of Horticulture, Turkey; and [4]Retired agricultural engineer. zeyneper07@gmail.com

In this study, we aimed to select promising higher yield and quality orange types from 'Valencia Late' orange cultivars grown in different parts of Mediterranean region. The selection studies were done between 1979 and 1983 and a total 13 promising types were selected from cv. 'Valencia Late' in different locations. An adaptation parcel was established with these selected types in 1991 in Antalya. Long term evaluation were done on these 13 types and among them 3 types were found more promising and one of them registered as new orange cultivar under the name 'BATEM Baharı' in 2011. In this study, some fruit and yield characteristics of this new cultivar were compared with original cv. 'Valencia late'. 'BATEM Baharı' had lower seeds, thinner shell and higher juice yield. Average fruit weight, shell thickness, juice yield were 204,17g, 5,16 and 48,11% in cv. 'Valencia Late' and these values were 229,25 g, 4,41 and 58,80% in 'BATEM Baharı'.

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CITRUS GENOMICS

S03O01

Genome sequence analysis and comparisons reveal ancestral hybridization and admixture events in the origins of some citrus cultivars

Gmitter Jr. F.G.[1], Ollitrault P.[2], Machado M.A.[3], Reforgiato Recupero G.[4], Talon M.[5], Roose M.L.[6], Navarro L.[7], Wu G.[8], Jaillon O.[9], Morgante M. [10], and Rokhsar D.S.[9]

[1]University of Florida - Citrus Research and Education Center, USA; [2]CIRAD, France; [3]Centro APTA Sylvio Moreira, Instituo Agronômico, Brazil; [4]C.R.A.-Istituto Superiore per l'Agrumicoltura, Italy; [5]IVIA, Centro de Genomica, Spain; [6]University of California at Riverside, Department of Botany and Plant Sciences, USA; [7]IVIA, Centro de Protección Vegetal y Biotecnología, Spain; [8]USDOE, Joint Genome Institute, USA; [9]Genoscope - CNS, France; and [10]University of Udine, Istituto di Genomica Applicata, Italy. fgmitter@ufl.edu

Since its inception, the International Citrus Genome Consortium (ICGC) has pursued development of freely available genomics resources and tools for the benefit of the citrus research community; these efforts were conducted by citrus scientists in concert with researchers from some of the world's major genome sequencing centers and corporations. An early ICGC goal was to produce a full-length genome assembly. Two reference genome sequences were made publicly available in January 2011, from a 'Clemenules' clementine-derived haploid and diploid 'Ridge Pineapple' sweet orange, produced using Sanger and 454 sequencing technologies respectively. Subsequent work has proceeded to improve the assemblies and their annotation; a brief summary of their characteristics and status of the assemblies will be presented. As sequencing technology has evolved and costs have dramatically decreased, other citrus genomes have been sequenced (the diploid 'Clemenules', 'Ponkan', 'Willowleaf', 'W. Murcott' mandarins; 'Siamese Sweet' and 'Chandler' pummelo; and sour orange, among others). A comparative analysis of these genomes has made it possible to describe the genomic contributions from ancestral outbred populations to major cultivar types, and to propose likely underlying models. The results confirmed previous reports that clementine arose from a hybridization event between 'Willowleaf' mandarin and sweet orange. Further, introgressions of *Citrus reticulata* and *Citrus maxima* germplasm, long implicated in its ancestry, clearly defined the segmented mosaic genome of sweet orange, through the ancient admixture of these species. Finally, sour orange was found to be a direct hybrid of a pummelo and mandarin. Interestingly, although the two pummelos sequenced appear to have their derivations from a single wild species (*C. maxima*), the mandarins sequenced reveal ancestral admixture of *C. maxima* with *C. reticulata*. Implications and future directions for collaborative research will be discussed.

S03O02

Sequencing of 150 citrus varieties: linking genotypes to phenotypes

Terol J.[1], Carbonell J.[2], Alonso R.[2], Tadeo F.R.[1], Herrero A.[1], Ibáñez V.[1], Muñoz J.V.[1], López-García A.[1], Hueso L.[1], Colmenero-Flores J.M.[3], Conesa A.[2], Dopazo J.[2], and Talón M.[1]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Genómica, Spain; [2]Centro de Investigación Príncipe Felipe (CIPF), Bioinformatics and Genomics Department, Spain; and [3]Instituto de Recursos Naturales y Agrobiología (IRNAS), Departamento de Biotecnología Vegetal, Spain. talon_man@gva.es

Elucidation of genomic sequences is an essential and necessary first step to identify genetic variants associated with agronomic traits of interest. The CITRUSEQ Consortium, a joint effort developed by public institutions and private companies has sequenced the genomes of 150 citrus varieties with the Illumina platform at an average coverage higher than 60x. Paired end genomic DNA libraries were constructed and average readings of (2x) 100 bp length with an average insert size of 500 bp were performed. For each variety, useful total average readings were higher than 220 million and total mapped readings approached 212 million. The haploid genome of *Citrus clementina* (www.phytozome.com) was used as a reference genome. Sequencing statistics indicate an average representation higher than 92% of the reference genome with coverage of 15x. More than 150 citrus genomes including the 3 ancestral taxa and many rootstocks and species of mandarins, oranges, lemons, grapefruits and limes were sequenced. The consortium has generated a web portal that allows searching and sequence comparison among the approximately 8 million genes found and the 45 billion base pairs read. Initial analyses suggest that the stored information can help unravel fundamental aspects of biological and evolutionary interest such as the origin of citrus, the mechanism of generation of spontaneous varieties, the identification of recombination hotspots, the effect of induced mutations, etc. Preliminary data suggest, for example, the presence of mitotic crossovers

of large genomic regions. Sequence analyses have also shown that natural and induced mutations that produce similar changes in the genome also generate similar phenotypes. From the commercial standpoint this information may help to authenticate varieties, to support marker-assisted breeding and to identify genes of major agronomic interest.

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S03O03

Whole genome sequencing and mapping analysis for identifying polymorphism among 11 citrus varieties

Shimizu T.[1], Yoshioka T.[1], Nagasaki H.[2], Kaminuma E.[2], Toyoda A.[2], Fujiyama A.[2], and Nakamura Y.[2]

[1]NARO Institute of Fruit Tree Science (NIFTS), Citrus Research, Japan; and [2]National Institute of Genetics (NIG), Genome Informatics Laboratory, Japan. tshimizu@affrc.go.jp

NIFTS has been conducting cross-breeding of citrus to develop new promising scions with unique and attractive features for consumers. Developing new scions, however, requires 14 to 20 years due to long juvenile period of citrus. Marker assisted selection of important traits at the seedling stage is anticipated to reduce the total cost of the breeding, and it requires sufficient numbers of genome-wide DNA markers to achieve efficient selection. In this study, we sequenced 11 citrus varieties that were widely used in our breeding program to obtain sufficient amounts of polymorphic loci among them. Nine of them include satsuma, 'Ponkan', sweet orange and clementine. Two hybrids were also selected to confirm inheritance of called polymorphisms. Next generation sequencing of the citrus varieties was conducted by paired-end analysis with HiSeq 2000 DNA sequencer (101 bp for both ends). A total of 100 x genome sequence coverage were obtained for each variety, and they were de novo assembled individually. Mapping analysis with the clementine haploid sequence as a reference detected more than 100,000 SNPs. Number of SNPs and ratios of homozygous SNPs to heterozygous SNPs were different among varieties. Obtained sequences for individual varieties with called SNPs would be useful to develop high-throughput genotyping platforms and to promote cross-breeding with less cost.

S03O04

Sweet orange genome: sequencing, annotation and beyond

Xu Q.[1], Ruan X.[2], Chen L.L.[2], Chen D.J.[2], Zhu A.D.[1], Chen C.L.[1], Ruan Y.[2], and Deng X.X.[1]

[1]Huazhong Agricultural University (HAU), Horticulture Department, China; and [2]Huazhong Agricultural University (HAU), Colleage of Life Science and Technology, China. xuqiang@mail.hzau.edu.cn

Sweet orange (*Citrus sinensis*) is one of the most important fruit crops in the world. Orange is responsible for approximate 70% of total citrus production, and is used both as fresh fruit and processed juice. Most oranges are diploids, with the haploid chromosome number x=9 and estimated genome size of 370 Mb. On a double-haploid line of 'Valencia' sweet orange, we employed the Illumina/Solexa platform to produce 785 million high quality paired-end-tag sequencing reads (2×100 bp) from various DNA fragment sizes (~300 bp, 2 Kb, 10 Kb, and 20 Kb). The sequence reads were assembled by SOAPdenovo and Opera, resulting in 16,890 assembled sequence contigs and 4,811 scaffolds (N50=1.7 Mb). The total contig sequence length covers nearly 90% of the estimated sweet orange genome. We also sequenced more than 5000 BAC-ends, the alignments between these BAC sequences and the citrus genome assembly showed an overall high identity. To annotate the citrus genome for protein coding genes, a comprehensive strategy that combined *ab initio* gene predictions, homology searches, and experimental supports (EST, RNA-seq and RNA-PET) was employed. We generated 965 million RNA-seq reads and 100 million RNA-PET reads from four citrus tissues (callus, leaf, flower and fruit) to characterize the orange transcriptomes. In total, 29,655 protein-coding loci (gene models) with 44,645 transcripts were identified, with 99% of predicted coding sequences (CDS) supported by RNA-seq data, demonstrating the high accuracy of gene annotation in the orange genome. The sweet orange genome, as a complementary source to the clementine genome released by the International Citrus Genome Consortium, represents a valuable resource for genetic understanding of orange biology and manipulation of many important traits for citrus breeding.

S03O05

A reference genetic map of *Citrus clementina*; citrus evolution inferences from comparative mapping

Ollitrault P.[1], Terol J.[2], Chen C.[3], Federici C.T.[4], Lotfy S.[5], Hippolyte I.[1], Ollitrault F.[6], Bérard A.[7], Chauveau A.[7], Cuenca J.[6], Costantino G.[8], Kacar Y.[9], Mu L.[4], García-Lor A.[6], Froelicher Y.[1], Aleza P.[6], Boland A.[10], Billot C.[1], Navarro L.[6], Luro F.[8], Roose M.L.[3], Gmitter Jr. F.G.[3], Talón M.[2], and Brunel D.[7]

[1]Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), BIOS, France; [2]Instituto Valenciano de Investigacion Agrarias (IVIA), Genomic Center, Spain; [3]Citrus Research and Education Center, University of Florida (CREC), USA; [4]University of California, Riverside (UCR), Botany and Plant science, USA; [5]Institut National de la Recherche Agronomique (INRA), Morroco; [6]Instituto Valenciano de Investigacion Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain; [7]Institut National de la Recherche Agronomique (INRA), UR EPGV, France; [8]Institut National de la Recherche Agronomique (INRA), UR GEQA, France; [9]Faculty of Agriculture, University of Çukurova, Department of Horticulture, Turkey; and [10]Centre de l'Energie Atomique (CEA), DSV/Institut de Génomique, France. patrick.ollitrault@cirad.fr

The availability of a saturated genetic map of clementine was identified by the ICGC as an essential prerequisite to assist the assembly of the reference whole genome sequence based on a 'Clemenules' clementine derived haploid. The primary goals of the present study were to establish a clementine reference map, and to perform comparative mapping with pummelo and sweet orange. Five parental genetic maps were established with SNPs, SSRs and InDels. A medium density reference map (961 markers for 1084.1 cM) of clementine was established and used by the ICGC to facilitate the chromosome assembly of the haploid genome sequence. Comparative mapping with pummelo and sweet orange revealed that the linear order of markers was highly conserved. The map should allow reasonable inferences of most citrus genomes by mapping next-generation sequencing data against the haploid reference genome sequence. Significant differences in map size were observed between species, suggesting variations in the recombination rates. Skewed segregations were frequent and higher in the male than female clementine. The mapping data confirmed that clementine arose from hybridization between 'Mediterranean' mandarin and sweet orange and identified nine recombination break points for the sweet orange gamete that contributed to the clementine genome. Moreover it appears that the genome of the haploid clementine used to establish the citrus reference genome sequence has been inherited primarily from the 'Mediterranean' mandarin.

S03O06

The chromosomes of citrus: from a unifying nomenclature to the evolution of karyotypes

Silva S.C.[1], Mendes S.[1], Moraes A.P.[1], Marques A.[1], Mirkov T.E.[2], Iglesias D.J.[3], Ibáñez V.[3], Talón M.[3], Soares Filho W.S.[4], Guerra M.[1], and Pedrosa-Harand A.[1]

[1]Universidade Federal de Pernambuco (UFPE), Departamento de Botânica, Brazil; [2]Texas AgriLife Research (Texas A&M System), Department of Plant Pathology and Microbiology, U.S.A.; [3]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Genómica y Postcosecha, Spain; and [4]Embrapa Cassava&Fruits (EMBRAPA), Brazil. andrea.pedrosaharand@pesquisador.cnpq.br

Most citrus species have a small genome compartmentalized in nine chromosome pairs (2*n*=18). Their small chromosomes were differentiated in types based on the distribution of heterochromatic blocks when the fluorochromes CMA and DAPI were applied. Several cultivars could then be distinguished and hybrids identified. Only recently, FISH (fluorescent *in situ* hybridization) of ribosomal DNA genes (5S and 45S rDNA) and BACs (bacterial artificial chromosomes) from *Poncirus trifoliata*, in combination with the CMA/DAPI staining, were used to establish chromosome-specific markers. We have used these markers to identify each chromosome pair and build cytogenetic maps of *P. trifoliata* cv. 'Flying Dragon', *Citrus reticulata* cv. 'Cravo', *Citrus maxima* cv. 'Pink' and *Citrus medica* var. 'Ethrog'. Because the same markers were applied to these species, a unifying chromosome nomenclature could be proposed. No breakdown of synteny was detected, but most homeologous chromosomes varied in type, indicating differential expansion and contraction of heterochromatin. While chromosomes 1, 4 and 8 were conserved, chromosome 3 was especially variable, suggesting additional structural rearrangements such as inversions. Those markers were also useful to identify the parental chromosomes 2, 3 and 6 in the hybrid *Citrus sinensis* cv. 'Valencia', corroborating *C. maxima* and *C. reticulata* as its putative ancestors. The present addition of anchored mandarin BACs to the cytogenetic map will integrate it to the citrus genome sequence.

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S03O07

Analysis of the clementine floral transcriptome uncovers candidate genes involved in self-incompatibility

Caruso M.[1], Lo Cicero L.[1], Distefano G.[1], Merelo P.[2], La Malfa S.[1], Tadeo F.R:[2], Talón M.[2], Lo Piero A.R.[1], and Gentile A.[1]

[1]University of Catania (DISPA), Department of Agriculture and Food Science, Italy; and [2]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Genómica, Spain. gentilea@unict.it

Self-incompatibility (SI), typical of many mandarin and pummelo varieties, is a valuable trait to obtain seedless fruits. Despite the importance of SI, the genetic basis of this trait in citrus is poorly understood. Two clementine genotypes showing divergent behaviour related to self-pollen rejection ('Comune', self-incompatible'; and 'Monreal', self-compatible) were chosen as model to study the molecular events occurring during pollen-pistil interaction and the self-incompatible response. The comparative analysis of the floral transcriptome of the two clementine genotypes led to identifying a set of candidate genes related to SI. The identified genes show no homology to SI genes of other plant species and lack annotation. Functional analyses are therefore needed to unravel their function and to confirm their role in the citrus progamic phase. Our attention focused on 4 genes over- represented in the' Comune' transcriptome whose expression drastically increased in concomitance with the halting of pollen tube elongation, namely a putative F-box protein gene and three aspartic-acid rich (Asp-rich) protein encoding genes. A first step of the functional characterization consisted in their over-expression in tobacco plants. Moreover, the Asp-rich proteins were expressed in a cell-free system and purified for a biochemical characterization. The preliminary results of the functional analyses are discussed.

S03O08

Citrus gene function analysis using a viral vector based on the *Citrus leaf blotch virus* genome

Agüero J., Velázquez K., Vives M.C., Pina J.A., Navarro L., Moreno P., and Guerri J.

Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Mocada, Valencia, Spain. jaguero@ivia.es

Genetic improvement of citrus by conventional breeding is hindered by their complex reproductive biology, high heterozygosis and long juvenile period (up to 6-8 years). Moreover, the genes responsible for each biological process are presently unknown. Since viral vectors allow rapid testing of gene function, they may be a better tool than transgenic expression for improvement of citrus, whose adult plants are difficult to transform. We have developed vectors based on the *Citrus leaf blotch virus* (CLBV) genome by i) introducing in a full-genome infectious cDNA clone a unique restriction site in the 3' untranslated region (*clbv*3´ vectors) or at the intergenic region between the movement protein and the coat protein (CP) genes (*clbv*IN vectors), and ii) duplicating in these positions a minimum promoter derived from the natural promoter sequence of the CP subgenomic RNA. When fragments of the *phytoene desaturase*, *magnesium chelatase* or *actin* genes were cloned in those vectors and then inoculated onto different citrus species all plants showed the silencing phenotype and reduced accumulation of the cognate mRNAs. This phenotype was observed in successive flushes for at least 2 years and the inserts remained stable as tested by RT-PCR with primers flanking the insertion site. Expression of the *FLOWERING LOCUS T* in young seedlings using those vectors incited flowering in just 4 months. This dramatic reduction of the juvenile period may be a useful tool for citrus genetic improvement.

S03P01

Expression analysis of genes differentially expressed in stem tissue of two lemon cultivars.

Koutsioumari E.M., and Voloudakis A.E.

Agricultural University of Athens (AUA), Laboratory of Plant Breeding and Biometry, Department of Crop Science, Greece. eva.kouts@yahoo.gr

Lemon (*Citrus limon*) cultivation in Greece and other Mediterranean countries suffers from two major problems: the destructive disease Mal Secco caused by the fungus *Phoma tracheiphila* and chilling injury caused by low temperatures. The knowledge of genes involved in resistance to the above mentioned stresses would be useful in breeding tolerant lemon cultivars. In order to determine the molecular basis of resistance to these stresses,

suppression subtractive hybridization (SSH) was applied on two lemon cultivars exhibiting differences in resistance to Mal Secco and cold, namely 'Lisbon' (susceptible to both stresses) and 'Adamopoulou' (tolerant Greek cultivar), which is believed to have derived from 'Lisbon'. Two subtractions were performed resulting in two subtractive cDNA libraries: 'Adamopoulou-Lisbon' containing sequences expressed preferentially in 'Adamopoulou" and 'Lisbon-Adamopoulou' containing genes expressed in 'Lisbon'. A total of 587 clones were sequenced and the obtained sequences were edited resulting in 81 non-redundant ESTs that have been deposited in GenBank EST database. The analysis of these sequences using BLAST software (NCBI) revealed homology to previously identified genes involved in defense mechanisms against biotic and abiotic stresses. ESTs from each subtractive library were selected for further analysis of their expression in both cultivars by performing semi-quantitative RT-PCR reactions. The results of this experiment will be discussed.

S03P02

Transcriptome profile analysis of Citrus Canker resistance of Chinese citron (*Citrus medica*) via massively parallel mRNA sequencing

Li D.Z.[1], Dai S.M.[2], Li N.[2], and Deng Z.N.[2]

[1]Provincial Key Laboratory of Crop Germplasm Innovation and Utilization (PKLCGIU), Hunan Agricultural University, China; and [2]National Center for Citrus Improvement, (NCCI), Hunan Agricultural University, China. ldazhi@163.com

Citrus Canker (*Xanthomonas axonopodis* pv. citri) has brought great damage to citrus industry worldwide. To control the disease, it seems the use of resistant genotypes is the only choice. A total of 14 accessions from three species were investigated by artificial inoculation on leaves with the pathogen, and it was found that only Chinese citron (*Citrus medica*) showed complete and active resistance to canker disease. Comparative transcript profiling of leaves after inoculating pathogen on resistant Chinese citron by using massively parallel signature sequencing (MPSS) was performed. Samples from 3 stages (RC1,RC2,RC3) according to the symptom development were chosen and they were divided into the susceptible stage (SS) and the resistant stage (RS). A total of 13,396,168,12,747,212 and 13,491,020 high quality clean reads were obtained for the samples, respectively. Interpretation of the MPSS signatures revealed that the total number of transcribed genes in the SS (18,045) was larger than that in the RS (17,394). Further comparison of the transcripts revealed that 2554 genes had nearly twofold expression difference in the RS compared with the SS (1409). Especially, the number of down-regulated genes in the RS (1984) is much higher than that in the SS (470), suggesting that many genes were restrained in the RS. In addition, we identified more than 20 genes that were involved in the plant-pathogen interaction pathway. These genes are highly conserved in citrus and other species, suggesting that the function of the related proteins in resistance to bacterial disease may be conserved as well. Our results provide a foundation for comparative gene expression studies about the incursive development of that citron was inoculated by pathogen. Additionally, a number of candidate genes that were involved in resistance to disease were identified. These results provide new insight into the molecular processes regulating resistance to Citrus Canker.

S03P03

Characterization of genes associated with two agronomically important traits in citrus: drought tolerance and fruit color

Costa M.G.C.[1], Cidade L.C.[1], de Oliveira T.M.[1], Martins C.P.S.[1], Mendes A.F.S.[1], Pereira S.L.S.[1], Pedrosa A.M.[1], Gesteira A.S.[2], Girardi E.A.[2], Coelho-Filho M.A.[2], Soares Filho W.S.[2], and Machado M.A.[3]

[1]State University of Santa Cruz (UESC), Center for Biotechnology and Genetics, Brazil; [2]Embrapa Cassava & Fruits (EMBRAPA-CNPMF), Brazil; and [3]Instituto Agronômico de Campinas, Centro APTA Citros Sylvio Moreira, Brazil. marciogc.costa@gmail.com

Drought tolerance and fruit color are important traits to citrus producers and consumers. Because citrus cultivation is mainly located in regions subjected to long periods of water shortage, drought is one of the main factors affecting citrus productivity worldwide. On the other hand, the color of citrus fruits provided by carotenoid pigments is one of the most important attributes of fruit quality and consumer acceptance. Within the scope of the National Science and Technology Institute of Genomics for Citrus Breeding, with support of Embrapa Cassava & Fruits, our group has focused on functional characterization of genes affecting drought tolerance and fruit color in citrus. To this end, we are using the tools of genomics, molecular biology,

and recombinant DNA technology. Some of the results already obtained include: (1) identification of novel members of the NAC, WRKY and NF-Y transcription factor gene families involved in drought stress response, (2) characterization of LEAs and a TIP2;1-like aquaporin conferring tolerance to dehydration, (3) description of an alternative mechanism of ABA biosynthesis regulation and abiotic stress tolerance provided by the phytoene synthase gene, (4) characterization of two novel lycopene beta-cyclase genes controlling beta-carotene biosynthesis in photosynthetic- or chromoplast-containing tissues, and (5) identification of several alternatively spliced isoforms of the *lycopene epsilon-cyclase* gene.

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S03P04

Expression of *Ptcor8* gene induced by low temperature as related to cold resistance in citrus

Long G.Y.[1], Song J.Y.[1], Luo K.[1], Deng Z.N.[2], Li N.[2], and Gentile A.[1]

[1]College of Horticulture and Landscape (CHL), Hunan Agricultural University, China; and [2]National Center for Citrus Improvement-Changsha Subcenter (NCCICS), Hunan Agricultural University, China. longgy63@163.com

*Ptcor*8 (EU077497) gene was previously isolated from the leaf of trifoliate orange (*Poncirus trifoliata*) naturally cold acclimated in field. In different investigations it was found that the expression of *Ptcor8* gene may not be directly related to the physiological defoliation of trifoliate orange, but rather closely related to low temperature induction. In the whole cold acclimation process in different citrus genotypes, when the temperature decreased to or below 5^{o}C, the expression of the gene significantly increased. The maximum expression level was observed in the most cold-tolerant trifoliate orange, followed by kumquat (*Fortunella crassifolia*), pummelo (*Citrus grandis*), sweet orange (*Citrus sinensis*), and the minimum in lemon (*Citrus limon*), indicating that *Ptcor8* expression was relatively closed to low temperature and related to cold tolerance of different citrus species. Then, the genomic DNA sequences of *Ptcor8* from *Poncirus* and lemon were cloned and analyzed. Results showed that the two homologous genes contain four exons and three introns, and shared high identity of 98.31% and 96.12% in nucleotide and amino acid sequence, respectively. So, we further cloned the promoter sequences from *Poncirus* and lemon. The size of consensus sequence from lemon was 2053bp. Promoter sequences from trifoliate orange are divided into two types and the sizes were 1973bp and 1991bp, respectively. Predication of *cis*-acting elements in promoter sequences demonstrated that no difference of cis-acting elements-is there-in two types of promoter sequences from trifoliate orange. There is low-temperature responsive cis-acting element (LTRE) in the promoter sequences of lemon and trifoliate orange, but some elements related to stress responsiveness only exist in promoter of trifoliate orange, whereas auxin-responsive element only in promoter of lemon.

S03P05

Cloning and characterization of a prolin-rich protein gene *CsPRP4* from citrus

Ma Y.Y.[1], Zhang L.Y.[2], Zhu S.P.[3], and Zhong G.Y.[4]

[1]Key Laboratory of Horticulture for Southern Mountain Regions, Ministry of Education, China; [2]Guizhou Normal University, Guiyang, China; [3]Citrus Research Institute, Southwest University, China; and [4]Key laboratory of South Subtropical Fruit Biology and Genetic Resources, Ministry of Agriculture, China. zhongyun99cn@163.com

Proline-rich proteins (PRPS) represent a class of cell wall proteins that possess repetitive proline and hydroproline residues. In this study, we isolated a novel PRP gene that was designated as *CsPRP4* from citrus. Expression analysis showed that the *CsPRP4* transcript was existed preferentially in leaves and significantly up-regulated by exogenous ethylene treatment. A 1.5kb promoter sequence of *CsPRP4* gene was cloned from *Citrus sinensis* genome and its activity was assayed by using a GUS reporter gene under its control. The promoter is shown to direct the expression of the GUS gene in leaves and cotyledons of the transgenic *Arabidopsis thaliana*. To study the function of *CsPRP4*, we transformed citrus with a binary vector containing a full length clone of *CsPRP4* in sense orientation and also a vector containing an RNAi construct to study the function of the gene. The RNAi plants exhibited a stunted phenotype by showing leaves significantly smaller than both the over-expression plants and the wild type plants. Electron microscopy revealed that blocking the expression of *CsPRP4* in the RNAi plants reduced the number and size of starch grains in chloroplasts, indicating that *CsPRP4* may be involved in development and carbohydrate metabolism of leaves.

S03P06

Ploidy and gene expression in clementine

Niñoles R., Aleza P., Castillo M.C., Navarro L., and Ancillo G.

Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain. ancillo@ivia.es

Polyploidy has long been recognized as an important force in the evolution of flowering plants as often results in novel phenotypes that are not present in their diploid progenitors or exceed the range of the contributing species. The new acquired phenotypes may have an advantage in adaptation that may also enhance their interest for agriculture. Many crops, including citrus, are bred to a higher level of ploidy in search of desirable traits. In previous works of our group, synthetic clementines with different levels of ploidy were generated, specifically haploid, dihaploid and autotetraploid lines were obtained. This plant material represents a valuable tool for studying the effects of polyploidization at a molecular level since important traits are expressed in a different manner. Interestingly, dihaploid lines have been not able to flower, while haploid, diploid and autotetraploid lines have. By using a microarray approach, we have accomplished a comprehensive analysis of transcriptome divergence among the newly created clementine lineages with different genome dosage and specifically have explored new gene expression patterns related to the ability to flower. Differences in expression in the bark tissue of the four lineages were found significant for ninety four genes. Clustering analysis results and expression profile of these genes in the 4 compared lineages were used as additional criteria to select 16 genes putatively involved in the flowering differences found in the lineages. To explore their potential involvement in the flowering event we have analyzed their expression in floral organs and the occurrence of differential expression in juvenile and adult bark tissues. Results and discussion will be presented.

S03P07

Expression of flowering genes in different shoot types in citrus

Muñoz-Fambuena N.[1], Mesejo C.[1], González-Mas M.C.[2], Iglesias D.J.[2], Primo-Millo E.[2], and Agustí M.[1]

[1]Universitat Politècnica de València (UPV), Instituto Agroforestal Mediterráneo, Spain; and [2]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Citricultura y Producción Vegetal, Spain. namuofam@upvnet.upv.es

In citrus, three main shoot types can be distinguished: vegetative shoots (VS), leafy inflorescences (LY) and leafless inflorescences (LS). This study aimed to determine the relationship between flowering genes expression and shoot type. To this end, shoots of 'Moncada' mandarin were collected at the beginning of bud sprouting, and-flowering genes expression was analysed by RT-PCR. LS and LY did not-significantly differ in the number of flowers per inflorescence. However, they significantly differed in flowering genes relative-expression. Thus, LS presented the highest relative expression of the-flowering genes *CiFT, CsAP1, CsLFY, CiSEP1* and *CiSEP3*, and the lowest of that flowering inhibitor *CsTFL*, whereas VS showed the opposite. LY showed intermediate relative expression between VS and LS of either flowering promoters or inhibitor. Results indicate an absence of relationship between the number of flowers per inflorescence and relative gene expression. On the other hand, the different levels of expression of flowering genes, *CiFT,* flowering-time, *CsTFL, CsLFY, CsAP1,* meristem identity and *CiSEP1* and *CiSEP3*, floral organ specification, seem to be related with the-appearance of different shoot types.

S03P08

Shortening the juvenile phase in *Arabidopsis* plants by ectopic expression of citrus transcription factors

Castillo M.C., Navarro L., and Ancillo G.

Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain. ancillo@ivia.es

In citrus, juvenility is a long period (5-20 years depending on the variety) during which floral initiation does not occur even under normally inductive environmental conditions. Efficient breeding is limited due to this long period of juvenility. Several transcription factors (TFs) were identified as differentially expressed in juvenile and adult phases in an approach involving the use of a microarray containing 1152 putative unigenes of citrus

TFs. Some of them are MADS-box genes, a family of TFs involved in developmental processes whereas others showed high partial sequence similarity restricted to specific domains but negligible outside those domains. Transgenic *Arabidopsis* plants overexpressing these TFs were generated and number of leaves without abaxial trichomes, flowering time and morphology of leaves, siliques and whole plants have been analysed. Transgenic lines for four of the TFs showed shortening of the juvenile phase, indicating that these genes are likely involved in determining the reproductive phase transition also in citrus. Lines which showed no phenotype differences corresponded to TFs that have not yet been characterized in other plants and therefore they might be novel genes that play specific roles during the juvenile-to-adult transition in citrus. This makes them good candidates to study the specific aspects of juvenility in citrus. Deciphering the molecular mechanisms involved in the juvenile-to-adult transition in citrus plants is crucial if we expect to reduce the generation time for genetic studies and breeding programs. Our results represent a step forward in the knowledge of these processes in citrus and offer the prospect of rapid transfer of information to other woody plants.

S03P09

Differential expression of proteins related to primary metabolism in 'Moncada' mandarin leaves with contrasting fruit load

Muñoz-Fambuena N.[1], Mesejo C.[1], Agustí M.[1], Tárraga S.[2], Iglesias D.J.[3], Primo-Millo E.[3], and González-Mas M.C.[3]

[1]Universidad Politécnica de Valencia (UPV), Instituto Agroforestal del Mediterráneo, Spain; [2]Universidad Politécnica de Valencia (UPV), Instituto de Biología Molecular y Celular de Plantas, Spain; [3]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Citricultura y Producción Vegetal, Spain. gonzalez_mde@gva.es

A proteomic study was used to know how the primary metabolism in citrus leaves is affected by the fruit load. To this end, we researched the differential expression of proteins related to these processes between on-crop and off-crop 'Moncada' mandarin leaves. Samples were collected in November and proteins were extracted. From 2D DIGE gel of these extracts, 33 spots related to primary metabolism were isolated: 26 spots being associated with photosynthesis and carbohydrate metabolism, 4 spots related to Krebs cycle, 1 spot related to pentose phosphate pathway, and 2 spots related to nutrient reservoir activity. These spots were identified by MALDI-MS or LC-MS-MS. Between the proteins related to photosynthesis and carbohydrate metabolism, 16 were up-expressed in off-crop leaves, such as Nadp-dependent glyceraldehyde-3-phosphate dehydrogenase, RuBisCO large subunit, ADP-glucose pyrophosphorylase, cinnamoyl-CoA reductase, and carbonic anhydrase; while 10 proteins were up-expressed in on-crop leaves such as NADP-dependent malic enzyme, and 3,4-dihydroxy-2-butanone kinase. The isolated proteins related to pentose phosphate pathway (6-phosphogluconate dehydrogenase) and to nutrient reservoir activity (Granule-bound starch synthase Ib precursor) were up-expressed in off-crop leaves. Regarding the proteins related to Krebs cycle, only malate dehydrogenase was up-expressed in off-crop leaves, while the other proteins were up-expressed in on-crop leaves (citrate synthase and NADP-isocitrate dehydrogenase). According these results, the primary metabolism seems to be more active in off-crop leaves, suggesting that fruit load inhibits the primary metabolism in 'Moncada' mandarin. This work has been supported by the Project INIA RTA2009-00147-C02 and Fundación Agro Alimed.

S03P10

Proteins related to stress and redox state of Moncada mandarin leaves with contrasting fruit load

Muñoz-Fambuena N.[1], Mesejo C.[1], Agustí M.[1], Tárraga S.[2], Iglesias D.J.[3], Primo-Millo E.[3], and González-Mas M.C.[3]

[1]Universidad Politécnica de Valencia (UPV), Instituto Agroforestal del Mediterráneo, Spain; [2]Universidad Politécnica de Valencia (UPV), Instituto de Biología Molecular y Celular de Plantas, Spain; [3]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Citricultura y Producción Vegetal, Spain. gonzalez_mde@gva.es

A proteomic study was used to know more about the citrus redox state and response stress related to fruit load. To this end, we researched expression differences of proteins related to these processes between on-crop and off-crop 'Moncada' mandarin leaves. Samples were collected in November, were immediately stored frozen at -80 ºC, and proteins were extracted in less than two weeks. From 2D DIGE gel of these extracts, 12 related spots were isolated: only 1 showed increased expression in the off-crop samples compared to on-crop

samples, while the other 11 showed increased expression in the on-crop samples against off-crop samples. These spots were identified by MALDI-MS or LC-MS-MS. The only protein with increased expression in the off-crop samples was putative monocopper oxidase precursor, while the up-expressed proteins in on-crop leaves were catalase, monodehydroascorbate reductase, Fe-superoxide dismutase, glutathione peroxidase, and three proteins related to response stress. Thus according to these results, generally, in the off-crop samples, proteins with oxidoreductase activity are down-regulated, while in the on-crop samples the proteins related to stress response and to oxidoreductase activity are up-regulated. Therefore, the redox state is different for off-crop and for on-crop *Citrus* leaves.

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S03P11

Genomic compositions and origins of *Citrus sinensis*, *Citrus clementina* and *Citrus poonensis* as revealed by genome-wide analysis of citrus ESTs

Zhong G.Y.[1], Wu B.[2], Zeng J.W.[1], Jiang B.[1], and Yang R.T.[2]

[1]Institute of Fruit Tree Research, Guangdong Academy of Agricultural Science, China; and [2]Citrus Research Institute, Southwest University, China. zhongyun99cn@163.com

The origins of *Citrus sinensis, Citrus clementina and Citrus poonensis* are largely unknown. A genome-wide analysis of citrus EST sequences was performed in this study to address this issue. A considerably high level of SNP polymorphisms in citrus was found by analyzing ESTs. The interference from the allelic expression bias associated with EST analysis was eliminated by using only the unigenes with enough sequence coverage. Two approaches, haplotype comparison and phylogenetic analysis were used to analyze the 359 different gene loci of citrus. It was shown that all the three species, *C. sinensis, C. clementina and C. poonensis* are genome-wide heterozygous. Inference from analyzing EST data was that *C. clementina* was derived from a cross between a mandarin and *C. sinensis*, and the mandarin was confirmed to be *Citrus deliciosa* by genotyping 25 representative SNPs. Statistically *C. sinensis* must be originated from three consecutive crosses, i.e., [(pummelo × mandarin) × pummelo or pummelo × (pummelo × mandarin)] × mandarin, and the supposed immediate mandarin parent was experimentally searched but not found. The evolution and domestication of citrus and the significance of high level of SNP polymorphisms were discussed. Information from ESTs provided some clues to the elucidation of the heterosis mechanism leading to the superiority of the three most important and widely cultivated citrus. EST based analysis and SNP genotyping are valuable in inferring the origins of cultivated *Citrus* species. The origins of *C. sinensis*, and *C. clementina* were revealed. The genome of *C. poonensis* was analyzed.

S03P12

Genomic sequencing and comparative analysis of *Citrus platymamma* (Byungkyool) by using next-generation sequencing technology

Chung S.J.[1], Han S.I.[2], Jin S.B.[3], Kim Y.W.[1], Yun S.H.[4], Riu K.Z.[2], and Kim J.H.[2]

[1]Research Institute for Subtropical Horticulture, Jeju National University, Korea; [2]Faculty of Biotechnology, College of Applied Life Science, Jeju National University, Korea; [3]Subtropical & Tropical Organism Gene Bank, Jeju National University, Korea; and [4]Citrus Research Station, National Institute of Horticultural & Herbal Science, Rural Development Administration, Korea. kimjh@jejunu.ac.kr

Next-generation sequencing (NGS) technologies are being utilized for de novo sequencing, genome re-sequencing, and whole genome and transcriptome analysis. *Citrus platymamma* (Byungkyool) has been known to be a native citrus species in Jeju Island, South Korea. In this study, we have sequenced *C. platymamma* genome to analyze genome structure and gene function. Genomic DNA was obtained from leaves of *C. platymamma* and about 25 Gb short-read sequences produced by the Illumina paired-end sequencing technology. The initial assembly was contained 321 Mbp of *C. platymamma* genome in 20,818 scaffolds with N50 size of 60Kbp. These scaffolds contained 176,765 contigs in which N50 size was 3.5 Kbp. Comparison with the sweet orange sequence, a total of 40,905 genes of *C. platymamma* were confirmed. This genome sequence data will be used to characterize potential roles of new genes in Byungkyool and can be used in comparative studies of other *Citrus* species.

S03P13

Fast and cost-effective DNA marker typing method with accuracy for citrus by direct PCR and multiplexed post-labeling

Ohta S.[1], Yano K.[1], Kurita Y.[2], Kita M.[1], Yoshioka T.[1], Nesumi H.[1], and Shimizu T.[1]

[1]NARO Institute of Fruit Tree Scie (NIFTS), Okitsu Citrus Research Station, Japan; and [2]Aichi Agricultural Research Center (Aichi Pref), Department of Horticultural Research, Japan. sohta@affrc.go.jp

DNA marker typing has been used in citrus for many purposes such as linkage mapping, marker-assisted selection, and cultivar identification. However, DNA marker typing is time-consuming and costly in citrus because of requiring highly purified DNA to eliminate compounds that strongly inhibit PCR. Fragment analysis with capillary DNA sequencer enables highly accurate genotyping analysis but it requires expensive fluorescently labeled primers that are another cost factor. We developed direct PCR method for citrus in order to achieve fast and cost-effective genotyping. This method consists of two steps; (1) pricking a leaf sample with a toothpick, and (2) dipping the toothpick into a PCR mixture or a TE buffer solution directly to prepare a DNA sample. The prepared DNA sample was then provided for PCR analysis. We were able to process 95 samples within 1.5 h, in contrast to 23 h by conventional DNA extraction method. Subsequent PCR amplification using five different STS markers was highly successful (96–100%) except for the 1200 bp marker using the DNA samples dissolved in TE buffer solution (86%). We combined the direct PCR method with multiplexed post-labeling method for fragment analysis with DNA sequencer, which enables multiplexed post-labeling of DNA markers (up to 6) in a single tube without laborious labeling step. As a consequence, total cost was decreased to one tenth or less than those using pre-labeled primers.

S03P14

Data mining and systems biology for identifying key genes involved in citrus quality

Silva E.M.A.[1], Bernardes L.A.S.[1], Ollitrault P.[2], Bonatto D.[3], and Micheli F.[1]

[1]Universidade Estadual de Santa Cruz (UESC), DCB, Brazil; [2]Centre de Cooperation Internationale en Recherche Agronomique pour le Developpement (CIRAD), BIOS - UMR AGAP, France; and [3]Universidade Federal do Rio Grande do Sul (UFRGS), DBMB, Brazil. fabienne.micheli@cirad.fr

Quality in citrus is mainly characterized by fruit and juice colour, fruit and skin size, juice percent, total soluble solids, titrable acidity, and carotenoid/flavonoid contents. Moreover, studies of biosynthetic pathway of the metabolites/proteins involved in quality at transcriptional and translational levels may give relevant information for subsequent functional studies and quality improvement. Data mining of ESTs from HarvEST database allowed the selection of 17 cDNA libraries from albedo, flavedo, peel, pulp and juice sac of different orange, mandarin, clementine and grapefruit varieties. In order to select key genes involved in quality we used systems biology that offers mathematical tools that include the analysis of the structure, clustering and centralities of the network. In order to have information regarding physical protein-protein interactions (PPPI) from citrus sequences, orthologous sequences of *Arabidopsis thaliana* were used (BLASTX; reciprocal BLASTP). Literature data mining was performed, and PPPI network design was obtained using the Cytoscape software. The interactome networks thus obtained were analyzed with MCODE. Gene ontology clustering analysis was performed using BiNGO. Specific algorithms were applied to identify modules and central nodes within the citrus libraries associated network. The obtained results will be used as a guideline to select specific genes/proteins from citrus for further functional studies as gene expression or plant transformation.

S03P15

Genotyping and mutation scanning by high resolution melting (HRM) analysis of citrus EST-SNPs and SSRs

Distefano G.[1], La Malfa S.[1], Caruso M.[1], Lo Piero A.R.[1], Nicolosi E.[1], and Shu-Biao W.[2]

[1]Università degli Studi di Catania (DISPA), Dipartimento di Scienze delle Produzioni Agrarie e Alimentari, Italy; and [2]University of New England, School of Environmental and Rural Science, Armidale, Australia. slamalfa@unict.it

Citrus taxonomy is very complex mainly due to specific aspects of its reproductive biology. A number of studies have been performed using various molecular markers in order to evaluate the level of genetic variability

in citrus. SNP markers were used for genetic diversity assessment using different methods; recently, the availability of EST database and whole genome sequences increased the information on sequence variability in citrus leading to wider employment of these markers so that reliable SNP detection methods are needed. In the present study, for the first time we applied high resolution melting curve analysis (HRM) in citrus to detect the presence of SNPs or SSRs polymorphisms. In citrus, the possibility of characterizing genetic diversity was drastically increased using this method since it allowed analyzing more polymorphisms in a single PCR fragment. The results suggest that HRM is an efficient, cost-effective and accurate method to detect sequence variations. In fact, HRM analysis provided a panel of primers for HRM genotyping in citrus representing also a useful tool for bridging functional and structural genomics.

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BIOTECHNOLOGY

S05O01

Unlocking the molecular secret of blood orange

Butelli E.[1], Licciardello C.[2], Reforgiato-Recupero G.[2], and Martin C.[1]

[1]John Innes Centre (JIC), Metabolic Biology, UK; and [2]Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee (CRA), Italy.
eugenio.butelli@jic.ac.uk

The derivation of blood orange has been an outstanding question in horticulture for several centuries and consumer interest in this variety has risen significantly in recent years with the demonstration that consumption of blood orange juice promotes health and prevents obesity. We have investigated the molecular basis for the blood orange trait and shown that all Mediterranean blood oranges are derived from the insertion of a *copia*-like retrotransposon in a gene (*Ruby*) encoding a transcription factor that regulates anthocyanin biosynthesis. We show that *Ruby* has come under the control of the retrotransposon LTR that provides a core promoter for *Ruby* expression. The cold-dependency of fruit pigmentation reflects the induction of expression of the retroelement by this abiotic stress. Amongst blood orange accessions in China, we have discovered a different molecular event; it was caused by a related retrotransposon that promotes anthocyanin biosynthesis through a parallel but distinct mechanism. Analysis of the *Ruby* locus in *citrus* indicated that many species and hybrids are unable to accumulate anthocyanins because they lack a functional copy of the *Ruby* gene while, in other *citrus* plants, anthocyanin pigmentation is dependent on the association between *Ruby* and retrotransposons. Our analysis provides a model for the evolution of the anthocyanin trait in *Citrus* and suggests that transposable elements may be responsible for generating much of the diversity available in this genus.

S05O02

A novel carotenoid cleavage activity responsible for the biosynthesis of *citrus* fruit-specific apocarotenoids

Rodrigo M.J.[1], Alquézar B.[2], Carmona L.[1], Alós E.[1], Al-Babili S.[3], and Zacarías L.[1]

[1]Instituto de Agroquímica y Tecnología de Alimentos-CSIC (IATA), Departamento de Ciencia de los Alimentos, Spain; [2]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain; and [3]University of Freiburg, Faculty of Biology, Germany. mjrodrigo@iata.csic.es

The C30 apocarotenoids, β-citraurin and 8-β-apocarotenal, are specific of the genus *Citrus* and accumulate in the peel of oranges and mandarins, providing an attractive orange-reddish coloration. However, the genes/enzymes involved in the biosynthesis of these compounds are currently unknown. The oxidative cleavage of carotenoids to produce apocarotenoids is catalyzed by a family of enzymes referred to as carotenoid cleavage dioxygenases (CCD). In this work, we report the identification of a novel *CCD* gene in citrus, named as *CsCCD*, whose expression was restricted to chromoplastic tissues and specifically to the fruit peel. *CsCCD* was induced during fruit maturation and stimulated by different postharvest treatments that enhanced fruit peel coloration, and, in good correlation with accumulation of C30 apocarotenoids. In order to determine the potential cleavage activity, *CsCCD* was coexpressed into E. coli strains accumulating different carotenoids and the production of apocarotenoids were analyzed by HPLC-PDA-MS and HS-SPME-GC-MS. Interestingly, only the strains accumulating β-carotene or zeaxanthin were able to produce the expected C30 and C10 apocarotenoids. Collectively, these results suggest that CsCCD may be an enzyme responsible for the biosynthesis of the *Citrus*-specific C30 apocarotenoids, and its involvement in fruit coloration, as well as the potential in vivo substrates will be discussed.

S05O03

Biosynthesis and regulation of carotenoids in citrus callus

Xu J., Gao H.J., Deng X.X., and Zhang H.Y.

Key Laboratory of Horticultural Plant Biology, Ministry of Education, (HZAU), College of Horticulture and Forestry, China.
xujuan@mail.hzau.edu.cn

Carotenoid profiles in citrus callus varied with genotypes and sub-cultures, while were stable within a genotype. Though their composition is different in fruit tissues and leaves, citrus callus is effective material in understanding

the regulation of carotenogenesis, due to its theoretically identical genetic background to other plant tissues. Light regulates the carotenoids synthesis. White light hampered the carotenoids biosynthesis in callus of 'Red Marsh' grapefruit (Citrus paradisi), while boosted it in 'Tarocco' blood orange (C. sinensis); the synthesis of carotenes seemed more sensitive to light than xanthophylls; PSY transcription was up-regulated in callus of two sweet oranges, while was down-regulated in 'Murcott' tangor (C. reticulata × C. sinensis); PSY was emphasized as a rate-limiting gene; however, in the callus of 'Red Marsh', PDS and ZDS might be the rate-limiting genes with an inhibited transcription. Sucrose supply affects carotenoids production. Sucrose starvation induced decreased carotenoid production in callus of 'Red Marsh'. Microarray analysis declared more than 1600 up- and down-regulated genes with 4-fold change. Among them, the expression of most carotenogenesis genes was boosted. Interestingly, the expression of transcription factors, such as AP2, was largely altered. The network began with sucrose starvation to the carotenoid decrease; the senescence signalling and the regulation of transcription factors will be discussed.

S05O04

Terpene downregulation triggers innate immunity and resistance to fungal pathogens in orange fruits

Rodríguez A.[1], Shimada T.[2], Cervera M.[1], Alquézar B.[1], Gadea J.[3], Gómez-Cadenas A.[4], de Ollas C.[4], Rodrigo M.J.[5], Zacarías L.[5], and Peña L.[1]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain; [2]National Institute of Fruit Tree Science. National Agricultural Research Organization, Okitsu Citrus Research Station, Japan; [3]Instituto de Biología Molecular y Celular de Plantas. CSIC-Universidad Politécnica de Valencia. (IBMCP), Spain; [4]Escuela Superior Ciencias Experimentales y Tecnología. Universidad Jaume I de Castellón, Departamento de Ciencias Agrarias y del Medio Natural, Spain; and [5]Instituto de Agroquímica y Tecnología de Alimentos-CSIC (IATA), Departamento de Ciencia de los Alimentos, Spain. arodrig@ivia.es

Volatile organic compounds, most of them being terpenes, are signals emitted by plants for communication with the environment. Orange fruit accumulates mainly terpenes in mature peel oil glands, D-limonene accounting for approximately 97% of terpene content. We have generated transgenic orange plants carrying a D-limonene synthase gene in antisense (AS) configuration. Transgenic expression caused a dramatic decrease in the accumulation of D-limonene in fruit peels, being about 80-100 times lower in AS samples than in empty vector (EV) transgenic ones. This affected the interaction of fruits with their biotic environment because they resulted to be resistant to different specialized pathogens. A gene expression analysis of these fruits linked the decrease of D-limonene to the upregulation of genes involved in the innate immunity response. Additionally, it caused an increase in the accumulation of jasmonates in AS orange peels, explaining their resistance to different fungi. Furthermore, this indicates for the first time an inverse correlation between volatile terpene accumulation upon fruit development and depletion of JA-mediated defense responses, thus stressing the importance of D-limonene for specialized pathogens to establish infections in mature orange peels. High D-limonene content in mature orange peels may be a signal for attractiveness of microorganisms which might be likely involved in facilitating the access to the pulp of seed dispersal frugivores.

S05O05

Improving *in vivo* antioxidant properties of orange fruits through increased β-carotene content

Pons E.[1], Alquézar B.[1], Rodríguez A.[1], Martorell P.[2], Genovés S.[2], Llopis S.[2], González N.[2], Ramón D.[2], Rodrigo M.J.[3], Zacarías L.[3], and Peña L.[1]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain; [2]Biópolis SL, Cell Biology Laboratory, Food Biotechnology Department, Spain; and [3]Instituto de Agroquímica y Tecnología de Alimentos-CSIC (IATA), Departamento de Ciencia de los Alimentos, Spain. elponba@ivia.es

β-Carotene is one of the most efficient carotenoid singlet oxygen quenchers and may interfere in free radical oxidation, typical of many degenerative diseases. Besides its activity as protective agent against oxidative stress, β-carotene also plays a nutritional role as precursor of vitamin A with twice the efficiency of any other pro-vitamin A carotenoid. Orange fruit is an excellent natural dietary source of carotenoids, as well as other nutrients and health-promoting compounds such as vitamin C, flavonoids and folic acid. However, the most abundant carotenoids present in the pulp of mature oranges are β-β-xanthophylls, while β-carotene is hardly detectable. In response to a growing public interest in healthy plant products, this work was aimed to strengthen the health benefits of orange fruits by increasing its β-carotene content through metabolic

engineering. We addressed this goal by blocking the expression of the endogenous β-carotene hydroxylase gene (β-CHX), involved in the conversion of β-carotene into xanthophylls, using RNA interference technology. Mature fruits of transgenic plants obtained showed changes in carotenoid content and composition in the pulp, with an increase in β-carotene accompanied by a general decrease of xanthophylls. The putative enhanced capacity of transgenic oranges for protection against oxidative stress and/or degenerative diseases may be due to an additive/synergistic effect of increased β-carotene content in a background matrix rich in vitamin C and other phytonutrients. This has been assessed by using Caenorhabditis elegans as experimental animal model. Results of in vivo assays will be presented and discussed.

S05O06

RNA interference against the three Citrus tristeza virus genes encoding silencing suppressors confers complete resistance to the virus in transgenic 'Mexican' lime plants

Soler N.[1], Plomer M.[1], Fagoaga C.[1], Moreno P.[1], Navarro L.[1], Flores R.[2], and Peña L.[1]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain; and [2]Instituto de Biología Molecular y Celular de Plantas, CSIC-Universidad Politécnica de Valencia (IBMCP), Spain. soler_nurcal@gva.es

Citrus tristeza virus (CTV), the causal agent of the most devastating viral disease of citrus, has evolved three silencing suppressor proteins acting at intra- (p23 and p20) and inter-cellular level (p20 and p25) to overcome host antiviral defense. RNA interference (RNAi), an approach based on using dsRNA to trigger RNA silencing, has been widely used for generating transgenic plant resistant against viruses. Considering the important role of p23, p20 and p25 in CTV pathogenesis, we transformed 'Mexican' lime plants with an intron-hairpin vector carrying full untranslatable versions of genes p25, p20, p23 and the 3'-UTR from the CTV strain T36, to attempt silencing their expression in CTV-infected cells. Complete resistance to viral infection was observed in three transgenic lines, with all their propagations remaining symptomless and virus-free after graft-inoculation with CTV-T36, either in the non-transgenic rootstock or directly in the transgenic scion. Accumulation of transgene-derived siRNAs was necessary but not sufficient for CTV resistance. When immune transformants were challenged with a dissimilar CTV strain the resistance was partially broken, stressing the importance of sequence identity in the underlying RNAi mechanism. This is the first evidence that it is possible to achieve full resistance to CTV in a highly sensitive citrus host by targeting simultaneously its three viral silencing suppressors through RNAi.

S05O07

Further verification of the resistance of 'C-05' citron to Citrus Canker disease

Liu L.P., Li D.Z., Li J.H., Li N., and Deng Z.N.

National Center for Citrus Improvement (NCCI), Hunan Agricultural University, China. deng7009@163.com

In the previous screening of citrus genotypes, a Chinese citron (nominated citron 'C-05') was found resistant to Canker disease (Xanthomonas axonopodis pv. citri). In order to verify its resistance, further tests were performed. Another 4 local citron genoytpes from Yunan, including 'Large' citron, 'Small' citron or 'Acid' citron, 'Java' citron and 'Indian Large Fruit' citron, were inoculated in vivo to test their reaction to Xac. The assay results indicated that all the 4 citron genotypes were less sensitive to the pathogen than sweet orange, but all showed typical symptoms, while citron 'C-05' remained symptomless. The results provided further evidence that citron 'C-05' is not non-host resistant. Growing leaves at different stages of 'Bingtang' sweet orange (highly susceptible) and citron 'C-05' were inoculated by spraying with 10^8 CFU/mL Xac suspensions. The leaves of 'Bingtang' sweet orange at 1/2 and 1/4 full size were highly susceptible, and leaves at other stages were less susceptible; while in the citron, there were only few untypical disease spots on the leaves at 1/2 and 1/4 full size stages, while no reaction appeared in the other leaf stages. Inoculation with infiltration of 10^{10}, 10^8, 10^6, 10^4, 10^2 and 10 CFU/mL bacterial solution all caused Canker disease on sweet orange leaves, while citron 'C-05' showed hypersensitivity reaction. Bacterial growth on the leaf surfaces was observed by electronic scanning microscope, and further by the use of a EGFP-labeled pathogen. The bacteria were randomly distributed on the leaves, but on the citron leaf, bacterial growth was much less than that on sweet orange leaf. Stomata observation revealed that the tested citrus genotypes were substantially similar. The

stoma density, size and opening differences between the genotypes were not enough to cause the reaction variability related to Xac infection. In conclusion, citron 'C-05' is actively resistant to Canker disease.

S05O08

Genetic transformation of citrus species aiming to increase tolerance to drought stress

Carlos E.F., Ariyoshi C., Felício M.S., Shimizu P., Souza G.V., Kudo S.A., Silva L.M., Silva A.J.M., Cação S.M.B., and Vieira L.G.E.
Instituto Agronômico do Paraná (IAPAR), Laboratory of Biotechnology, Brazil. efcarlos@iapar.br

Most of the citrus trees grown in Brazil are extensively managed with no irrigation, leaving trees more vulnerable to extended dry season, which may reach 90 days without rain in some areas. Thus, utilization of rootstocks more tolerant to drought, such as 'Rangpur' lime (Citrus limonia), is widely employed, while other rootstocks are not. Our laboratory has produced 'Carrizo' and 'Swingle' rootstocks that accumulate the osmoprotectant aminoacid proline, and the present work has continued those efforts aimed to obtain new transgenic rootstocks and scions more tolerant to drought stress. We employed Agrobacterium tumefaciens carrying a gene encoding a mutant enzyme of P5CS (D1-pyrroline-5-carboxylate synthetase), a key enzyme in the biosynthesis of proline, from Vigna aconitifolia, under control of either the constitutive CaMV 35S promoter, or the stress induced RD29A promoter. We worked with sweet orange cultivars (Citrus sinensis) and trifoliate rootstocks (*Poncirus trifoliata* cv. 'Flying Dragon' and P. *trifoliata* cv. 'Rubidoux'). Two sweet orange plantlets were micropropagated and further acclimated in greenhouse conditions. One of these trees was PCR positive for the transgenes and presented a concentration of free proline 2.6 times higher than its non-transgenic control plant. Four plantlets of juvenile 'Flying Dragon' were also obtained and trifoliate 'Rubidoux' plantlets are now under selection procedures.

S05O09

Targeted cybridization in citrus and transcript profiling of a male sterile cybrid pummelo containing cytoplasm from satsuma mandarin

Guo W.W.[1], Zheng B.B.[1], Xiao S.X.[1], Grosser J.W.[2], and Deng X.X.[1]

[1]Huazhong Agricultural University, Key Laboratory of Horticultural Plant Biology, Ministry of Education, China; and [2]University of Florida, Citrus Research and Education Center, USA. guoww@mail.hzau.edu.cn

Seedlessness is an elite fruit quality trait. By symmetric fusion of embryogenic callus protoplasts of satsuma mandarin and mesophyll protoplasts of elite seedy cultivars, diploid cybrid plants containing sterile cytoplasm from satsuma were regenerated and characterized from several fusions, and the cybrid pummelo between satsuma 'Guoqing No. 1' and 'Hirado Buntan' pummelo showed male sterile trait. To gain new insight into the underlying mechanism, the nuclear gene expression profiles of floral buds of the cybrid with that of fertile pummelo were compared by RNA-Seq. Gene expression profiles which identified a large number of differentially expressed genes were captured at both petal and stamen primordia distinguishable stages. In addition, agreeable with flower morphology of the cybrid, expression of PISTILLATA (PI) was reduced in stamen-like structures, even though it was restricted to correct floral whorls. Down-regulated expression of APETALA3 (AP3) coincided with that of PI. These finding indicated that, due to their whorl specific effects in flower development, citrus class-B MADS-box genes likely constituted 'perfect targets' for CMS retrograde signaling, and that dysfunctional mitochondria seemed to cause male sterile phenotype in the cybrid pummelo.

S05O10

In vitro anther culture of several cultivars of *Citrus sinensis* and *Citrus clementina*

Cardoso J.C.[1], Abdelgallel A.[2], Chiancone B.[2], Rocha Latado R.[3], Lain O.[4], Testolin R.[4], and Germanà M.A.[2]

[1]Centro APTA Citros Sylvio Moreira - Instituto Agronômico, Cordeirópolis, Centro de Energia Nuclear na Agricultura - Universidade de Sao Paulo, Brasil; [2]Facoltà di Agraria. Università degli Studi di Palermo, Dipartimento DEMETRA, Italy; [3]Centro APTA Citros Sylvio Moreira - Instituto Agronômico, Cordeirópolis, Instituto Agronômico de Campinas, Brasil; and [4]Università degli Studi di Udine. Dipartimento di Scienze Agrarie e Ambientali, Italy. mariaantonietta.germana@unipa.it

Citrus breeding goals can be achieved by traditional (hybridization, selection, mutation) or biotechnological methods. Efficient procedures of in vitro regeneration are required, in conjunction with the recent techniques of cellular and molecular biology, to achieve significant improvement in a brief time. Moreover, particularly in

citrus, somatic embryogenesis is an efficient method of plant regeneration and embryogenic callus is valuable for propagation as well as for genetic improvement. Anther culture, among the in vitro regeneration systems, is a widespread method to produce haploids (Hs) and doubled-haploids (DHs), drastically reducing the time needed to produce homozygous lines compared to conventional breeding methods, that include several generations of self-pollination. In addition, in vitro anther culture is also utilized to obtain somatic embryos and clonal plant propagation in many woody plants. Actually, in vitro anther culture is, in citrus, an efficient approach to obtain both gametic and somatic embryogenesis. In this study, anther culture has been applied to several citrus genotypes: four cultivars of *C. sinensis* and two of *C. clementina*, testing two temperature shocks. The strong influence of the genotype on the response of anthers in vitro cultured has been confirmed. Actually, the same treatments applied to the explants in culture resulted in the production of gametic embryos in clementines and of somatic embryos in sweet oranges. Clementine confirmed its tendency to regenerate homozygous and tri-haploids embryos and plantlets. Further studies should be performed, in other citrus genotypes aimed to obtain in vitro regeneration procedures, suitable for different applications, as new opportunities for genetic improvement and for the innovation in propagation methods.

S05O11

Bioethanol production from mandarin (*Citrus unshiu*) peel waste

Choi I.S.[1], Wi S.G.[1], and Bae H.J.[2]

[1]Bioenergy research center (BRC), Department of Wood Science and Landscape Architecture, Chonnam National University, South Korea; and [2]Bioenergy resecenter (BRC), Department of Bioenergy Science and Technology, Chonnam National University, South Korea. is.troy@hotmail.com

Mandarin peel waste (MP), waste biomass recovered after juice extraction, is the main mandarin juice process waste. In this study, MP was studied for the potential to produce bioethanol. We designed a new popping pretreatment method to produce ethanol from MP. Popping pretreatment was performed at 150°C for 10 min without chemical treatment. Popping pretreatment reduced the size of MP particles to less than 1 mm and decreased the concentration of D-limonene, a yeast fermentation inhibitor, from 0.21% to 0.01%. Enzymatic hydrolysis of pretreated MP was performed in sodium acetate buffer (50 mM and pH 4.8) at 45°C for 6 h, and the total saccharification rate was approximately 95.6%. The vacuum evaporation process increased the fermentable sugar concentration to 10%. Subsequent fermentation at 30°C at pH 5.0 for 12 h in a laboratory bioreactor increased the ethanol yield to 90.6%, compared to 78% at 36 h from raw MP.

S05P01

Screening and analyzing the root-specific genes of *Poncirus trifoliata*

Yao L.X., Chen S.C., He Y.R., Lei T.G., Xu L.Z., Liu X.F., and Peng A.H.

Citrus Research Institute, Chinese Acadamy of Agricultural Science (CRIC), China. syaolixiao@126.com

Poncirus trifoliata is a close relative of citrus species and has been widely used as a rootstock for citrus production in China. To isolate root-specific genes from this good genetic source, a root cDNA library of *P. trifoliata* was constructed by polymerase chain reaction (PCR)-based suppressive subtractive hybridization (SSH). The cDNA of seedling roots was used as a tester and cDNA of leaves was used as a driver. A total of 1362 randomly picked clones were sequenced, and 1177 sequences with over 100 base pairs were obtained. The majority of these expressed sequence tags (ESTs) showed sequence homology to previously identified genes in GenBank, which came from *Citrus sinensis* (34.5%), *Populus trichocarpa* (19.1%), *Ricinus communis* (16.2%) and *Vitis vinifera* (12.2%). The ESTs with gene ontology took part in biology processes such as metabolic process (29.8%), cellular process (19.8%), biological regulation (9.8%), developmental process (8.6%), and response to stimulus (8.4%), with the biological function of binding (42.1%), catalytic activity (36.6%), transcription (8.6%), transporter activity (5.1%) and so on. Through clusting the ESTs with DNAstar software, 455 unigenes were accepted, including 121 contigs and 334 singlets. 53 unigenes were selected and analyzed by real time RT-PCR. Singlet 52, contig 56, singlet 297, contig 22, singlet 86, contig 28, and singlet 283 showed significantly higher expression in roots than in leaves. As well, the full-length cDNA sequences and DNA sequences of these genes were obtained by RT-PCR and PCR. Singlet 52 and contig 56 belong to cytochrome P450 family, and were named as CYP88A36 and CYP88A37, respectively by Cytochrome P450 Nomenclature Committee. Singleton 297, contig 22, and singleton 86 belong to bet_v_I protein family.

S05P02

Comprehending crystalline β-carotene accumulation by comparing engineered cell models and natural carotenoid-rich system of citrus

Cao H.B., Zhang J.C., Xu J.D., Ye J.L., Yun Z., Xu Q., Xu J., and Deng X.X.

Key Laboratory of Horticultural Plant Biology, Ministry of Education, Huazhong Agricultural University, China. xuqiang@mail.hzau.edu.cn

Citrus fruits, which exhibit extensive diversity of carotenoid patterns, can serve as a unique system to improve the understanding of carotenogenesis. Herein, we engineered embryogenic calli from four citrus genotypes as engineered cell models (ECMs) by overexpressing a bacterial *phytoene synthase* gene (*CrtB*). Ripe flavedos (the colored outer layer of citrus fruits) were offered as a comparative system to the ECMs. In the ECMs, carotenoid patterns showed diversity depending on the genotypes and produced additional carotenoids, such as lycopene, that were absent from the wild-type lines. However, the carotenoid pattern in the ECMs of one genotype seems not to correlate with that in its flavedo. In the ECMs, there emerged a favored β,β-pathway characterized by a striking accumulation of β-carotene, which was dramatically unique from those in the wild-type calli and ripe flavedos. Unlike flavedos that contained a typical chromoplast development, the ECMs sequestered most carotenoids in the amyloplasts in crystal form, which led the amyloplast morphology to show chromoplast-like profile. Transcriptional analysis revealed a markedly flavedo-specific expression of β-*carotene hydroxylase* gene (*HYD*), which was suppressed in the calli. Coexpression of *CrtB* and *HYD* in the ECMs confirmed that *HYD* predominantly mediated the preferred carotenoid patterns between the ECMs and flavedos, and also revealed that the carotenoid crystals in the ECMs were majorly composed of β-carotene.

S05P03

Analyses of Expressed Sequence Tags (ESTs) of 'Barnfield' navel orange via constructing of a subtractive cDNA library between oleocellosis and healthy rind

Wei Z.X., Hong L., Cheng C.F., Wu C.Q., and Qi J.R.

Chongqing Academy of Agricultural Sciences (CAAS), Fruit Research Institute, China. chengchf@hotmail.com

A cDNA suppression subtractive hybridization (SSH) library was successfully constructed, which was synthesized from RNA extracted from a between oleocellosis and healthy rind of 'Barnfield' navel orange. Results showed that most of the clones had inserting fragments which length ranged from 100 bp to 700 bp. 292 ESTs were successfully sequenced from 300 of positive clones. Among the ESTs, 205 ESTs, involving in 105 genes, were found sharing homology with known genes, while the others did not show homology with known genes. These ESTs were mainly involved in metabolic pathways, biosynthesis of secondary metabolites, microbial metabolism in diverse environments, drug metabolism, phenylpropanoid biosynthesis, phenylalanine metabolism, naphthalene degradation, beta-lactam resistance, penicillin and cephalosporin biosynthesis and glyoxylate and dicarboxylate metabolism. Relative quantitation of expression of a number of genes, analyzed by real-time PCR, also confirmed the patterns predicted by SSH.

S05P04

Control of flowering time in citrus through ectopic overexpression of *flowering locus T* (*FT*) and *apetala1* (*AP1*) from sweet orange

Rodríguez A., Cervera M., and Peña L.

Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain. arodrig@ivia.es

Citrus trees have a long juvenile phase that delays their reproductive development by more than 6 years. This represents a major obstacle for citrus breeding. The key genes regulating floral transition have been studied extensively in species such *Arabidopsis thaliana* and poplar. In recent years, a growing number of genes related to flowering have been identified in crop species. With the aim of accelerating their flowering time, we have transformed juvenile citrange and sweet orange plants to constitutively overexpress the sweet orange homologues of *A. thaliana* genes *flowering locus T* (*FT*) and *apetala1* (*AP1*), which have been shown to accelerate the onset of flowering in a number of plant species including citrus. Both types of transgenic

citrus plants produced fertile flowers and fruits as early as the first year after genetic transformation. They have been characterized at the phenotypical and molecular level and compared to transgenic citrus plants over-expressing *FT* and *AP1* from *A. thaliana*, previously obtained in our laboratory. Additionally, zygotic and nucellar *CsFT* and *CsAP1* seedlings have been obtained, generally showing flowering soon after germination in *CsFT* lines or within months after transformation in *CsAP1* lines, thus demonstrating the stability and inheritance of this trait. These results offer new tools and possibilities for genetic improvement and functional genomics research in citrus species.

S05P05

Overexpression of a chromoplast-specific *lycopene* β-*cyclase* (β-*LCY2*) gene in orange fruit

Alquézar B.[1], Pons E.[1], Rodríguez A.[1], Rodrigo M.J.[2], Zacarías L.[2], and Peña L.[1]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain; and [2]Instituto de Agroquímica y Tecnología de Alimentos-CSIC (IATA), Departamento de Ciencia de los Alimentos, Spain. alquezar_ber@gva.es

Orange fruit is an excellent natural dietary source of phytochemicals and nutrients (as vitamin C, carotenoids, flavonoids and folic acid, among others) contributing to its quality, nutritional value and health benefits. In recent years, considerable advances in the understanding of the molecular basis regulating carotenoid biosynthesis have been accomplished and biotechnological strategies to modify carotenoid content or composition in orange fruits are now feasible. For example, β-carotene (provitamin A) content in orange has been increased by blocking the expression of an endogenous β-*carotene hydroxylase* gene, involved in the conversion of β-carotene into xanthophylls. β-lycopene cyclase activity is a key metabolic step in the pathway as determinates a branching point for the accumulation of β,β-xanthophylls and may be also implicated in lycopene accumulation in some citrus species. Therefore, the chromoplast-specific β-*LCY2* gene is an excellent candidate for the metabolic engineering of carotenoid biosynthesis and its promoter may be also a good target to drive fruit-specific expression. Thus, with the aim of increasing β,β-carotenoid levels in orange tissues we are over-expressing β-*LCY2* using its own promoter. Transgenic 'Pineapple' and 'Valencia' oranges carrying this construction are being generated. Detailed molecular and biochemical analysis of the transgenic lines will allow us to gain insights into carotenoid biosynthesis regulation in citrus.

S05P06

Genetic transformation of *Citrus sinensis* with *attacin* A gene driven by phloem tissue specific promoters for resistance to *Candidatus* Liberibacter spp.

Tavano E.C.R.[1], Harakava R.[2], Vieira M.L.C.[3], Mourão Filho F.A.A.[4], and Mendes B.M.J.[1]

[1]Universidade de São Paulo (USP), Centro de Energia Nuclear na Agricultura, Brazil; [2]Instituto Biológico (IB), Secretaria de Agricultura e Abastecimento do Estado de São Paulo, Brazil; [3]Universidade de São Paulo (USP), Escola Superior de Agricultura "Luiz de Queiroz" - Departamento de Genética, Brazil; and [4]Universidade de São Paulo (USP), Escola Superior de Agricultura "Luiz de Queiroz" - Departamento de Produção Vegetal, Brazil. ectavano@cena.usp.br

Huanglongbing (HLB) associated with sieve tube-restricted bacteria *Candidatus* Liberibacter spp., is one of the most destructive disease affecting citrus. The aim of this study was to produce transgenic sweet orange plants (*Citrus sinensis*) expressing antimicrobial *attacin* A gene (*att*A) under the control of phloem tissue specific promoters, in order to obtain resistant plants to *Candidatus* Liberibacter spp. Genetic transformation of 'Hamlin' and 'Valencia' sweet orange cultivars was performed via *Agrobacterium tumefaciens*. The gene construct for genetic transformation included the *attacin* A gene controlled by either sucrose transporter gene (*AtSuc2)* or phloem protein 2 gene promoters (*AtPP2*) from *Arabidopsis thaliana*, or phloem protein 2 gene promoter (*CsPP2*) from *C. sinensis*. Epicotyls segments collected from *in vitro* germinated seedlings were used as explants for genetic transformation. Regenerated shoots were micrografted onto 'Carrizo' citrange seedlings for plant development. Transgenic plants were identified by PCR analyses. The plants were acclimatized and transferred to a greenhouse. Integration of the transgene was confirmed for 'Hamlin' and 'Valencia' cultivars containing the *AtSuc2*, the *AtPP2* or the *CsPP2* promoters controlling the expression of *att*A. The transgene transcription is been studied by northern blot or RT-qPCR analyses.

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S05P07

D-limonene downregulation in transgenic sweet orange provides protection against Citrus Black Spot symptoms induced in detached fruits

Kava-Cordeiro V.[1], Rodríguez A.[2], Vicent A.[2], Glienke C.[1], and Peña L.[2]

[1]Universidade Federal do Paraná (UFPR), Genética, Brazil; and [2]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain. vanessagenetica@gmail.com

Citrus Black Spot (CBS) is a disease affecting sweet orange, lemons and mandarin varieties in Africa, Oceania and Americas. In Europe, CBS is a quarantine disease, limiting the import of fresh fruits from citrus-producing countries of those continents. CBS is caused by the fungus *Phyllosticta citricarpa*, which induces damages in fruit flavedo affecting their appearance and additionally causing premature fruit drop, thus reducing productivity. Its field control is currently based on the use of expensive fungicides. CBS symptoms are the consequence of fungal induced cell lyses and collapse around oil cavities. Such cells are responsible for the synthesis of essential oil. Orange fruit accumulates mainly terpenes in peel oil glands and D-limonene accounts for about 97% of oil glands content. In order to improve our understanding on the role of D-limonene and related terpenes in the interaction between orange flavedo tissues and the pathogen, we have used a *P. citricarpa* strain transformed with the reporter gene *GFP* to first develop a procedure to inoculate detached fruits in laboratory conditions and reliably get and follow fungal infection and symptoms in sweet orange. This has then served to compare the response to CBS challenge of fruits from transgenic orange plants carrying a *D-limonene synthase* gene in antisense (AS) configuration *vs*. empty vector (EV) transgenic controls. AS downregulation leads to a dramatic decrease in the accumulation of D-limonene in fruit peels, being about 80-100 times lower in AS samples than in EV transgenic ones. EV and AS transgenic fruits (141 and 140 mature fruits, respectively) were inoculated in four points, equally spaced intervals, with 5 μL of a conidia suspension (10^6). Fungal infection was evaluated daily and resulted to be very low or even absent in AS fruits while *P. citricarpa* readily developed in EV fruits. These results encourage the establishment of field trials with the transgenic events in CBS-affected areas.

S05P08

Resistance to *Citrus tristeza virus* in *Citrus sinensis* transgenic plants

Muniz F.R.[1], Harakava R.[2], Rezende J.A.M.[3], Moore G.A.[4], Febres V.J.[4], Mourao Filho F.A.A.[3], and Mendes B.M.J.[1]

[1]Centro de Energia Nuclear na Agricultura/Universidade de Sao Paulo (CENA/USP), Brazil; [2]Instituto Biologico de Sao Paulo (IB-SP), Brazil; [3]Escola Superior de Agricultura Luiz de Queiroz/Universidade de Sao Paulo (ESALQ/USP), Brazil; and [4]University of Florida (UF), USA. fabianaufla@yahoo.com.br

Citrus tristeza virus (CTV) control relies mainly on the use of tolerant rootstocks and scion cross protection. The aim of this work was to evaluate transgenic *C. sinensis* lines for resistance to CTV, in order to offer another alternative for virus control. Transgenic lines of *C. sinensis* cv. 'Valencia' and 'Hamlin' containing one of 3 different genetic constructs (pCTV-*CP*: coat protein gene of CTV; pCTV-*CS*: conserved sequence of CTV; pCTV-*dsCP*: hairpin with sense and antisense sequences of the *CP* gene) were evaluated, totalizing 60 transgenic lines. The lines were grafted into *C. limonia* and *C. aurantium*, totaling 360 plants. The plants were challenged 4 times with CTV by means of *T. citricida*. Indirect ELISA using a monoclonal antibody against the CTV coat protein or Real-time PCR using primers to amplify CTV genes were used to detect the virus. Variation in the virus resistance was observed among different transgenic constructs and different clones of the same line. The virus was not detected in 20 clones of different lines even after the fourth inoculation. For 'Hamlin', it was possible to identify 1 transgenic line (CS9 grafted into *C. limonia*) with no virus multiplication. For 'Valencia', 1 clone of each transgenic line CP1, CP2, CP4, CP9, CS1, CS8, DS3 and DS10 grafted into *C. aurantium*, 1 clone of CP7, CS1, CS5, CS10 and DS3 and 2 clones of CP6, CS6 and CS7 grafted into *C. limonia* did not amplify the virus genes. This indicates possible resistance to the pathogen.

S05P09

Production of a recombinant miraculin protein in transgenic citrus cell suspension cultures

Jin S.B.[1], Sun H.J.[2], Kim Y.W.[2], Bachchu M.A.A.[3], Yun S.H.[4], Riu K.Z.[3], and Kim J.H.[3]

[1]Subtropical and Tropical Organism Gene Bank, Jeju National University, Korea; [2]Subtropical Horticulture Research Institute, Jeju National University, Korea; [3]Faculty of Biotechnology, College of Applied Life Science (SARI), Jeju National University, Korea; and [4]Citrus Research Station, National Institute of Horticultural and Herbal Science, Rural Development Administration, Korea.
kimjh@jejunu.ac.kr

An efficient system for the production of a recombinant miraculin protein in transgenic citrus plant cells was developed. Miraculin is a taste-modifying protein that can transform a sour taste into a sweet taste. A miraculin gene containing a *N*-terminal signal peptide was introduced into navel orange (*Citrus sinensis*) callus cells by *Agrobacterium*-mediated transformation. Transgenic green color somatic embryos were generated on SIM medium containing 25 mg/L hygromycin and then embryogenic callus cell lines were produced from green color somatic embryos by using RCIM (Callus Reproduction Induction Medium) medium supplemented with 1 mg/L of GA_3, 34 mg/L of adenine, 5% of sucrose, 500 mg/L of malt extract, and 0.2% of gelrite. The insertion of the miraculin gene in the transformants was confirmed by genomic-PCR, using specific primers of the miraculin gene. Cell suspension cultures were kept in MT liquid medium, pH 5.2, containing 5% sucrose. In order to overproduce the miraculin protein, the transgenic cells were transferred to fresh liquid suspension medium at 5% (weight of wet callus/volume of medium) density and were cultured at 15°C with rotation speed of 120 rpm. Recombinant miraculin expression and secretion in transgenic citrus suspension cells was confirmed by Western blot analysis. The recombinant miraculin protein was secreted into the culture medium and formed a disulfide-linked dimer similarly to the native miraculin with the taste-modifying activity. This production system could be a good alternative for production of the recombinant miraculin.

S05P10

Transgenic sweet orange rootstocks overexpressing a osmotin like PR-5 protein show resistance against Phytophthora Foot Rot in the field

Peris J.E., Vicent A., and Peña L.

Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain. lpenya@ivia.es

Phytophthora spp. causes foot rot and gummosis, the most serious soilborne diseases of citrus worldwide. Sweet orange (*Citrus sinensis*) might be an excellent rootstock but it cannot be used as such in many citrus areas due to its high sensitivity to *Phytophthora* spp. The development of sweet orange that possess durable genetic resistance provides the best prospect for full recovery of this citrus type as rootstock. A wide range of plant defensive antifungal proteins has been identified and is being used in attempts to provide protection via expression in transgenic crops. Proteins of the PR-5 group (osmotin and thaumatin-like proteins) have antifungal activity *in vitro* against fungi and oomycetes. We demonstrated the antifungal properties of tomato P23 (PR-5) *in vivo* by overexpressing the *PR-5* coding sequence under the control of the 35S promoter from CaMV in sweet orange plants, and testing plant response to *P. citrophthora* infections in excised bark pieces and in potted plants. One transgenic line (9.1a) resulted to be clearly resistant in both experiments, while other 9 (including 2.31a) resulted susceptible to *P. citrophthora* as the empty vector transgenic control (2.15). Promising results with transgenic line 9.1a encouraged us to conduct a field trial to test resistance to *Phytophthora*-induced foot rot. Transgenic sweet orange seedlings from lines 9.1a, 2.31a and 2.15 were used as rootstocks and non-transgenic clementine mandarin was budded on top and used as scion. Thirteen trees per transgenic event were randomly disposed in the field in 2009 and let to become naturally infected. Three years after planting, more than 50% of 2.31a and 2.15-trees are showing severe foot rot caused by *Phytophthora*, while all 9.1a trees remain symptomless.

S05P11

Evaluation of transgenic oranges expressing the *stx IA* gene for resistance to Citrus Canker

Marques V.V.[1], Miller A.M.[2], Bagio T.Z.[2], Souza G.V.[3], Meneguin L.[2], Vieira L.G.E.[4], Pereira L.F.P.[5], and Leite J.R.R.P.[2]

[1]Fundecitrus, Scientific, Brazil; [2]Instituto Agronômico do Paraná (IAPAR), Plant Protection, Brazil; [3]TMG, Scientific, Brazil; [4]Instituto Agronômico do Paraná (IAPAR), Biotechnology, Brazil; and [5]Embrapa, Biotechnology, Brazil. viviani@fundecitrus.com.br

Citrus Canker caused by *Xanthomonas citri* subsp. *citri* (*Xcc*) is an important disease for citrus production around the world. Several highly valuable commercial citrus cultivars are moderately to highly susceptible

to this disease. Development of transgenic plants for disease resistance in economically important citrus cultivars may represent a unique and efficient strategy for control of Citrus Canker. In this work, we report the reaction to Citrus Canker of five independent events (STX-3, STX-5, STX-11, STX-12 and STX-13) of sweet orange cv. 'Pera' (*Citrus sinensis*), transformed with the vector pST10 containing the antibacterial peptide gene *stx IA* under control of CaMV 35S promoter and the signal peptide from tobacco PR 1a. For inoculation, young expanded citrus leaves were injection-infiltrated or sprayed with a suspension of strain 306 of *Xcc*. Reduction in disease incidence was observed for the transgenic events as compared with the non-transformed control citrus plants. The lowest levels of disease incidence were observed for the events STX-3 and STX-5. Furthermore, significant differences were also observed in disease severity between the transgenic plants and the non-transformed control plants. The events STX-3 and STX-12 showed the lowest populations of *Xcc* in the leaf tissue. A slower growth of *Xcc* was observed in the *stx IA* transgenic plants, with differences of up to 2 log units in bacterial populations between transgenic plants and non-transformed control plants.

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S05P12

Transgenic 'Mexican' lime plants expressing the gene *p23* from *Citrus tristeza virus* (CTV) under the control of a phloem-specific promoter display symptoms closely similar to those accompanying virus infection

Soler N.[1], Fagoaga C.[1], López C.[2], Moreno P.[1], Navarro L.[1], Flores R.[2], and Peña L.[1]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain; and [2]Instituto de Biología Molecular y Celular de Plantas, CSIC-Universidad Politécnica de Valencia (IBMCP), Spain. soler_nurcal@gva.es

Citrus tristeza virus (CTV) is phloem-restricted in natural citrus hosts. The 23 kDa protein (p23) encoded by the virus is a RNA silencing suppressor and an important pathogenicity factor. Constitutive expression of *p23* in transgenic citrus plants under the control of the 35S promoter incites, in addition to aberrations resembling CTV symptoms, others likely related to the ectopic expression of this protein in cells usually not infected by the virus. Moreover, transgenic expression of a fragment comprising only the N-terminal 157 amino acids (p23Δ158-209) elicit CTV-like leaf symptoms similar to, albeit milder than, those resulting from expression of the complete p23 protein. To discriminate between symptoms induced by p23 in CTV infection and other pleiotropic effects of this protein, we restricted expression of *p23*-derived transgenes to phloem-associated cells in 'Mexican' lime plants by means of the phloem-specific promoter from *Commelina yellow mottle virus* (CoYMV). The complete gene *p23* from either the severe T36 or the mild T317 CTV isolates, or the fragment p23Δ158-209 from the former, were used. Expression of these constructs in the phloem incited CTV-specific symptoms like vein clearing in 'Mexican' lime leaves, but not the unspecific symptoms (epinasty, growth cease and apical necrosis) observed when p23 was expressed under the control of the 35S promoter. Moreover, vein necrosis was exclusively associated to phloem-specific expression of the transgenes derived from CTV T36.

S05P13

Efficient deletion of the selectable marker gene from transgenic citrus via the Cre/loxp system

Zou X.P., Peng A.H., Liu X.F., He Y.R., Xu L.Z., and Chen S.C.

Citrus Research Institute, Chinese Academy of Agricultural Sciences (CRIC), China. zxping11230@126.com

Selectable marker genes conferring antibiotic- or herbicide- resistance are in general required to efficiently recover transgenic plants from transformed cells during plant transformation. However, the presence of marker genes raises public concerns regarding the field release of transgenic plants. Removal of marker genes prevents the risk of its release into the environment and hastens the public acceptance of transgenic products. In this study, an efficiency system for deletion of a selectable marker gene from transgenic citrus by Cre/loxp site-recombination has been constructed via conventional *Agrobacterium*-mediated transformation. Our results showed that about 20% of transformation efficiencies could be obtained using *ipt* (isopentenyltransferase) gene from *A. tumefaciens* as a selectable marker gene. Molecular analysis demonstrated that 20-100% complete deletion occurred in transgenic plants and that Cre/loxp-mediated excision was always precise in citrus. This approach provides a reliable strategy for deleting selectable marker genes from transgenic citrus after transformation and thus producing marker-free transgenic plants.

S05P14

Assessment of pollen-mediated transgene flow in citrus under experimental field conditions

Pons E.[1], Navarro A.[1], Ollitrault P.[2], and Peña L.[1]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain; and [2]Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), UPR amélioration génétique des espèces à multiplication végétative, France. elponba@ivia.es

Despite potential benefits granted by genetically modified (GM) citrus trees, their release and commercialization raises concerns about their potential environmental impact. The transfer via pollen of transgenes to cross-compatible cultivars is deemed to be the greatest source for environmental exposure. In this work, three different citrus genotypes carrying the *uidA* (GUS) tracer marker gene (pollen donors) and a non-GM self-incompatible contiguous citrus genotype (recipient) were used in conditions allowing natural entomophilous pollination to occur. The examination of 603 to 2990 seeds per year showed unexpectedly low frequencies (0.17-2.86%) of transgene flow. Paternity analyses of the progeny of subsets of recipient plants using 10 microsatellite (SSR) loci demonstrated a higher mating competence of trees from another non-GM pollen source population that greatly limited the mating chance of the contiguous cross-compatible and flowering-synchronized transgenic pollen source. This mating superiority could be explained by a much higher pollen competition capacity of the non-GM genotypes, as was confirmed through mixed-hand pollinations, indicating that pollen competition strongly contributed to transgene confinement. This is the first study on transgene flow in citrus. It provide crucial information on the safety and field performance of GM citrus that can serve as a basis for further field trials and as a guide for (case-by-case) regulatory policies.

S05P15

Functional analysis of a citrus transcription factor with mature fruit-specific expression using transgenic tomato

Endo T.[1], Shimada T.[1], Fujii H.[1], Sugiyama A.[1], Nakano M.[1], Ikoma Y.[1], and Omura M.[2]

[1]NARO Institute of Fruit Tree Science (NIFTS), Citrus Research Division, Japan; and [2]Shizuoka University, Faculty of Agriculture, Japan. tomoen@affrc.go.jp

Citrus fruit changes color drastically at maturation with reduction of chlorophyll and accumulation of carotenoids in peel. This color change is known to be suppressed with exogenous gibberellin and, in contrast, promoted with ethylene treatment. In order to identify genes regulating metabolisms involving citrus fruit color change, we have screened transcription factor genes with the profile as their expression is induced by ethylene and reduced by gibberellin treatment in mature fruit peel using a microarray. Among them, a *bHLH* gene, *TF-BFC*, with mature fruit-specific expression has been selected, introduced and overexpressed in tomato 'Micro Tom'. Three independent transgenic lines with high transgene expression were selected and analyzed. Transgenic plants were dwarf, had deep-green and curled leaves and orange-colored fruit. The chlorophyll content in transgenic leaves was about two times higher than in controls. Microarray analysis of transgenic plants showed that over 3,000 genes had significant expression changes (>2-fold) in red fruit, but in green fruit, the number of genes with significant expression changes was only about half. Gene homologs probably encoding enzymes of carotenoid biosynthesis, such as phytoene synthase, phytoene desaturase, z-carotene desaturase and b-ring hydroxylase were shown to be highly expressed in transgenic red fruit, in accordance with the results from RT-PCR analysis. These results suggest that *TF-BFC* induced gene expression of carotenoid biosynthesis and suppressed genes involving photosynthesis in transgenic tomato mature fruit, showing important roles in carotenoid biosynthesis.

S05P16

Effect of the citrus *lycopene* β*-cyclase* transgene on carotenoid metabolism in transgenic tomato fruits

Guo F., Zhou W.J., Zhang J.C., Xu Q., and Deng X.X.

Key Laboratory of Horticultural Plant Biology of Ministry of Education (KLHPBME), Huazhong Agricultural University, China. guofei_121@163.com

Lycopene β-cyclase (*LYCB*) is the key enzyme for the synthesis of β-carotene, a valuable component of the human diet. In order to evaluate the effect of constitutively expressing a citrus *Lycb-1* gene on carotenoid synthesis,

tomato constitutively express *Lycb-1* was engineered in this study. The β-carotene level of transformant increased 4.1 fold, and the total carotenoid content increased by 30% in the fruits. In the transgenic line, the downstream α-branch metabolic fluxes were repressed during the three developmental stages while α-carotene content increased in the ripe stage. Microarray analysis in the ripe stage revealed that the constitutive expression of *Lycb-1* affected a number of pathways including the synthesis of fatty acids, flavonoids and phenylpropanoids, the degradation of limonene and pinene, starch and sucrose metabolism and photosynthesis. This study provided insight into the regulatory effect of *Lycb-1* gene on plant carotenoid metabolism and fruit transcriptome.

S05P17

Role of the sweet orange *tau* glutathione transferases (*csgstu*) in transgenic tobacco plant detoxification from xenobiotics

Lo Cicero L.[1], Madesis P.[2], Tsaftaris A.[3], Lo Piero A.R.[4]

[1]University of Catania (DISPA), Dipartimento di Scienze delle Produzioni Agrarie e Alimentari, Italy; [2]The Centre for Research and Technology Hellas (CERTH), Institute of Applied Biosciences, Greece; [3]The Centre for Research and Technology Hellas (CERTH), Institute of Agrobiotechnology, Greece; and [4]University of Catania (DISPA), Dipartimento di Fitopatologia e genetica vegetale, Italy. lociceroluca1981@libero.it

The glutathione S-transferases (GSTs) catalyse the attack of glutathione (GSH) on a variety of hydrophobic toxic chemicals. The conjugation of GSH to such molecules increases their solubility and facilitates further metabolic detoxifying processes. Two *csgst* genes (*gstu1* and *gstu2*) encode for proteins which differ only for three amino acids located in the hydrophobic co-substrate binding site (*gstu1*: R89, E117, I172; *gstu2*: P89, K117, V172). Previously, site-directed mutagenesis experiments generated several cross-mutate enzymes and among them the GST-RKV mutant derives from the P89R substitution upon the *gstu2* isoform. In this work, both wild-type (*gstu1* and *gstu2*) and the GST-RKV mutant were incorporated into the tobacco genome under the control of the strong constitutive CaMV 35S promoter via *Agrobacterium* mediated transformation. PCR and Southern blot analysis was performed to confirm the presence of the *nptII* gene only in the genomic DNA from transformed tobacco plants. The expression of the transgenes was also assessed by RT-PCR experiments. The GST activity of tobacco leaves transformed with wild type GSTs turned out to be similar with that exhibited by untransformed plants, whereas plants harbouring the GST-RKV mutant gained a 2-fold increase of the GST activity assayed against several pollutants, thus suggesting that the RKV-transformed tobacco plants acquired novel favourable traits which might provide a more efficient contribution in the detoxification system.

S05P18

An alternative transformation method in citrus using cell-penetrating peptides (CPPs)

Jensen S.P., Febres V.J., and Moore G.A.

University of Florida (UF), Plant Molecular and Cellular Biology, United States of America. jensensp@ufl.edu

Citrus transformation is typically *Agrobacterium tumefaciens*-mediated, in which citrus tissues are cultivated with the bacterium and regenerated on selection media. Part of the bacterial plasmid, with the gene of interest and resistance genes, is then inserted into the citrus genome. Due to the slow growth of citrus, this long process must be optimized for each cultivar and ultimately the transformation efficiency in citrus is substantially less than with other model systems. Commercialization of transgenic citrus is even slower because of the regulations on genetically modified produce worldwide. In order to decrease the dependence upon bacterial transformation and increase transformation efficiency, we propose an alternative method of transformation using cell penetrating peptides (CPPs) that does not involve *Agrobacterium*. CPPs are positively charged short amino acid sequences able to simultaneously bind proteins and nucleic acids and deliver them across cellular membranes and cell walls. CPPs are used currently in plants in transient expression assays and gene silencing, but have not been used in citrus or in stable transformation experiments. We have developed a standard method for the transient expression of reporter genes (*gus* and *gfp*) in citrus. Our data indicate that up to 50% of treated explants express GUS when CPPs are used alone. Several optimization steps have been tested and the efficiency is increased to 100% when CPPs are used in conjunction with a lipid reagent. We have transformed several explant segments which survived kanamycin selection, produced shoots and roots, and were planted. PCR and reporter gene analysis will confirm stable integration. Further experiments with CPPs, comprising RNAi and protein trafficking, will also be performed.

S05P19

Establishment of a transient expression system in citrus via agro-infiltration

Dai S.M., Li F., Yan J.M., Li D.Z., Li R.H., and Deng Z.N.

Hunan agricultural university, China. dsm531@126.com

Genetic transformation is a traditional tool for both functional genomics and molecular breeding in plants, but it is time-consuming and low efficient in citrus due to its low transformation rate. It is essential to establish a transient expression system to speed-up gene function identification. Here we report a gene transient expression by using agro-infiltration for citrus. A full comparison was performed between different parameters including *Agrobacterium* injection buffer solutions, expression vectors and concentrations of the bacterial solution. Results showed that in the transient expression system with injection buffer solution composed of 50mM MES pH5.6, 10mM $MgCl_2$, 150μM acetosyringone, pCAMBIA-2301 expression vector and OD_{600} = 0.4 as ideal concentration of bacterial solution, a foreign gene could be effectively expressed in citrus. With this system, it is possible to analyze gene function in citrus rapidly.

S05P20

Improvement of transformation efficiency in citrus

Yang L.[1], Hu W.[1], Xie Y.M. [1], Li Y.[2], and Deng Z.N.[1]

[1]National Center for Citrus Improvement (Changsha) (NCCI), Hunan Agricultural University, China; and [2]Department of Plant Science (DPS), University of Connecticut, USA. yangli526526@126.com

Internodal stem segments from citrus seedlings have been widely used as explants in transformation research, due to their ability of easy regeneration. Since 1990, genetic transformation has been applied on different citrus species including sweet oranges (*Citrus sinensis*), mandarins (*C. reticulata*), trifoliate orange (*Poncirus trifoliata*), sour orange (*C. aurantium*), pummelo (*C. grandis*), citrange (*C. sinensis* × *P. trifoliate*), and lemon (*C. limon*) in our biotechnology program. But the successful transformation rates are low, thus impeding the realization of transgenic cultivar breeding. Consequently, the development of an efficient transformation system becomes essential. A series of factors was tested to improve transformation rates in sweet orange and kumquat (*Fortunella crassifolia*). The results indicated that using seedlings grown 20 to 25 days in dark and 10 days in light as explants could provide higher frequencies of GUS positive regenerated buds. Adding auxin and placing sterile filter paper during the co-cultivation estimulated the growth of regenerated buds, and increased about 13% the frequency of GUS positive regenerated buds. Lower pH and co-cultivation temperature under acetosyringone induction enhanced transformation competence. With all the improved factors, the transformation rate was in average 18.6%, and in the best case, the transformation rate reached 23%.

S05P21

Improvement of genetic transformation with mature explants of sweet orange (*Citrus sinensis*)

Xie Y.M.[1], Yang L.[1], Hu W.[1], Li Y.[2], and Deng Z.N.[1]

[1]National Center for Citrus Improvement (Changsha) (NCCI), Hunan Agricultural University, China; and [2]Department of Plant Science (DPS), University of Connecticut, USA. xym9366@163.com

High level of contamination, low morphogenetic potential and recalcitrance to *Agrobacterium tumefaciens* infection are the main problems in the transformation of adult citrus tissues. A series of experiments was performed to solve these problems. 'Newhall', 'Succari' and 'Bingtang' sweet orange cultivars were tested. Adult shoots from the 3 cultivars were grafted and re-grafted on *Poncirus trifoliata* rootstocks in the greenhouse. Internodal stem segments of about 1 cm in length from 30-day old mature shoots of were taken as explants. Through re-grafting mature shoots, the contamination rate of explants was much lower, even 0%. The adventitious bud regeneration rate was obviously improved, approximately 80%, 67.5% and 55% in 'Succari', 'Bingtang' and 'Newhall' sweet oranges, respectively. After *Agrobacterium* infection, the highest adventitious bud regeneration rate of 'Bingtang' sweet orange reached 25%. The transformation rate was 3.8%, 4.6% and 3.2% in 'Succari', 'Bingtang' and 'Newhall' sweet orange cultivars, respectively. The 'gene-

deletor' technology is an efficient system to remove all the functional transgenes from the pollen and seeds of the transgenic plants, activated by specific promoters. Two 'gene-deletor' constructs containing pLF_polB-FLP and pLF_polL-Cre boxes, respectively, were transferred into mature explants of sweet orange by the improved procedures, and 10 transformed plants were confirmed to be positive by GUS expression and PCR analysis. The transformed plants will be tested for the gene-deletor function later on.

S05P22

Efficient propagation and rooting of citrus rootstocks adult explants using different basal medium and plant growth regulators

Tallón C.I., Porras I., and Pérez-Tornero O.

Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA), Citricultura, Spain. olalla.perez@carm.es

The influence of the basal medium and different plant growth regulators on the efficient micropropagation of nodal explants from mature trees of sour orange and 'Cleopatra' mandarin citrus rootstocks was studied. The preference of all two citrus rootstock shoot cultures for high-salt media, like MS or DKW, was observed. Several combinations of BA and AD, KIN or GA were made in an attempt to optimize the proliferation phase. The addition of BA and AD to the culture medium improved the proliferation in sour orange or 'Cleopatra' mandarin in the same way as BA/GA. The use of different combinations of BA/KIN did not improve any of the studied variables. The transfer of *in vitro* shoots to rooting media, containing different concentrations of IBA and IAA, produced complete plantlets of 'Cleopatra' mandarin, but very low rooting percentages in sour orange were obtained. To improve the rooting results different combinations of NAA and IBA were tested and higher rooting percentages than in the IBA/IAA combinations were produced. In sour orange percentages nearly 100% of rooted explants were obtained.

S05P23

Optimization of *in vitro* organogenesis and plantlet regeneration of sweet orange from mature tissue

Castro L.M.[1], Mendes B.M.J.[2], and Mourão Filho F.A.A.[3]

[1]Universidade de São Paulo (USP), Departamento de Produção Vegetal, Brazil; [2]Universidade de São Paulo (USP), Centro de Energia Nuclear na Agricultura - Laboratório de Biotecnologia Vegetal, Brazil; and [3]Universidade de São Paulo (USP), Departamento de Produção Vegetal, Brazil. liviamdecastro@yahoo.com.br

The use of mature tissue for citrus genetic transformation is important for the precocious evaluation of the genetically modified characteristics. However, an efficient organogenesis protocol with high rates of plant regeneration is necessary. This work aimed to optimize a protocol for *in vitro* organogenesis of 'Hamlin', 'Pera', and 'Valencia' sweet orange cultivars (*Citrus sinensis*) using as explant internodal segments collected from adult plants cultured in greenhouse. Organogenesis was induced by culturing internodal segments in Petri dish (100 x 15 mm) contanining MS medium supplemented with sucrose (30 $g \cdot L^{-1}$) and different concentrations of benzylaminopurine (BAP; 0.0, 1.0, 2.0, 3.0, and 4.0 $mg \cdot L^{-1}$). The material was incubated in the dark at 27°C ± 2°C for 6 to 8 weeks. After adventitious bud development, the shoots were transferred to Petri dish (100 x 20 mm) containing MS medium with half concentration of BAP and incubated under 16-h photoperiod. The highest percentage of responsive explants was obtained with explants cultured in medium supplemented with 1 $mg \cdot L^{-1}$ BAP. The explant responsiveness varied among cultivars. 'Hamlin' was the most responsive followed by 'Valencia' and 'Pera'. Adventitious shoots were grafted *ex vitro* onto decapitated 'Rangpur' lime rootstocks (*C. limonia*). This protocol will be utilized for citrus mature tissue genetic transformation.

S05P24

Applications of citrus shoot-tip grafting *in vitro*

Juárez J., Aleza P., and Navarro L.

Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain. jjuarez@ivia.es

The main application of shoot-tip grafting (STG) is the control of graft-transmissible pathogens that requires the use healthy trees in the new plantings. The routine application of STG using 0.1-0.2 mm shoot tips is very

efficient for elimination of all citrus graft-transmissible pathogens from local or imported varieties. It has allowed worldwide the recovery of hundreds of healthy cultivars and the planting of hundreds of millions of healthy certified trees. Only in Spain about 135 million certified nursery plants propagated from micrografted plants have been planted. STG is also a very useful technique for regeneration of elite genotypes in several areas of research. *In vitro* grafting for these purposes may be done using larger shoots (up to 1 cm). STG is being routinely used for the following purposes: (i) Regeneration of somatic hybrids from embryos difficult to germinate; (ii) Regeneration of plants from irradiated shoots to produce seedless varieties; (iii) Regeneration of plants from haploid embryos that are very difficult to germinate. STG was used to regenerate the 'Clemenules' haploid plant that has been used by the International Citrus Genome Consortium to sequence the whole citrus genome; (iv) Production of stable tetraploid plants of monoembryonic genotypes, which are very useful for triploid breeding; (v) Regeneration of transgenic plants from shoots that are very difficult to root *in vitro*. STG has become a routine application in citrus genetic transformation.

S05P25

Microshoot tip grafting *in vitro*-a technique for establishment of disease free scion bank of *Citrus reticulata* var. 'Khasi' mandarin

Sanabam R.S., Huidrom S.D., and Handique P.J.

Institute of Bioresources and Sustainable Development (IBSD), Medicinal Plants and Horticultural Resources Division, India. sbnm_rakesh@yahoo.com

This study has been carried out to produce viral disease free scion bank of 'Khasi' mandarin (*Citrus reticulata*) using four citrus cultivars namely 'Kachai' lime, 'Champra maounthabi', 'Solom' and 'Phouheiree' through micrografting techniques as viral disease affects the longevity of citrus orchards and quality of fruits. Success of micrografting depends on the type of cut for scion insertion, age of seedlings of rootstock, conditions of light or darkness, concentration of sucrose used and also on cultivar used for micrografting. Frequency of success improved considerably from 36.24% success at 3% sucrose to 40.05 % success at 6% sucrose with 0.5 mg/L benzyl amino purine (BAP) in Murashige and Tucker (MT) liquid media. Response of micrografting also significantly improved when inverted T type of cut was used for scion insertion giving 37.07% compared to 28.13% of success in that of wedge type of cut. Highest rate of success was obtained in 'Champra maounthabi' with success rate of 40.74% followed by 'Phouheiree', 'Solom' and 'Kachai' lime of 34.26%, 32.24% and 27.53% success respectively. Analysis of 8-10 months old micrografted plants for the presence of graft-transmissible *Citrus tristeza virus* (CTV) following double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) technique was performed giving negative results for the virus.

S05P26

Elimination of *Spiroplasma citri* by somatic embryogenesis from citrus stigma and style culture: preliminary results

Frasheri D.[1], Moujahed R.[1], Djelouah K.[1], Carra A.[2], Carimi F.[2], Valentini F.[1], and D'Onghia A.M.[1]

[1]CIHEAM-Mediterranean Agronomic Institute of Bari (CIHEAM-MAIB), Integrated Pest Management, Italy; and [2]Consiglio Nazionale delle Ricerche, UOS Palermo (CNR-UOS Palermo), Istituto di Genetica Vegetale, Italy. frasheri@iamb.it

Spiroplasma citri, the causal agent of Citrus Stubborn disease, is spread by infected budwood and, in nature, by phloem-feeding homoptera insects, predominantly leafhoppers. This pathogen can be usually eliminated by shoot-tip grafting. In this work, somatic embryogenesis from stigma and style culture, which proved to be effective in the elimination of several infectious agents of citrus, has been firstly applied in the elimination of this pathogen. Flowers were collected from an infected Egyptian source of sweet orange 'Washington' navel. Pistils were tested by DAS ELISA using monoclonal antibodies (SEDIAG-INRA, France) and PCR using specific primers targeting the spiralin; 5 out of 70 pistils were found infected by *S. citri*. About 75 flowers were surface sterilized in ethanol and vertically placed in Petri dishes in contact with Murashige and Skoog (MS) medium supplemented with 6- benzylaminopurine. Explants were *in vitro* cultured and subculturing was carried out every 3-4 weeks. Thirty-nine explants produced callus about 20-30 days after culture initiation, twenty-eight of which regenerated embryos 4 months later. Embryos were individually cultured in test tube and *in vivo* acclimatized after 6 months from embryo formation by minigrafting regenerated plants onto 6 month-old

sour orange seedlings. The embryogenic callus and leaf midveins of 3 month-old regenerated plants were cultured in SP4 and LD8 media, which were used for PCR assays. Results of sanitary tests showed a 30% infection rate in the callus while no infection was detected in the regenerated plants.

S05P27

The study on callus induction of citrus anther culture *in vitro*

Luo J.Q., Xu J.G., Wang P., Ke F.Z., and Nie Z.P.

Zhejiang Citrus Research Institute (ZCRI), Research, China. xujg@mail.zaas.ac.cn

Anther culture has special significance for breeding of citrus. It has been proved as one effective way of obtaining haploid and heteroploid materials. Citrus anthers mostly at middle and late uninucleate stage were cultured to study the influence of genotype, hormone composition of the culture medium, cold pretreatment, culture temperature and light conditions on callus formation. Results showed that the sequence of increasing difficulty on callus induction started with 'Meyer' lemon (*Citrus limon*), followed by '439' tangor (*C. reticulata* × *C. sinensis),* calamondin (*C. madurensis*), 'Changshou' kumquat (*Fortunella obovata*) and 'Zaoju' (*Citrus compressa*), regarding genotype. MT 6-BA 0.5mg/L, TDZ 0.2 mg/L, 2,4-D 0.2 mg/L, sugar 30-40g/L was determined to be a suitable culture medium for anther callus induction of citrus types. Cold pretreatment at 6±2°C for 5-10 d, and then cultivation at 23-25°C were beneficial for callus induction of citrus anthers *in vitro*. Cultivation in darkness was also helpful to mitigate the maturation and browning of anthers, promoting their survival and then callus induction. More than 3000 citrus calli were obtained during 2 years of anther culture, more than 2900 pieces from 'Meyer' lemon, and 35, 39, 2 and 2 callus pieces from '439' tangor, calamondin, 'Changshou' kumquat and 'Zaoju', respectively. 'Meyer' lemon calli is being subcultivated to study ploidy, embryoid induction and plant regeneration.

S05P28

Induction of tangerine embryogenic calli from unfertilized or immature ovules

Machado M.P.[1], de Bona C.M.[2], and Biasi L.A.[1]

[1]Federal University of Parana (UFPR), Plant Production, Brazil; and [2]Agronomic Institute of Parana (IAPAR), Pomology, Brazil. debona@iapar.br

Triploid hybrids may be obtained by interploid crosses between diploid and tetraploid individuals, but it is difficult to obtain tetraploid lineages because of the high frequency of chimerical polyploids. It is possible to induce polyploidy in embryogenic calli or cell suspensions by using antimitotics. Embryogenic calli may be obtained from abortive, unfertilized or fertilized ovules, or from nucellus. Aiming to establish suspension cell cultures for further treatment with antimitotics, ovules of 'Ponkan', 'Montenegrina', 'Mexerica-do-Rio' and 'Cravo' tangerines were used. After asepsis in sodium hypochlorite (3:1) solution, the ovules were inoculated on Petri dishes (100 x 15 mm) (20 ovules per plate) containing 20 mL MT basal medium, 500 $mg \cdot L^{-1}$ malt extract, 50 $g \cdot L^{-1}$ saccharose and 5,0 $mg \cdot L^{-1}$ kinetin and maintained in the dark at 25°C ± 2°C, being subcultivated every four weeks. The experimental design was completely randomized with four repetitions. After two months, the percentage of embryogenic calli and somatic embryos was recorded. The data were submitted to variance analysis and mean deviation was calculated. Calli and embryo induction occurred at the 8th week. 'Mexerica-do-Rio', 'Cravo', 'Ponkan' and 'Montenegrina' presented 25%, 35%, 15% and 9% calli, respectively. Somatic embryos developed rapidly. 'Ponkan' and 'Montenegrina' presented the highest percentage of somatic embryos, 45% and 30%, respectively. The different cultivars presented different responses to calli and somatic embryos induction.

S05P29

Chromosome instability in 'Carrizo' citrange × *Citrus macrophylla* somatic hybrids

Pensabene G.[1], Ruíz M.[1], Aleza P.[1], Olivares-Fuster O.[1], Ollitrault P.[2], and Navarro L.[1]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain; and [2]Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), BIOS, France. lnavarro@ivia.es

Somatic hybridization by protoplast fusion is a very useful technique for citrus rootstock breeding. Indeed, it theoretically allows the accumulation of all genes of the parents, irrespective of their heterozygosity level and

therefore the addition of complementary dominant traits. In this work, protoplasts were isolated from callus of *Citrus macrophylla* and from leaves of 'Carrizo' citrange (*C. sinensis* × *P. trifoliata*). Chemical, electro-chemical and electric fusions were performed and the ploidy of regenerated plants was evaluated by flow-cytometry. Five tetraploid plants, one pentaploid plant, one mixoploid (3x-6x) plant and one heptaploid plant were recovered. All these plants were analyzed with SSR and SNP markers, distributed in the nine chromosomes of citrus. Cytoplasmic genomes were characterized with chloroplastic and mitochondrial markers. Mitochondrial genome was inherited from *C. macrophylla* for all plants while segregation was observed for the chloroplastic genome. Nuclear genome analysis revealed the loss of parental alleles in most of the regenerated plants. In tetraploids, it affected mainly *C. macrophylla* alleles while *P. trifoliata* alleles were mostly lost in 5x and 7x plants. The results indicate chromosome instability in this complex intergeneric combination with apparent non random loss of some chromosome fragments. Two allotetraploid somatic hybrids have been selected for evaluation as potential rootstocks.

S05P30

Preliminary results on biotization of encapsulated *in vitro*-derived propagules of 'Carrizo' citrange (*Citrus sinensis* × *Poncirus trifoliata*)

Germanà M.A.[1], Micheli M.[2], Chiancone B.[1], Bianco C.[3], Casales F.[1], and Defez R.[3]

[1]Università degli Studi di Palermo, Dipartimento DEMETRA, Italy; [2]Università degli Studi di Perugia, Italy (DSAA), Dipartimento di Scienze Agrarie e Ambientali, Italy; and [3]Consiglio Nazionale delle Ricerche (CNR), Istituto di Genetica e Biofisica, Italy.
mariaantonietta.germana@unipa.it

The encapsulation technology represents a new tool to integrate micropropagation into the nursery activity. It allows combine the advantages of zygotic or gamic seeds with those of micropropagation. The synthetic seed or artificial seed, described as "artificially encapsulated somatic embryos, shoots or other tissues which can be used for sowing under *in vitro* or *ex vitro* conditions", will be a powerful propagation tool in the nurseryman hands, if the levels of the synthetic seeds conversion will be increased also in the nurseries, without the asepsis of *in vitro* laboratories and with the presence of many parasitic microorganisms, like bacteria and fungi, responsible for contamination and/or for trophic competition. This research has been carried out in order to introduce the biotization to the synthetic seed technology of 'Carrizo' citrange (*C. sinensis* × *P. trifoliata*), one of the most widespread citrus rootstocks, because of its resistance to the *Citrus tristeza virus* (CTV). With this goal, preliminary experiments to set up protocols for biotization, through the introduction of Plant Growth Promoting Bacteria (PGPB) into calcium alginate capsules of 'Carrizo' citrange *in vitro*-derived encapsulated microcuttings, have been carried out, in order to protect the plantlets from abiotic and biotic factors and to promote their growth during the first stages of development. Specifically, the *Sinorhizobium meliloti* wild type strain 1021 and its derivative RD64, that synthesizes 39-fold more IAA as compared to the wild type strain, have been used to evaluate their performances in inducing rooting of synthetic seeds.

S05P31

Biodegradable films made from PLA-limonene blends for food active packaging applications

Arrieta M.P.[1], López J.[1], Ferrándiz S.[1], and Peltzer M.[2]

[1]Polytechnic University of Valencia (UPV), Technological Institute of Materials, Spain; and [2]University of Alicante (UA), Analytical Chemistry, Nutrition and Food Sciences Department, Spain. marrieta@itm.upv.es

Active packaging films are increasingly being investigated for their application in food packaging industry. Among these, an increasing proactive attitude towards a reduction on the environmental impact produced by food packaging materials has focused research on bio-based polymers. In this sense, polylactic acid (PLA) films have increasingly received attention due to its biodegradation, biocompatibility, overall good mechanical property, superior transparency, being obtained from renewable resources, and labeled as Generally Recognized as Safe material by the FDA. Packaging has a prominent role in packaged food products, being a key component in the food preservation. Since active agents could have an important effect on shelf-life extension of foods, a way to develop actives packaging is by adding antioxidants components into the packaging system. Essential oil rich in monoterpens contain natural antioxidants and are recognized as food preservatives. Additionally, natural antioxidants are of great interest as stabilizers for polymers and the fact that natural antioxidants are

biodegradable makes them attractive for use in the stabilization of food packaging. Limonene is present in a variety of citrus fruits and is one of most important contributors to citrus flavor. Also, it have been reported that limonene shows good antioxidant activity. The aim of this work was to develop biodegradable active films made from PLA and limonene blends as an antioxidant compound for food packaging applications. Films were prepared and full characterization was carried out by using several techniques and methods. The incorporation of limonene in PLA matrix was evaluated and quantified by Pyrolysis Gas Chromatography Mass Spectrometry. Flexibility of the films was evaluated by tensile tests. The polymer stabilization was studied by the kinetic parameters using thermogravimetric analysis.

S05P32

PLA and PLA-PHB incorporated with limonene for biodegraable food packaging

Arrieta M.P.[1], López J.[1], Ferrándiz S.[1], and Peltzer M.[2]

[1]Polytechnic University of Valencia (UPV), Technological Institute of Materials, Spain; and [2]University of Alicante (UA), Analytical Chemistry, Nutrition and Food Sciences Department, Spain. marrieta@itm.upv.es

An increasing environmental concern towards a reduction on the environmental impact produced by plastics is contributing to growth the biopolymer industry. Polylactic acid (PLA), polyhydroxybutyrate (PHB) and their copolymers are biodegradable materials widely investigated for food packaging applications. One of the potential application fields of these materials is as a film. However, amorphous PLA and PHB films are rigid and need to be plasticized. The composition of materials in contact with foodstuffs is tightly regulated so food grade plasticizers are required. On the other hand, when consumers decide which product to purchase, the packaging of a product could influence the decision, therefore transparency in food packaging is required. In this sense, essential oil rich in monoterpens, such as limonene, are of great interest as stabilizers for polymers. Limonene is one of the most important residues in the citrus industry, so, it would be positive to increase its added value from a low-grade by-product to a useful plasticizer. In this work, PLA and PLA-PHB were blended with limonene as plasticizers, in a mixer. The films were obtained by compression-moulding at 180°C in a hot press. Optical properties using CIELab color space were determined. The influence of plasticization process on mechanical properties was determinate by tensile testing according to ASTM-D882 Standard, where average percentage elongation at break and elastic modulus were determined. Oxygen transmission rate (OTR) was also measured to evaluate the barrier properties of these films to oxygen.

S05P33

Shoot tip grafting (STG) in commercial production of disease free, quality-bud grafts of citrus in Central India: the present status

Vijayakumari N.

National Research Centre for Citrus (NRCC), Tissue Culture, India. narukullav@gmail.com

Citrus growers are generally facing a serious problem of decline that is attributed to prevalence of citrus viruses and virus like diseases. The average life span of citrus orchards in tropical countries is less than 10 years because of these diseases. Hence, the need for healthy certified quality planting stock is recognized as basic to the establishment of a viable citrus industry. Use of healthy superior clonal planting material for establishing the new orchards will help in controlling the diseases and increasing the productivity. Shoot tip grafting (STG) has been proved effective in elimination of diseases and in establishment of healthy citrus orchards worldwide (*viz*. Brazil, USA, Spain, etc.) since decades. Keeping this in view, a comprehensive program based on STG to produce, multiply, maintain and distribute the disease free planting stock to citrus growers/nurserymen was initiated nearly 10 years ago in Central India. The program named as citrus variety improvement program has the following functions: 1) Restricted movement of budwood. 2) Introduction of citrus exotic scion cultivars and cleaning through STG. 3) Maintenance of healthy mother trees in protected foundation block under insect proof condition. 4) Continued testing and growth quality evaluation. 5) Discarding infected trees. Through successful transfer of STG technology from lab to land, so far 2.75 lakhs of certified quality bud grafts of *Citrus reticulata* cv. 'Nagpur' mandarin were produced and distributed to citrus growers which facilitated establishment of around 800 hectares of healthy citrus orchards in farmer's fields of Central India, thus paving the way for a sound and sustainable citrus industry. In this paper, the authoress will present the detailed role of STG in the citrus improvement program, and the procedure and technical requirements for the production of healthy foundation stock.

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FRUIT PHYSIOLOGY

S06O01

Effect of male-female interaction and temperature variation in citrus pollen performance

Distefano G.[1], Hedhly A.[2], Las Casas G.[1], La Malfa S.[1], Cicala A.[1], La Rosa G.[1], Herrero M.[3], and Gentile A.[1]

[1]University of Catania (DISPA), Department of Agriculture and Food Science, Italy; [2]University of Zurich, Institute of Plant Biology, Switzerland and [3]Consejo Superior de Investigaciones Científicas (CSIC), Departmento de Pomología, Estación Experimental de Aula Dei, Spain. gentilea@unict.it

Characterizing pollen performance is especially relevant for some economically important genus like *Citrus*, in which failure of the sexual reproductive process resulting in parthenocarpic fruit development and seedlessness is a prized character. Pollen-pistil interaction in the three ancestral true-type *Citrus* species was characterized in order to determine the influence of the male and female genotypes, as well as of the temperature regime on pollen performance. Specifically, temperature effect on pollen grain germination *in vitro*, and on pollen tube growth either *in vivo* in cut flowers under controlled temperature chambers or *in planta* under field temperature conditions was characterized. Results obtained showed that temperature variation has a strong effect on pollen germination, on pollen tube kinetics, and on their population size depending on the specific male-female combination. Furthermore, temperature appears to have an effect on the self-incompatibility reaction by affecting the place where pollen tubes are arrested. Overall, our results show that pollen performance is not only an inherent characteristic of the pollen genotype, but is largely dependent on the particular male-female combination and on genotype-temperature interactions.

S06O02

Abscission of reproductive structures in citrus and its control with brassinosteroids and girdling

Pérez M.C.[1], Almenares G.[2], Torres W.[3], Aranguren M.[2], Betancourt M.[2], García M.E.[2], Núñez M.[3], Llauger R.E.[4], Mahouachi J.[5], Iglesias D.J.[6], and Talón M.[6]

[1]Instituto Nacional de Ciencias Agrícolas (INCA), Dirección General, Cuba; [2]Instituto de Investigaciones en Fruticultura Tropical (IIFT), Fisiología, Cuba; [3]Instituto Nacional de Ciencias Agrícolas (INCA), Fisiología, Cuba; [4]Instituto de Investigaciones en Fruticultura Tropical (IIFT), Dirección General, Cuba; [5]Instituto Canario de Investigaciones Agrarias (ICIA), España; and [6]Instituto Valenciano de Investigaciones Agrarias (IVIA), Genómica, España. mcperez@inca.edu.cu

A study was done to characterize the abscission of reproductive structures under tropical conditions using brassinosteroids and girdling for its control. The trial included 'Valencia Late' and 'Washington' navel orange trees. The tendency to abscise was determined since the early stage of flower development until the end of fruit-set. Fallen reproductive structures were classified through the BBCH scale, foliar levels of gibberellins during fruit-set were also determined and the effect of foliar sprays of analogues of brassinosteroids during petal fall and branch girdling from the anthesis until petal fall on fruit-set, foliar gibberellin levels and yield were also evaluated. Results indicate that the abscission of reproductive structures was interrupted since the beginning by flower development until the end of fruit-set with a maximum during the stage 69 of the BBCH scale, when abscission is high and the fall of reproductive structures is increased in initial phenological stages, the sink capacity reduces, foliar gibberellin levels rise and vice versa. The spray of the analogue of brassinosteroid BB-6 (0.1 $mg \cdot L^{-1}$) reduced the accumulated abscission in 9% and increased yield in 38%, while branch girdling also reduced accumulated abscission and increased yield in 54%. This girdling was preceded by a reduction of total carbohydrates and increased gibberellin levels in leaves during the stage II of fruit growth and development.

S06O03

"To fall or not to fall, that's the question!" Molecular mechanisms underlying organ abscission in citrus

Tadeo F.R., Agustí J., Merelo P., Estornell L.H., Cercós M., Terol J., Domingo C., and Talón M.

Instituto Valenciano de Investigaciones Agrarias (IVIA), Genómica, España. tadeo_fra@gva.es

During their life cycle, citrus plants can experience different processes involving separation of cells by dissolution of cell adhesion materials and breakdown of cell walls at specific sites on the plant body. Abscission enables both vegetative and reproductive organs to be shed in response to developmental, hormonal and

environmental cues. From an evolutionary point of view, abscission is a highly favorable process that has several advantages such as fruit and seed dispersal as well as the shedding of no longer needed, infected, damaged or senescent organs. In an agricultural context, however, abscission is a major limiting factor for crop productivity. In this regard, understanding the regulation of abscission is an important item for the citrus fruit industry. Deeper understanding on the mechanisms underlying abscission would help to regulate crop production and improve current cultural and management practices such as mechanical fruit harvesting or removal of undesirable fruit production. In addition, further molecular research on abscission could help to elucidate new molecular markers to improve genetic breeding. This review highlights the current status of research on genes specifically associated with organ abscission that have been uncovered so far in citrus.

S06O04

Carbohydrate control over carotenoid build-up in citrus is conditional on fruit ontogeny

Fanciullino A.L.[1], Poiroux-Gonord F.[1], Poggi I.[1], and Urban L.[2]

[1]French National Institute for Agricultural Research (INRA), UR 1103 GEQA, France and [2]Université d'Avignon et des Pays du Vaucluse (UAPV), Laboratoire de Physiologie des Fruits et Légumes, France. fanciullino@corse.inra.fr

The final contents of primary and secondary metabolites of the ripe fruit depend on metabolic processes which are tightly regulated during fruit ontogeny. Here, we hypothesized that the sensitivity of clementine (*Citrus clementina*) fruit metabolism to carbohydrate supply was conditional on fruit developmental stage. Defoliation or temporary shading were used as irreversible and reversible means, respectively, of obtaining three levels of fruit load or leaf-to-fruit ratio. Treatments were applied at three key stages of fruit development: early after cell division (S1), at color break (S2), and near maturity (S3). The highest fruit load obtained by defoliation at S1 had the highest impact on fruit growth, maturity and metabolism, followed by the highest fruit load obtained by shading at S1. Soluble sugars decreased by 18% in the first treatment, final fruit size by 21% and 18% in the irreversible and reversible treatments respectively, while organic acids increased by 46% and 29%, respectively. Interestingly, total carotenoids increased by 50% and 18%, respectively. Changes in leaf starch content and photosynthesis supported that these early treatments triggered a carbon starvation in the young fruits, with irreversible effects. Furthermore, our observations on the early treatments challenge the common view that carbohydrate supply influences positively carotenoid accumulation in fruits. We propose that early carbon starvation irreversibly promotes carotenoid accumulation.

S06O05

Largely altered terpenoids production in red-fleshed fruits of 'Cara Cara' navel orange (*Citrus sinensis* L. Osbeck) in comparison with pale-fleshed Seike navel orange

Xu J., He M., Liu X., Gao H.J., Wang Z., Deng X.X., Cheng Y.J., and Zhang H.Y.

Key Laboratory of Horticultural Plant Biology, Ministry of Education (HZAU), Postharvest and fruit quality, China. xujuan@mail.hzau.edu.cn

For two growing seasons, the synthesis of carotenoids, limonoids and abscisic acid were investigated in fruits of red-fleshed 'Cara Cara' and pale-fleshed Seike navel oranges (*Citrus sinensis*). 'Cara Cara' accumulated large amount of phytoene, phytofluene, lycopene and thus total carotenoids in its flesh, while β-carotenoids were dominant in flesh of 'Seike' navel orange. Real time-PCR analysis revealed that lycopene accumulation in the juice sacs of 'Cara Cara' was more likely attributed to the up-regulated expression of phytoene synthase gene than the expression level of lycopene cyclase genes. However, the lack or the decrease of lycopene in leaves and fruit flavedo implied the different regulation mechanism in different tissues. In comparison with 'Seike', throughout the fruit development, 'Cara Cara' contained lower contents of abscisic acid and higher contents of limonin and nomilin in its juice sacs. Additionally, contents of other compounds such as naringin (the main bitter flavonoids in the citrus), soluble sugars and organic acids were found significantly different between two oranges for both seasons. Furthermore, RNA-seq and bioinformatics' analysis declared the global differences in the processes of carotenoids biosynthesis, starch and sucrose metabolism, citrate cycle and glycolysis pathways between the two navel oranges.

S06O06

Endogenous factors affecting fruit color development in navel sweet oranges

Gambetta G.[1], Gravina A.[1], Fasiolo C.[1], Martínez-Fuentes A.[2], Reig C.[2], Mesejo C.[2], Bentancur O.[3], and Agustí M.[2]

[1]Facultad de Agronomía. Universidad de la República (UDELAR), Producción Vegetal, Uruguay; [2]Universidad Politécnica de Valencia (UPV), Instituto Agroforestal Mediterráneo, España; and [3]Facultad de Agronomía. Universidad de la República (UDELAR), Biometría, Estadística y Computación, Uruguay. cfasiolo@gmail.com

Fruit colour change is positively associated to endogenous abscisic acid (ABA), soluble carbohydrates (CHOs), and negatively related to nitrogenous (N) application, but its endogenous evolution is not clear at all. The objective of this work was to study the effect of two growth regulators on fruit colour evolution and on the time course of endogenous ABA, CHOs and N concentration in the flavedo of 'Navelina', 'Washington' navel and 'Navelate' sweet oranges. Foliar sprays of GA3 (20 mg L^{-1}) or norflurazone, an ABA inhibitor (NFZ, 1 mM), were applied prior to colour break, to adult plants, during two years. In the three cultivars, GA3 delayed colour break and its final intensity, while NFZ did not affect it. In control fruits, CHOs concentration increased during colour development and N (total and proteinaceous fraction) concentration diminished. In all experiments, GA3 reduced flavedo CHOs concentration; however, results on N concentration were inconsistent. In 'Navelina' oranges, GA3 reduced ABA concentration in the flavedo along with colour delay. However, in the other two cultivars, flavedo ABA concentration of GA3-treated trees was slightly but not significantly lower than control fruit. NFZ did not affect ABA, CHOs or N concentration in the flavedo according to colour evolution. Possible roles of these endogenous substances on colour development are discussed.

S06P01

Effect of continuously high temperature throughout a growth period on flowering, physiological fruit drop and fruit quality in satsuma mandarin

Sato K.S., Fukamachi H., and Ikoma Y.

Institute of Fruit Tree Science, National Agriculture and Food Research Organization (NARO), Citrus Research Division, Japan. satokei@affrc.go.jp

In Japan, some reports suggest that annual mean temperature in future (2081-2100) will be 2-3 °C higher than that in recent times (1981-2000). However, in satsuma mandarin the influence of continuous high temperature throughout a growth period was not well understood though the influence of high temperature during short terms (i.e., physiological fruit drop, ripening) has been well studied. Thus, we investigated the effect of continuous high temperature from pre-sprouting to fruit maturation on flowering, physiological fruit drop and fruit quality in satsuma mandarin.

Temperature treatment for satsuma mandarin was conducted with a temperature gradient greenhouse (TGG) in which two temperature zones were established. One zone was maintained at ambient temperature (control). The other zone was maintained at 2°C above ambient temperature (plus 2°C treatment).

Flowering in plus 2°C treatment occurred earlier than that in control. There was little difference between plus 2°C treatment and control in fruit set rate after physiological fruit drop although physiological fruit drop occurred earlier in plus 2°C treatment than in control. At optimum harvesting time, color development in plus 2°C treatment was similar to that in control. However, peel puffing was enhanced in plus 2°C treatment. There was little difference in sugar and acid content between plus 2°C treatment and control. These findings suggested that the continuous temperature rise of 2 °C might have a bad influence on peel puffing in satsuma madanrin.

S06P02

Evaluations of potential fruit abscission and characteristics of seeded and seedless fruit abscission in several citrus species during physiological fruit drop

Kitajima A.[1], Li X.[2], Habu T.[1], Kataoka K.[1], Nakazaki T.[1], and Yonemori K.[1]

[1]Experimental Farm, Graduate School of Agriculture, Kyoto University (EFGAKU), Experimental Farm, Japan; and [2]Graduate School of Agriculture, Kyoto University (GAKU), Plant Production Control Lab., Japan. kitajima@kais.kyoto-u.ac.jp

We developed an evaluation method for the potential fruit abscission and investigated the characteristics of seeded and seedless fruit abscission during secondary fruit drop in several citrus species.

The experiment was conducted at 6 weeks after full bloom using satsuma mandarin and tangelo. As an evaluation method for the potential fruit abscission, the fruits with peduncle were detached from a tree and incubated on 1% agar medium (detached). As a control, the fruits on a tree were treated ringing on the peduncle to prevent the translocation of photo-assimilates to the fruit (on tree). The ratios of fruit abscission (separation of fruit from disk) at 168 h after treatment in both "detached" and "on tree" were over 70% in tangelo and were below 20% in satsuma mandarin. The time course of abscission pattern in "detached" was similar to that in "on tree" in both species. The result indicates that the fruit detachment method is useful for an evaluation of the potential fruit abscission in citrus.

The flowers of 7 citrus species were treated with non-pollinated and open-pollinated. These fruits were detached and incubated on agar medium at 5 weeks after full bloom. The ratios of fruit abscission at 96 h after detachment in both open-pollinated and non-pollinated were over 80% in 'Ponkan', navel orange and Iyo, and were below 30% in satsuma mandarin, hyuga-natsu and pummelo, respectively. The non-pollinated fruits of satsuma mandarin, hyuga-natsu, pummelo were seedless. The results indicate that the potential of citrus fruit abscission is different in species and the both of the seeded and seedless fruit abscission potential is lower in satsuma mandarin, hyuga-natsu and pummelo. It is suggested that these species would be useful for seedless citrus breeding.

S06P03

Cross-pollination and control of seed formation in 'Afourer' mandarin

Gravina A.[1], Gambetta G.[1], Fasiolo C.[1], Espino M.[2], and Rivas F.[3]

[1]Facultad de Agronomía. Universidad de la República (UDELAR), Producción Vegetal, Uruguay; [2]URUDOR (URUDOR), Departamento Técnico, Uruguay; and [3]Instituto Nacional de Investigación Agropecuaria (INIA), Programa Nacional de Cítricos, Uruguay. agravina@fagro.edu.uy

'Afourer' mandarin, a self-incompatible variety, produce under Uruguayan conditions high number of seeded fruits. In order to reduce seed presence, four field experiments were performed, including plant net coverage (exp. 1 and 2), and application of different concentrations of copper sulphate and gibberellic acid, one, two or three times during the flowering period (exp. 3 and 4). Additionally, artificial pollinations were performed with 'Valencia' orange and 'Clemenules' clementine pollens. Net covered trees had 99% of seedless fruit. In experiment one, in two year-old plants, no differences in yield components between open pollination and covered plants was registered, but in experiment two, with seven year-old trees, a significant fruit number reduction on covered trees was verified. The most efficient treatments to diminish seeds was three applications of GA_3 (50 mg L^{-1}) alone or combined with $CuSO_4$ (25 mg L^{-1}), that increased seedless fruit from 19 to 31% (exp. 3) and from 26% to 52% (exp.4). Additionally, the average number of seeds per fruit decreased from 3.7 and 2.9 in the controls to 2.3 and 1.8, in experiment three and four respectively. Single flowers self pollinated or artificially pollinated with 'Afourer' pollen, set similar seedless fruit number. 'Afourer' flowers pollinated with 'Valencia' had the highest percentage of seeded fruit (88%) and seeds per fruit (3), while pollinated with 'Clemenules' clementine only 21% of the fruits had seeds.

S06P04

Changes of nutrient element contents and accumulation in fruit of navel orange during fruit growth and development

Peng L.Z., Fa X., Cailun J., Li C., and Lili L.

Citrus Research Institute of Chinese Academy of Agricultural Sciences,Chongqing (CRICCAAS,CQ), China. penglz809@163.com

The changes of nutrient element contents and accumulation of N, P, K, Ca, Mg, S, Mn, Zn, Cu, Fe and B in fruit of 30 years old 'Seike' navel orange (*Citrus sinensis*) on trifoliate orange (*Poncirus trifoliata*) rootstock were studied. Results showed that: (1) During the fruit development, N, P, Mg, S, Mn and Zn contents in fruit reached the highest levels in April, the highest K and Fe contents in July and the highest Ca content in October. However B and Cu contents in fruit showed only small changes from April to November. With the fruit growing and developing, N, P, K, Mg, S, Zn and B contents in fruit decreased to or near the

lowest levels at fruit mature stage in November, but Ca and Fe contents in fruit showed their lowest levels in young fruit stage from April to June. (2) N, P, K and Cu contents were significantly higher in fruit pulp than in fruit peel. Ca, Mn and B contents were significantly lower in fruit pulp than in fruit peel. Mg and S contents were very close in fruit pulp and peel. Contents of Zn and Fe were significantly higher in pulp than in peel before September but turned into opposite after September. (3) The main period for accumulation of N, P, K and Mg in fruit was in June and July. During this period of time in mature fruit, 45.6%, 44.6%, 53.7% and 57.2% of total N, P, K and Mg were accumulated, respectively. The main accumulation period of micro-nutrient elements in fruit was from June to September. (4) At mature stage, there were about 560mg N or K, 274.9 mg Ca, 42.3-57.3 mg P, Mg or S, and 0.17-3.3mg micro-nutrient elements in each fruit.

S06P05

The effects of source-sink ratio on leaf photosynthetic characteristics of 'Newhall' navel orange

Chun C.[1], Wenwen Z.[2], Liangzhi P.[3], Lili L.[1], Xingzheng F.[1], Li C.[3], Xiaohuan M.[2], Fa X.[2], Cai L.[2], and Cailun J.[1]

[1]Citrus Research Institute, Southwest University, Chongqing (CRIC,SWU,CQ), China; [2]College of Horticulture and Landscape,Southwest University,Chongqing (CHL,SWU,CQ), China; and [3]National Citrus Engineering Research Center,Chongqing, (NCERC,CQ), China. chuncpzi@163.com

Leaf growth and photosynthetic characteristics were measured on 30-year-old 'Newhall' navel orange (*Citrus sinensis*) mature leaves on three types of shoots, including no fruit-bearing shoots (NFS), one fruit-bearing shoots (OFS) and two fruit-bearing shoots (TFS), during fruit development period in 2010. Leaves of NFS were larger, thicker, higher relative chl and higher nitrogen (N), phosphorus (P), potassium (K) concentration than the leaves of fruit-bearing shoots (FBS). The net photosynthetic rates (Pn) of leaves on the top 2nd and 4th nodes on NFS were significantly or very significantly higher than those of the same position leaves on FBS, as well as the average Pn of the 2nd, 4th and 6th leaves (except for on 150th day after full bloom). As to the leaf Pn of the two types of FBS, the OFS was slightly higher, and the significant difference of the average Pn of the 3 position leaves between the two types of FBS occurred on 130th day after full bloom. With the fruits bearing increased on a shoot, the leaf thickness, leaf area, leaf N level, leaf P level, leaf K level, relative chl content and average leaf stomatal conductance (Gs) decreased along with the intercellular CO_2 concentration (Ci) and transpiration rate (E) increasing. For all the 3 types of shoots, as the position of the leaves on a shoot located lower, the leaf N, P and K level increased. For the area, thickness, relative chl content and Pn of leaves located on different shoot positions. Fruits from the OFS were larger with thicker peel, significantly higher Vc content than fruits from the TFS. Therefore, the relatively poor differentiation and development of leaves on FBS resulted in their lower Pn. Compared with the leaf differentiation and development, source-sink ratio had slighter effects on leaf Pn.

S06P06

Anatomy of abnormal 'HB' pummelo caused by boron deficiency

Li S.[1], Liu Y.Z.[1], Yang C.Q.[1], and Peng S.A.[1]

[1]Key Laboratory of Horticultural Plant Biology (KLHPB), Ministry of Education, Huazhong Agricultural University, Wuhan, 430070, P. R. China. ganjuli_2002@mail.hzau.edu.cn

Boron (B) deficiency is a worldwide nutritional disorder in citrus industry. Under B deficient condition, symptoms of corky split veins in mature orange leaves, weak tree vigor and low yield are always visible. Moreover, B deficiency could reduce fruit size (shrunk fruit) and increase fruit peel thickness. In this study, we used paraffin section technique to investigate the anatomic structure of mature-fruit albedo between normal and B deficient fruits, by using 'HB' pummelo (*Citrus grandis*) as research material. As compared with in the normal fruit, the spongy cells arranged compactly and the average number of vascular bundle per eyeshot increased in the albedo of B deficient fruit. Moreover, vascular bundles could be found to be linked and the trachea density or trachea number in vascular bundle was obviously increased in the spongy tissue of the abnormal fruit. In addition, we also isolated vessel elements from the spongy tissue and compared their difference between the normal and abnormal (B deficient) fruits. In general, normal vessel elements from spongy tissue are plump. However in the spongy tissue of B deficient fruits, they deformed with hyperplasia and uneven vessel wall; moreover, much perforation could be found in the vessel side wall and the number of pitted vessel increased while the numbers of scalariform and reticulated vessels decreased.

S06P07

Pre-harvest rind breakdown in New Zealand and a comparison with similar problems reported from other countries

Pyle K.R.

Pyle Orchards and Consulting Pty Ltd, New Zealand. kpyle@xtra.co.nz

Pre-harvest rind breakdown is an occasional problem on mandarins, tangelos and oranges in New Zealand. It usually affects fruit at or soon after colour break and is often referred to as 'on-tree oleocellosis'. It is normally associated with extreme wet, cold weather events and with gusty winds. In the Gisborne region in July 2011, some exposed, wet orchards, mainly navel oranges, were adversely affected with up to 50% fruit marking and 60% fruit drop in the worst cases.This prompted a more detailed examination of the condition, a review of the local meteorological and other factors that may have caused the breakdown and some recommendations for growers. These are described, together with a brief comparison of similar pre-harvest rind defects and their management obtained from international literature and correspondence. The condition most closely resembles pitting of 'Pineapple' orange described in Florida, pitting of 'Fortune' mandarin reported in Spain, rind staining of navels and tangelos in Morocco, mandarin rind disorder in California, rind spotting of clementines in Greece and 'pateta de rata' or 'colapso de la corteza' on 'Navelate' in Spain and Chile.

S06P08

The effect of combined spraying of gibberellin and prohydrojasmon on peel puffing in satsuma mandarin

Ikoma Y., Matsumoto H.M., and Sato K.S.

NARO Institute of Fruit Tree Science (NIFTS), Citrus Research Division, Japan. yoshino@affrc.go.jp

Peel puffing of satsuma mandarin is a serious physiological disorder in which the pericarp and flesh separate. This disorder causes increased fruit rot and decreases eating quality. Because peel puffing is promoted by high temperatures and humidity, there is a fear that global warming will cause frequent occurrence of peel puffing. To reduce peel puffing effectively, combined spraying of the plant hormones gibberellin (GA) and prohydrojasmon (PDJ) was examined. In the present study, 3 chemical sprays (1 $mg{\cdot}L^{-1}$ GA spray, 1 $mg{\cdot}L^{-1}$ GA plus 25 $mg{\cdot}L^{-1}$ PDJ spray and 1 $mg{\cdot}L^{-1}$ GA plus 50 $mg{\cdot}L^{-1}$ PDJ spray) were prepared just before treatment. On August 29, the selected branches of satsuma mandarin trees were treated with these chemical sprays, respectively. At optimum harvesting time, fruit qualities were investigated. Peel puffing was significantly inhibited by combined spraying of GA and PDJ but not by spraying of GA without PDJ. There was no difference between two combined sprays (1 $mg{\cdot}L^{-1}$ GA plus 25 $mg{\cdot}L^{-1}$ PDJ spray and 1 $mg{\cdot}L^{-1}$ GA plus 50 $mg{\cdot}L^{-1}$ PDJ spray) in the intensity of the peel puffing inhibition. Coloring was delayed by combined spraying of GA and PDJ but not by spraying of GA without PDJ. The intensity of the delay of coloring was smaller in 1 $mg{\cdot}L^{-1}$ GA plus 25 $mg{\cdot}L^{-1}$ PDJ spray than in 1 $mg{\cdot}L^{-1}$ GA plus 50 $mg{\cdot}L^{-1}$ PDJ spray. In TSS, there was no difference among treatments. In contrast, citric acid content became high by combined spraying of GA and PDJ. Especially in 1 $mg{\cdot}L^{-1}$ GA plus 50 $mg{\cdot}L^{-1}$ PDJ spray, the increase in citric acid content was remarkable. These results suggested that the optimum concentrations of GA and PDJ for inhibition of peel puffing were 1 and 25 $mg{\cdot}L^{-1}$, respectively.

S06P09

Production cost reduction of Shiranuhi mandarin hybrid in plastic house cultivation using underground air and air mixer.

Han S.H.

Jeju National University (JNU), Horticulture, Korea, Republic. sangheon@jejunu.ac.kr

The Jeju citrus area is located in a cold area, but cultivation is generalized using plastic film houses. The late maturing mandarin variety 'Shiranuhi' started to be grown in 1990 and it has reached 1,188 ha

(5.6% of the total citrus area) with a production of 22.000 tons, that represent 3.7% of the whole citrus production. The fruit price of this variety is high compared with imported citrus, as a consequence of FTA recent reduction of customs duties, and domestic consumption is decreasing, and thus, production cost reduction is needed. This research attempted production cost reduction using underground aerial use and an air mixer in plastic film house cultivation. The system maintained a similar temperature than conventional systems that use a heavy oil hot air during the winter period, but CO_2 content was about 5 times higher and the size of the fruit was better. The fruit quality was similar in the two houses. After the annual production cost was compared, the underground aerial use house could reduce the production cost about 50 %. We expect to utilize such production cost reduction technique and to be able to compete with imported citrus.

S06P10

Abscission study during citrus fruit maturation in Corsica: unfavorable environmental conditions for fruit shedding

Khefifi H.[1], Ben Mimoun M.[2], Luro F.[3], and Morillon R.[4]

[1]Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD)/Institut National de la Recherche Agronomique (INRA)/ Institut National Agronomique de la Tunisie (INAT) (CIRAD/INRA/INAT), France/Tunisia; [2]Institut National Agronomique de Tunisie (INAT), Production végétale, Tunisia ; [3]Institut National de la Recherche Agronomique (INRA), Génétique et Ecophysiologie des Agrumes, France; and [4]Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Amélioration des plantes à multiplication végétative, France. khefifihajer@yahoo.fr

Citrus fruit development and ripening are complex processes involving physiological and biochemical changes that are under hormonal, nutritional and environmental control. One of the most evident phenomena in late maturation is shedding of ripe fruit. A previous study on sweet orange abscission supposed that fruit shedding was related to the increase of sugars content of pulp juice. To investigate this potential relationship between fruit abscission and internal maturity parameters we investigated the fruit maturity process of 10 mandarin x clementine hybrids and 9 commercial sweet orange varieties with different maturity time. Morphological and biochemical analyses (acidity, total soluble solids, fruit weight thickness of the flavedo, thickness of the peduncle and external color) were carried out on fruit sets of the different varieties during the maturation process, from December to June. The abscission initiation was evaluated by measuring the force required to detach the fruit of the peduncle. Analysis showed that under our local conditions, abscission was generally not expressed even though fruit maturation was evolving. Therefore, we suppose that even though the local conditions have an important effect, abscission of citrus fruit also results from environmental-genotype interactions. Moreover, evolution of abscission and fruit parameters are not correlated, suggesting the independence in the processing of maturation and abscission.

S06P11

Fruit quality characteristics of very early satsuma mandarin by soil mulching with poly porous reflective sheet

Han S.G., Kim Y.H., and Moon Y.E.

Citrus Research Station, National Institute of Horticultural and Herbal Science, RDA. skhan@korea.kr

This experiment was carried out to determine the effect of soil mulching with a porous water-proof sheet (PWPS) on fruit quality of the very early maturing satsuma 'Nichinan I go' at citrus orchards in Jungmun, Seogwipo, Jeju, Korea. Soil was mulched with a porous water-proof sheet on early June and started irrigation when the fruits reached 8.5oBrix. At maturity, soluble solid content was12.8º Brix in the mulching plot and 10.2º Brix in the control, showing a significant increase of mulching of 2.6o Brix . Acidity in fruits of the mulching plot was slightly lower than in the control plot. Peel coloration (Hunter parameters) also was significantly higher in the mulching plot than in the control.

S06P12

Fruit growth and sugar and acid characteristics in leafy and leafless fruits of satsuma mandarin

Kim S.B.[1], Oh E.U.[1], Yun S.H.[2], Oh H.W.[3], and Song K.J.[1]

[1]Jeju National University (JNU), Faculty of Bioscience and Industry, Republic of Korea; [2]National Institue of Horticultural and Herbal Science (NHHS), Citrus Research Station, Republic of Korea; and [3]Jeju Special Self-governing Province Agricultural Research & Extension Services (JSSPARES), Citrus Breeding Center, Republic of Korea. kwansong@jejunu.ac.kr

The study was conducted to investigate the possible differences of fruit bearing type in satsuma mandarin, with leafy (LY) and leafless (LS) fruits, on fruit growth and sugar and acid content. Fruit growth including fruit length, diameter, shape index (diameter/length), weight, and size distribution was not different between LY and LS fruits at ripening time. Total soluble solids (TSS) of fruit juice increased and acidity decreased continuously and then TSS: acidity ratio increased with fruit development from 100 days after anthesis (DAA) to ripening time. Soluble sugar was continuously increased, whereas organic acid decreased. Theses tendencies were related to increase of sucrose and decline of citric acid, respectively. However, there was no effect of fruit bearing type on TSS, acidity, and TSS: acidity ratio, and soluble sugar and organic acid composition. Also, photosynthetic rate and chlorophyll index with SPAD value were not different between leaves adjacent to LY and LS fruits. The results indicate fruit bearing type in satsuma mandarin do not affect fruit growth, and sugar and acid content.

S06P13

Physiological investigation of June drop in Thomson navel orange (*Citrus sinensis*) in the north of Iran

Akhlaghi Amiri N.[1], Asadi Kangarshahi A.[2], and Arzani K.[3]

[1]Mazandaran Agricultural and Natural Resources Research Center, Seed and Plant Improvement, Iran; [2]Mazandaran Agricultural and Natural Resources Research Center, Soil and Water, Iran; and [3]Tarbiat Modares University (TMU), Horticultural Science, Iran. neginakhlaghi@yahoo.com

One of the reasons for high losses in some varieties of citrus like 'Thomson' navel orange is summer physiological drop. Recognition of physiological events in the fruit abscission zone and application of some nutritional treatments can reduce or control abscission, so affect on decreasing losses. This experiment was conducted to investigate auxin, cellulase and poly-galacturonase content in the abscission zone of 'Thomson' navel orange and its relationship with fruit abscission percent. The experiment was performed in a randomized complete block design with 8 treatments: 1.Control; 2.Urea (1%); 3. Urea 2,4-D (15 mg L^{-1}); 4.Urea GA3 (15 mg L^{-1}); 5.Urea Sucrose (1%); 6.Urea $ZnSO_4$ (0.5%); 7.Urea $ZnSO_4$ 2,4-D and 8.Urea $ZnSO_4$ GA3, with four replications. Foliar application was done in late May. Results showed that auxin level in treatments 5, which included sucrose in its composition, was the highest among all treatments. This treatment also provided the lowest amount of hydrolytic enzymes. Decreasing cellulase and poly-galacturonase activity also reduced fruitlet abscission in treatment 5. Reduced fruitlet competition for carbohydrates could be the main reason explaining the reaction of trees to this treatment.

S06P14

Reducing alternate bearing index in satsuma mandarin by nitrogen management and pruning in north of Iran

Asadi Kangarshahi A.[1], Akhlaghi Amiri N.[2], and Savaghebi Gh.R.[3]

[1]Mazandaran Agricultural and Natural Resoueces Research Center, Soil Science, Iran; [2]Mazandaran Agricultural and Natural Resoueces Research Center, Seed and Plant Improvement, Iran; and [3]Tehran University (TU), Soil Science, Iran. kangarshahi@gmail.com

In order to reducing alternate bearing in satsuma mandarin (*Citrus unshiu*), an experiment was conducted in randomized complete block design with 5 replications and 6 treatments during 4 years. Treatments include: 1. Control; 2. Autumn nitrogen application (in on years based on leaf analysis and yield); 3. Spring pruning (in on years); 4. Summer urea sprays (4 g L^{-1} in on years); 5. Winter urea spraying (10 g L^{-1} after on years); 6. Urea spraying with autumn nitrogen using and spring pruning. To obtain alternate bearing index, the ratio of yield difference in each two consecutive years to total yield of the same two years in each treatments was calculated then the average of all was taken. Results showed that in all treatments, alternate bearing cycle was reduced compared to control trees. But in treatment 6, alternate bearing cycle

almost disappeared and total yield in four years reached to an acceptable level. Also index of alternate bearing was calculated for all treatments and results showed that treatment 6 had the lowest and control had the highest index among other treatments. So, as in treatment 6 (urea spraying with autumn nitrogen application and spring pruning), alternate bearing almost was balanced and total yield increased and in each year reached to an acceptable amount. Use of this treatment in citrus plantings in north of Iran could be economical and profitable.

S06P15

Possibility of adjusting alternate bearing cycle in satsuma mandarin (*Citrus unshiu*) in north of Iran

Asadi Kangarshahi A.[1] and Akhlaghi Amiri N.[2]

[1]Mazandaran Agricultural and Natural Resources Research Center, Soil Science, Iran; and [2]Mazandaran Agricultural and Natural Resources Research Center, Seed and Plant Breeding, Iran. kangarshahi@gmail.com

Alternate bearing is one major problem in some citrus varieties like satsuma mandarin. An alternate bear tree does not produce a regular crop in consequence years. Reduced fruit quality in “on year” and decreased quantity in “off year” cause economic loss to producer. To evaluate possibility of reducing this disorder, an experiment was conducted in randomize complete block design with 5 treatments: 1. Control; 2. Optimum using of chemical fertilizers (based on leaf analysis and annual yield prediction); 3. Optimum using of chemical fertilizers and NAA spraying (300 mg L^{-1} during June drop in on years); 4. Optimum using of chemical fertilizer and 2,4-D spraying (50 mg L^{-1} during June drop in on years) and 5. Optimum using of chemical fertilizer and sucrose spraying with 4 replications in 4 consecutive years on satsuma mandarin mature trees. Control trees had the most alternate bearing during 4 years, as showed one “on” year, two consecutive “off” years and one “on” year, respectively. On the other hand, other 4 treatments reduced number of “off” years to one, during 4 years. Treatment 2 had the highest yield in “off” year and the lowest yield in “on” year. The smallest and lightest fruit in first, second and fourth years was allocate to control. During 4 years, the lowest total yield was recorded in control plants. In conclusion, treatment 2 by reducing yield in “on” year, increasing yield in “off” year and reduce number of “off” years compare to control and treatments 3, 4 and 5 by reducing number of “off” years and increasing fruit size in on years caused adjusting alternate bearing cycle, increase total yield in consecutive years, decrease fruit losses and improve marketability in satsuma mandarin.

S06P16

Reproductive behavior of plants of ‘Valencia’ sweet orange under two soil moisture conditions

Laskowski L.L.

Universidad Lisandro Alvarado (UCLA), Agronomía - Cs. Biológicas, Venezuela. llaskowski@ucla.edu.ve

Drought in the tropics impose cessation of reproductive growth in citrus. In Venezuela has been established a minimum requirements of 1200 mm of precipitation per year to maintain a profitable citrus farm. Otherwise, irrigation is absolutely necessary to sustain productivity. During this investigation we evaluated the fruit set, growth and fruit quality in plants of ‘Valencia’ sweet orange (*Citrus sinensis)* subject to the application of deficient irrigation. Plants of 8 years of experimental orchard at the University Lisandro Alvarado, Lara, Venezuela, were used. Starting from the first sprouting induced by the rains applied two irrigation treatments: 50 and 100% of the water requirement, considering the losses by evapotranspiration. In each plant were selected four branches of 30 nodes and each month we proceeded to determine: number of flowers, fruit number and diameter. After reaching fruit maturity, we took between 20 and 15 fruits of each plant to determine: fresh mass, skin thickness, volume of juice, ° Brix, TSS and juice acidity. As a result, the fruits of plants under 100% irrigation during the first 11 daa increased growth rate and shown higher percentage of abscission compared to 50% irrigation. Next, until the end of study (244 daa), fruit set, fruit diameter and quality were similar in both treatments and were comparable to reported for variety. The quality of the juice shown to be significantly more palatable under deficient irrigation. In conclusion, the 50% decrease in the amount of irrigation water considering the weekly evaporation losses did not affect plant productivity and fruit quality under the environmental conditions studied.

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REGULATION OF GROWTH AND DEVOLOPMENT

S07O01

Citrus developmental research: a historic, conceptual perspective

Goldschmidt E.E.

The Hebrew University of Jerusalem, The Robert H. Smith Faculty of Agriculture, Food and Environment, Israel.
goldsmit@agri.huji.ac.il

As long as microscopes were the major research tool, developmental anatomy and morphology led the way. But then, towards the middle of the 20th century, horticulturists were enchanted by the plant hormone concept. The hormonal thinking dominated citrus developmental physiology for a long time; flowering, fruit development and ripening, abscission, rooting and vegetative growth were all attributed to one, specific plant hormone or to the perplexing 'hormonal balance' concept. The synthetic growth regulators were exploited in every possible way to solve all practical problems. A major representative of this 'Hormonal' era was the renown Israeli citrus researcher S.P. Monselise (1920-1986). The emergence of the source-sink concept (ca. 1980) partially replaced the hormonal hypothesis. The source-sink hypothesis claims that carbohydrate levels control the major stages of the fruiting process –flowering, fruit set and fruit enlargement– and are, therefore, responsible also for the 'alternate bearing' phenomenon. Although the effects of girdling and fruit thinning seem to support the sink-source concept, direct control of flowering and fruit set by carbohydrate levels was not unequivocally demonstrated. The Spanish researcher J.L. Guardiola (1943-2010) played a major role in this debate. But then, rather soon, came the molecular-genetic era, forcing us all to acquire a new language and follow its logic. Citrus research is still a few steps behind *Arabidopsis* but, here we are, still doing our best in order to solve the remaining mysteries of citrus.

S07O02

Stock-scion interactions in grafted citrus: a role for microRNA

Tzarfati R.[1], Ben-Dor S.[2], Sela I.[1], and Goldschmidt E.E.[1]

[1]The Hebrew University of Jerusalem, Institute of Plant Science and Genetics in Agriculture, Israel; and [2]The Weizmann Institute, Biological Services, Israel. goldsmit@agri.huji.ac.il

Grafting is a widely used plant propagation technique in horticultural crops, but the physiological basis of stock-scion interactions is as yet, incompletely understood. Recent studies indicate that movement of proteins and small RNAs through the graft union might be involved. MicroRNAs (miRNAs) are known to play a significant role in regulation of higher plants' developmental and metabolic traits. We would like to hypothesize that changes in expression of mRNAs play a role in mediating the effects of grafting. The objective of the present study was to examine this hypothesis in citrus. We have determined the expression of a broad range of mRNAs in citrus leaf petioles, as affected by grafting. Four stock/scion combinations ('Merav' mandarin and 'Star Ruby' grapefruit scions X 'Troyer' and 'Volkamer' rootstocks), rootstock auto-grafts and non-grafted rootstock controls were examined. Microarray comparisons of mRNA expression revealed significant differences in major as well as minor mRNAs. Grafting caused a dramatic reduction in the expression of the major miR156 (and miR157) which appear to be associated with reduction of juvenility in woody plants. Expression of miR894 also declined after grafting. Differential rootstock and scion-dependent expression of the minor miR397 was found in petioles of grafted scions, while in non-grafted rootstocks, the expression of miR397 was barely detectable. Bioinformatic analysis confirmed the presence of miR397 in the citrus genome, validated its sequence and demonstrated its ability to form a stem loop. The differences in miR397 expression might be related to copper and other micronutrient requirements of citrus stock-scion combinations. Thus, our results support the hypothesis, suggesting an involvement of specific mRNAs in engendering physiological effects of grafting. The precise mechanism remains to be elucidated.

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S07O03

Alternate bearing in *Citrus reticulata* - An overview

Verreynne J.S.[1], Faber B.A.[2], and Lovatt C.J.[3]

[1]Nulandis (Nulandis), South Africa; [2]University of California Cooperative Extension (UCCE), Ventrua and Santa Barbara Counties, United States of America; and [3]University of California-Riverside (UCR), Department of Botany and Plant Sciences, United States of America. carol.lovatt@ucr.edu

Alternate bearing, cycling of heavy on-crops with light off-crops, reduces mandarin (*Citrus reticulata*) grower income. On-trees produce numerous small fruit of reduced commercial value; Off-trees produce too few fruit for a good income. The on-crop causes correlative inhibition (auxin>cytokinin) of buds that produce summer/fall vegetative shoots, reducing the number of nodes that bear inflorescences at bloom, and also inhibits spring bud break. The effect of crop load on return bloom is strongest on bearing shoots on on-crop trees compared to nonbearing shoots on on-crop trees and nonbearing shoots on off-crop trees, which are least affected. Thus, return yield is a function of the number of nonbearing shoots per tree. Trunk injecting (to avoid the problem of poor leaf uptake) of on-crop 'Clemenules' clementine trees with an auxin-transport inhibitor and/or a cytokinin in July and again in January, mitigated the effects of the on-crop on summer/fall shoot growth and spring bud break and successfully increased return bloom. One treatment tested significantly increased return yield relative to untreated on-crop (Year 1) control trees and equal to that of untreated off-crop (Year 1) control trees. Our goal is to develop a cost-effective strategy that mitigates the effect of the on-crop and increases 'Clemenules' clementine 2-year cumulative total yield, yield of commercially valuable large fruit and grower net income.

S07O04

Relationship between a citrus *FLOWERING LOCUS T* expression and fruit bearing

Nishikawa F.[1], Iwasaki M.[1], Fukamachi H.[1], and Endo T.[2]

[1]National Agriculture and Food Research Organization, Institute of Fruit Tree Science (NIFTS), Kuchinotsu Citrus Research Station, Japan and [2]National Agriculture and Food Research Organization, Institute of Fruit Tree Science (NIFTS), Okitsu Citrus Research station, Japan. fumien@affrc.go.jp

Citrus trees often show alternate bearing where a high yielding ON year is followed by a low yielding OFF year. This behavior results from a suppression of subsequent flowering by high fruit bearing. In this study, we investigated whether a flowering-related gene, citrus *FLOWERING LOCUS T* (*CiFT*) is related to the suppression of flowering by fruit bearing. In satsuma mandarin trees with different amounts of fruit, *CiFT* expression in November, which is the period of floral induction, was negatively and highly correlated with the fruit weight per leaf area ratio at harvest ($R=-0.857$, $p<0.01$). *CiFT* expression in November was positively and highly correlated with the flower number in the following spring ($R=0.843$, $p<0.01$). The correlation coefficient between fruit weigh per leaf area ratio at harvest and the flower number in the following spring was -0.62. Thus, *CiFT* expression in November showed a close correlation with the leaf to fruit ratio at harvest and with the flower number in the following spring. These results suggest that excess fruit bearing reduces flower number in the following spring via the suppression of *CiFT* expression.

S07O05

Managing drought stress of oranges under Sao Paulo-Mina Gerais, Brazil conditions to optimize flower bud induction and productivity

Albrigo L.G.[1], and Carrera R.R.[2]

[1]University oof Florida Citrus Research and Educatiion Center (UF CREC), US; and [2]Sucocitrico Cutrale Ltda, Production Dept., Brazil. albrigo@ufl.edu

Winter weather in the Sao Paulo-Minas Gerais citrus production area often varies from adequate cold induction in the south to excessive drought in the north. Cool temperature induction hours (19o C or less) ranged from less than 200 to more than 1300 hours depending on location and year. Using an ultralow volume irrigation scheme, deficits in cool temperature induction were compensated for by drought stress to achieve adequate flower bud induction without excessive drought stress. Soil moisture in the surface 20 cm was kept at adequate moisture levels to prevent severe drought with as little as 0.25 to 1 mm/day. These treatments kept trees in adequate condition without bud sprouting for up to 120 days.

S07O06

Exploring microRNA target modulation in citrus somatic embryogenesis by high-throughput small RNA and degradome sequencing

Wu X.M., Liu M.Y., Xu Q., Deng X.X., and Guo W.W.

Key Laboratory of Horticultural Plant Biology, Ministry of Education, Huazhong Agricultural University (HZAU), China.
ema@webmail.hzau.edu.cn

Somatic embryogenesis (SE) from callus is an important approach of *in vitro* regeneration, as well as a model for studying mechanisms of nucellar polyembryony in citrus. In our study, small RNA (sRNA) and degradome (the collection of molecules that result from the incomplete degradation of mature, functional RNAs) sequencing were implemented on embryogenic callus (EC) and non-embryogenic callus (NEC) of 'Valencia' sweet orange, as well as on EC cultured on glycerol medium for 1-4 weeks (E1-4) on which somatic embryos were induced. A total of 64 previously known and 191 novel microRNAs (miRNAs) were identified. Quite a few miRNAs were found to be highly expressed in NEC, while only 25 miRNAs were more abundant in embryogenic tissues than in NEC. miRNA expression was detected in preserved callus of seven citrus varieties with different SE capability. Five miRNAs, miR156, 164, 390, 171 and 059, were found to be expressed higher in callus with SE capability compared to callus that had lost SE capability. In addition, 1,150 transcripts were identified to be cleaved by 129 miRNAs in the three tissues (EC, NEC, E1-4). A total of 347 phased siRNA (phasiRNA) were identified and most highly expressed in NEC and only two expressed higher in EC or E1-4 tissue than in NEC. For the over-accumulation of one of the most conserved miRNAs, miR156 in EC and SE processes of 'Valencia', the *Csi-MIR156 gene* was overexpressed by a *35S* promoter in 'Guoqing No. 1' satsuma mandarin (*Citrus unshiu*), which had lost SE capability over years. Although the SE capability was not recovered, the overexpression lines tended to accumulate significantly more amyloids, which resembled EC cells of 'Valencia' with strong SE capability.

S07P01

Periods of normal and induced vegetative and reproductive shoots of 'Persian' lime (*Citrus latifolia*)

Medina-Urrutia V.M.[1], Durán-Martínez C.M.[2], Virgen-Calleros G.[2], Robles-González M.M.[2], and Rendon-Salcido L.A.[2]

[1]Universidad de Guadalajara (CUCBA-UDG), Centro Universitario de Ciencias Biológicas y Agropecuarias, México; and [2]Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP-Colima), CIRPAC, México. vmmedinau@gmail.com

'Persian' lime fruit is mainly produced under tropical conditions in the State of Veracruz in México. As the demand in international markets has increased, new lime orchards also were established in subtropical regions. Information about the phenological performance of 'Persian' lime trees under this climate is scarce. The objective of this work was to determine the time of vegetative (VEG) and reproductive (REP) sprouting of 'Persian' lime trees under subtropical conditions of Jalisco State in México. Two orchards of lime trees budded on 'Volkamer' lemon (*C. volkameriana*) rootstock and planted on clay soils were selected. The first site was located at San Martin Hidalgo (SMH) at 1518 m of altitude, mean temperature of 16.7 to 24.4 °C and average annual rainfall of 908 mm. The second orchard was planted in Atotonilco (ATO) at 1606 m of altitude, mean temperature of 15.7 to 22.6 °C and with 856 mm of rainfall. Tree age was six- and four-years-old at SMH and ATO respectively. VEG and REP shoots were recorded biweekly during two years. Results showed that periods of VEG and REP shoots were very similar in both regions. Two peaks of VEG shoots were detected, one initiated in middle winter (February) and the second in middle summer (end of June-beginning of July). The production of VEG shoots during the summer season was 3 fold greater than the winter sprouting. VEG sprouting extended 15-40 days more during winter than in the summer season. Both mixed and REP shoots were also produced in a higher proportion during summer than in the winter season. VEG and REP shoots emerged in summer time were induced by a treatment of water stress applied before the initial rainy season. Normal REP and VEG shoots emerged during winter were associated with lower night temperatures (6 to 13 °C) and higher day temperatures (24 to 29 °C) recorded during this period. Fruits initiated in the summer bloom required 130-145 day to harvest. Flowering during winter time preceded harvest by more than 150 days.

S07P02

Daily temperature amplitude affects the vegetative growth and carbon metabolism of orange trees in a rootstock-dependent manner

Bueno A.C.R., Prudente D.A., Machado E.C., and Ribeiro R.V.

Instituto Agronomico (IAC), Laboratorio de Fisiologia Vegetal Coaracy M Franco, Brazil. rafael@iac.sp.gov.br

Both instantaneous and average growth temperatures affect plant metabolism, and the physiological importance of daily variations in temperature is frequently underestimated. In this study, we hypothesized that vegetative growth would be stimulated in orange trees subjected to large daily temperature variations even without changes in the average daily air temperature or the amount of energy. This hypothesis was tested with orange trees grafted onto 'Rangpur' lime or 'Swingle' citrumelo rootstocks and grown for 20 days under day/night thermal regimes of 25/25 ºC or 32.5/17.5 ºC. Such regimes imposed daily temperature variations of 0 ºC and 15 ºC. Plant growth, photosynthesis, respiration and carbohydrate availability in leaves, stems and roots were measured under both thermal conditions. The daily temperature variation affected the carbon metabolism of young citrus trees; plants grown with 15 ºC of daily variation used more of the carbon stored in mature leaves and roots and the energy generated by respiration for the biosynthesis of vegetative structures, such as leaves and branches. Thus, there was a significant increase in the leaf area of plants subjected to the high daily temperature variation. Current photosynthesis was similar in the two thermal regimes; however, the photosynthetic rates increased under the 15 ºC variation when measurements were normalized to 25 ºC. In addition to the stimulatory effect of the source-sink relationship on photosynthesis, we suggest a probable involvement of hormonal regulation of plant growth through gibberellin metabolism. The rootstock affected the response of the canopy to daily temperature amplitude, with the Rangpur lime improving plant growth through higher carbohydrate availability in roots. This is the first report that highlights the importance of daily temperature variations for citrus growth and physiology under non-limiting conditions.

S07P03

The effects of paclobutrazol and fruit on flowering and carbohydrate accumulation in roots, branches and leaves of alternate bearing clementine mandarin

Martínez-Fuentes A.[1], Mesejo C.[1], Muñoz-Fambuena N.[1], Reig C.[1], González-Mas M.C.[2], Iglesias D.J.[2], Primo-Millo E.[2], and Agustí M.[2]

[1]Instituto Agroforestal Mediterraneo. Universitat Politècnica de València (IAM-UPV), España; and [2]Instituto Valenciano de Investigaciones Agrarias (IVIA), Citricultura y Producción Vegetal, España. carmeco@upv.es

This study aimed to determine the effects of paclobutrazol (PCB) in on- and off-bearing trees on flowering and carbohydrate accumulation in roots, branches and leaves of 'Hernandina' clementine mandarin. Application of PCB (1 g tree^{-1}) to the roots during the flower bud induction period (November) significantly increased bud sprouting and flowering in off-trees. However, this effect was prevented in on-trees. One month before the treatment with PCB, hexose concentrations were significantly reduced in roots from both on- and off-trees compared to untreated trees, but sucrose concentration was only reduced in on-trees. At the moment of bud sprouting, sugar concentrations significantly increased in leaves from all treatments. PCB significantly increased hexose concentrations in leaves from on-trees compared to untreated trees. On the other hand, sucrose concentration was significantly higher in leaves from off-trees. No significant differences in carbohydrate concentrations were found in branches. Neither carbohydrate contents in roots nor in leaves and branches due to PCB treatment or fruit was related to flowering intensity.

S07P04

Fruit set in orange with phytohormones and its relation to engogeneous levels of GA 3

Galván J.J.[1], Martínez A.[1], López N.[1], and Salazar O.[2]

[1]UAAAN (UA), Horticultura, México; and [2]Universidad Autónoma de Tamaulipas (UAT), UAMAC, México. juan.galvan@uaaan.mx, doctorgalvanluna@hotmail.com

This research work aims to contribute to increasing the fruit set given the importance fruit set has in determining the harvest. This work evaluated the effects of phytohormones (auxins, gibberellins and cytokinins) in fruit

set of the orange varieties 'Washington' navel and 'Thomson' (*Citrus sinensis*).The design was a split plot in a randomized complete block with five treatments and four replications. Flowering tissue samples were stored in liquid N until the extraction of gibberellins and the identification and quantification of GA3. The results show statistically significant differences ($P \leq 0.05$) between treatments in the number of fruits retained on the 129 d after flowering and the percentage of final set. GA3 content ranged from 1.66 mg g^{-1} in the control to 20.79 mg g^{-1} dry weight in the high dose. The mean doses of 32.2 mg L^{-1} of auxins, 32.2 mg L^{-1} of gibberellins and 83.2 mg L^{-1} of cytokinins, resulted in the largest increases in fruit set.

S07P05

Analysis of expressed proteins in the pollen tube occuring in the self-incompatible response using mass- or single-culture system of mature pollen in citrus.

Abe A.[1], Uchida A.[2], Hoshino Y.[3], Sakakibara Y.[1], Suiko M.[1], and Kunitake H.[1]

[1]University of Miyazaki (UOM), The Graduate School of Agriculture, Japan; [2]University of Miyazaki (UOM), The Interdisciplinary Graduate School of Agriculture and Engineering, Japan; and [3]Hokkaido University (HU), Field Science Center for Northern Biosphere, Japan. ae11003@student.miyazaki-u.ac.jp

To analyze protein expression of pollen tubes having self-incompatible reactions in hyuganatsu (*Citrus tamurana*), mature pollen was cultured and treated with crude extracts from styles using the mass or single liquid culture system and pollen tubes were collected for proteome analysis. The relative expression of each identified protein was quantified by Imager and revealed as a value relative to the pollen grain. Eleven identified proteins were remarkably up-regulated (above 1.2-fold) or down-regulated (under 0.8-fold) in the treatment with crude extract from self-pollinated styles. Seven of these proteins were predicted to be related to the SI reaction between the reported function of these seven proteins. In this study, F-box protein was identified as showing minimum expression in the treatment with extract from styles of wild-type hyuganatsu. The *S-locus* gene product expressed in pollen has been identified as an F-box protein in Solanaceae and Rosaceae. It is not clear whether a mechanism similar to that in Solanaceae and Rosaceae exists in the SI reaction in hyuganatsu, however, the reduced expression of F-box protein may induce an SI reaction.

S07P06

Hormone complex and micronutrients in production and fruit quality of 'Valencia' sweet orange

Galván J.J., López N., and Tolentino A.

UAAAN (UA), Horticultura, Saltillo. juan.galvan@uaaan.mx, doctorgalvanluna@hotmail.com

A complex hormone (Biozyme ® TF) was applied with micronutrients (Zinc poliquel and multi) at different concentrations and we evaluated the effects on both quality and performance of the fruit of 'Valencia' sweet orange in 20 year-old trees. Tree were located near the Eugenia Huerta Montemorelos Monterrey road Km 66, Gil de Leyva Montemorelos Nuevo Leon, Mexico, between 25° 11' 24" north latitude and 99° 41' 33" west longitude of Greenwich, with an altitude of 423 m and a rainfall of 600 to 1.000 mm. The prevailing weather was semi-warm with an annual average temperature of 18º C to 22º C and humid with rains intermediate in summer and winter. The variables evaluated were fruit weight (FW), equatorial diameter (DE), polar diameter (DP), thickness of the peel (GC), fruit firmness (F), % juice content (CJ), volume of juice (VJ), weight of juice (PJ), º Brix (GB), pH of the juice, citric acid % (AC), vitamin C, color ratio L * a * b * and number of seeds per fruit. The results show that treatment of Biozyme Poliquel Zn), positively affected the majority of the variables evaluted.

S07P07

Effects of Triclopyr (3,5,6-trichloro-2-pyridyloxyactetic acid) applications on fruit quality of SRA63, SRA85, SRA88 and SRA92 clementines

Zurru R.[1], Deidda B.[1], Dessena L.[2], and Mulas M.[2]

[1]AGRIS-Sardegna (AGRIS), Department of Wood and Fruit Tree Research, Italy; and [2]University of Sassari (DIPNET), Department of the Nature and Land Sciences, Italy. mmulas@uniss.it

In order to improve the yield and quality of clementine fruit, many treatments with hormonal compounds were carried out during the last 8 years in the south of Sardinia (Italy). The main objectives of the research

program were to increase fruit set, control fruit thinning, increase fruit size at harvest, reduce fruit drop during maturation, and to maintain on-tree fruit quality storage particularly of the peel. Four different cultivars of clementine were tested: 'SRA63', 'SRA85', 'SRA88', and 'SRA92'; as well as two rootstocks: sour orange and 'Carrizo' citrange. In this paper, the effects of Triclopyr (3,5,6-trichloro-2-pyridyloxyactetic acid), as an alternative to 2,4-D (dichlorophenoxyacetic acid), were evaluated for the control of the pre-harvest natural drop. Triclopyr treatments were more effective to control fruit drop and to protect the peel of the mature fruit when applied early on fruit of 18-22 mm of diameter (about the middle of July) with respect to treatments made at peel colour break time. This treatment in middle July alone determined the increase in fruit size and sometimes the total yield per tree. Lower yield was generally observed in trees grafted on sour orange than on 'Carrizo' rootstock. Triclopyr showed a strong thinning effect when applied to fruit of 6-13 mm of diameter, thus negatively affecting quantity and quality of the yield. On the contrary, Triclopyr treatments at peel colour break time allowed normal colour development.

S07P08

Foliar application effect of the 3,5,6-TPA on Tarocco red orange yield and fruit size in "on years" of eastern Sicily orchard

Tumminelli R., Marano G., Carta Cerella D., and Sinatra V.

Servizio Fitosanitario Regionale Siciliano (SFRSICILY), Quarantine and crop protection service, Italy.
riccardo.tumminelli@regione.sicilia.it

In the last four years (2009-2012), a method of reducing alternate bearing of red oranges (*Citrus sinensis*) of eastern Sicily was investigated. In 2009 and 2011, 3,5,6-trichloro-2-pyridyloxyacetic acid (3,5,6 TPA) treatments were tested for their ability to increase yield of commercially valuable large size red orange fruit (6 - 8 cm transverse diameter). Commercially bearing 15-year-old 'Tarocco' red orange scion on sour orange rootstock (*C. aurantium*) was used in this research. 3,5,6 TPA (30 g a.i./ha) plus 0.7% of paraffinic narrow range mineral oil were applied in 3000 L of water (pH 7.0) per ha with a low-profile air-blast sprayer to the foliage of one individual tree per treatment with 4 replicate trees per treatment when fruit averaged 10 mm transverse diameter. Four untreated trees served as the control. Averaged across the 4 years of the experiment, 3,5,6-TPA treatments in 2009 and 2011, reduced total yield and increased yield of commercially valuable large size fruit in the 'on' years 2010 and 2012, and increased total yield and decreased commercially valuable large size fruit in the 'off ' year 2011. Flower induction in 2012 was increased in treated trees in set fruit that will reach maturity during the 'off' year 2013.

S07P09

Fruit thinning of 'Okitsu' satsuma mandarin

Rivadeneira M.F[1], Gómez C.[1], and Silva-Muller W.[2]

[1]Instituto Nacional de Tecnología Agropecuaria. Estación Experimental Agropecuaria Concordia (INTA EEA Concordia), Entre Ríos, Argentina; and [2]Ando Cia. frivadeneira@correo.inta.gov.ar

The plant growth regulator naphthalene acetic acid (NAA) is used as a fruit thinning agent before the physiological drop stage of citrus fruit. Chemical and manual fruit thinning of 'Okitsu' satsuma mandarin (*Citrus unshui*) was studied in an experiment carried out at Concordia, Entre Ríos, Argentina. In the spring of 2010 and 2011, fourteen-year-old mandarin trees grafted on *Poncirus trifoliata* rootstock were sprayed with NAA (at 0, 100, 200 mg L^{-1}) 40 days after full bloom (DAFB). Manual thinning was done at 80 DAFB. Treatments were: control (0 NAA), manual thinning (MT), 100 mg L^{-1} NAA, 100 mg L^{-1} NAA with MT, 200 mg L^{-1} NAA and 200 mg L^{-1} NAA with MT. Fruit number, cross sectional shoot area, and fruit diameters were measured at 40, 70, 120 and 160 DAFB in two shoots per tree. Yield per tree was recorded at commercial harvest time in three trees per treatment. Physiological fruit drop at 70 DAFB was 20-26% and with NAA fruit drop was 30-48%. In the first year, there was no significant effect in yield per tree or diameter of fruit at harvest, although NAA increased the number of fruits with larger diameters. Chemical thinning might be a useful tool to improve commercial harvests in 'Okitsu' satsuma mandarin.

S07P10

Transcript accumulation of flowering genes in response to water deficit and gibberellins during floral induction in *Citrus sinensis*

Chica E.J., and Albrigo L.G.

University of Florida Citrus Research and Education Center (UF/IFAS CREC), Horticultural Sciences, United States. jchica@espol.edu.ec

Temperature, tree water status, crop load and gibberellins are the only factors known to modify floral induction in *Citrus sinensis*. Recently, several groups have characterized the accumulation of transcripts of flowering-related genes in response to temperature and crop load, but reports on the effect of tree water status and gibberellins on the expression of these genes are lacking. Here we report the patterns of accumulation of *CsFT*, *CsSL1*, *CsAP1* and *CsLFY* transcripts in response to water deficit and gibberellin treatments. Under water deficit, the accumulation of *CsFT* transcripts increased as the time under water deficit increased. Conversely, the accumulation of *CsSL1*, *CsAP1* and *CsLFY* transcripts was reduced while trees remained under water deficit. After the water deficit was interrupted, accumulation of *CsFT* transcripts returned to initial stress levels whereas the accumulation of *CsSL1*, *CsAP1* and *CsLFY* transcripts increased. A similar response was observed when trees were exposed to water deficit and floral-inductive temperatures. In general, application of gibberellins (GA3) reduced the accumulation of transcripts of all 4 genes. These results show that exposure to water deficit induces a response in the accumulation of transcripts of flowering-related genes similar to that induced by low ambient temperatures and that gibberellins partially inhibit the up-regulation of these genes.

S07P11

The effect of ethyclozate on tree water stress and fruit quality of satsuma mandarin

Okuda H.O[1], Ichinokiyama H.I.[2], and Noda K.N.[3]

[1]Faculty of bioresources, Mie University, Japan; [2]Mie prefecture agricultural research institute, Japan; and [3]Center for environment, health and field sciences, Chiba University, Japan. okudat@bio.mie-u.ac.jp

The fruit quality of satsuma mandarin strongly depends on drought stress during the maturation period. Most commercial orchards in Japan receive significant amounts of rain during the maturation period. To obtain the highest quality fruit, water vapor-permeable sheets are being used to cover the ground (mulching) in approximately 20 percent of the very early satsuma mandarin orchards in Japan. However, such sheets are ineffective in citrus fields converted from paddy fields. Chemicals, such as ethyclozate, might be used to improve fruit quality as a substitute for mulching for applying water stress because this chemical could reduce water uptake through roots. This effect was notable only in young trees but there was no report of effects in mature trees. In this experiment, seasonal changes in the volumetric water content (VWC) in the trunk of mature trees were monitored along with the effects of the chemical. The results suggested that VWC decreased in the treated trees from early July immediately after treatment until early September. In addition, coloring began to occur one week earlier, and the brix increased by about one degree at harvest. These results suggest that the application of ethychlozate in early summer might play a role for applying water stress to improve fruit quality.

S07P12

Relationship between flower intensity, oxidative damage and protection in citrus under water stress conditions

Manzi M.[1], Borsani O.[2], Díaz P.[2], and Rivas F.[3]

[1]Universitat Jaume I (UJI), Ciencias Agrarias y del Medio Natural, Spain; [2]Facultad de Agronomía - Universidad de la República (FAgro - UdelaR), Biología Vegetal, Uruguay; and [3]Instituto Nacional de Investigación Agropecuaria (INIA), Programa Nacional de Investigación en Producción Citrícola, Uruguay. mmanzif@gmail.com

Stress conditions are known to promote reproductive sprouting and flowering in citrus. However, signalling involved in stress perception and flowering induction remains obscure. To gain insight into the mechanism

involved in stress signalling, the aim of this work was to study the physiological and biochemical changes triggered by water stress and their relationship with budbreak in 'Valencia' sweet orange (*Citrus sinensis*) and 'Ellendale' tangor (*C. reticulata* x *C. sinensis*). Photoinhibition was promoted by subjecting plants to 70-days of water deficit (water potential: Ψw <-4.0 MPa), thereby increasing photoinhibition (decreasing *Fv*/*Fm*, ΦPSII and increasing *Fo)*. An increase in oxidative damage was achieved. Water stress raised proline content in both cultivars and was higher in 'Ellendale'. After rewatering, plants of both cultivars increased sprouting and flowering. Under these conditions, 'Valencia' trees produced 55% more flowers (160 flowers/100 nodes) than 'Ellendale'. This was explained by an increase in multiflowered shoots. These results suggest that citrus flowering in response to stress conditions is variety dependent and that the active compounds that increased in response to stress such as proline, are positively correlated with flowering intensity. Overall, the intensity of oxidative damage in citrus leaves during stress could act as an indicator of flowering intensity in each variety.

S07P13

The influence of climate on the components of yield and quality for Cuban citrus fruits. Adaptation to climate change.

Pérez M.C.[1], Almenares G.[2], Betancourt M.[2], Aranguren M.[2], García M.E.[2], Sistachs V.[3], Núñez M.[4], Torres W.[4], Oliva H.[2], Noriega C.[2], and Llauger R.E.[5]

[1]Instituto Nacional de Ciencias Agrícolas (INCA), Dirección General, Cuba; [2]Instituto de Investigaciones en Fruticultura Tropical (IIFT), Fisiología, Cuba; [3]Universidad de La Habana (UH), Facultad de Matemáticas, Cuba; [4]Instituto Nacional de Ciencias Agrícolas (INCA), Fisiología, Cuba; and [5]Instituto de Investigaciones en Fruticultura Tropical (IIFT), Dirección General, Cuba. mcperez@inca.edu.cu

A study to determine the influence of climate on the components of yield and quality of citrus fruits to predict production and fruit quality in the present and future growing environments, was conducted in Valencia Late, Washington navel orange and Marsh Seedless grapefruit. The following were evaluated: flowering, fruit-set, fruit growth, fruit development and internal quality. All variables were correlated to the meteorological variables and climate decisively influenced on the components of yield and internal fruit quality. The beginning of flowering was directly related to the spectrum of extreme temperatures and inversely related to rainfall and to mean, minimum and maximum air temperatures. Meteorological conditions that favored flower induction retarded the beginning of flowering; the duration of flowering was subject to mean temperature. During fruit-set, the highest loss of reproductive structures occurred under water stress periods. During fruit growth and development, mean and maximum high temperatures prior to the 121-180 days of fruit development, caused a reduction of the fruit equatorial diameter, fresh weight and juice percentage but higher acidity content. The stage II of fruit growth and development was sensitive to water stress with implications on fruit quality. Predictive models were developed for fresh weight and juice content according to meteorological variables for the present and future growing environments subject to climate change.

Session 08

ABIOTIC STRESS

S08O01

Polyamines: a key player in stress response

Liu J.-H.

Key Laboratory of Horticultural Plant Biology, Ministry of Education, College of Horticulture and Forestry Sciences, Huazhong Agricultural University, Wuhan, China. liujihong@mail.hzau.edu.cn

Polyamines, mainly putrescine, spermidine and their diamine precursor spermine, are polycationic low-molecular-mass aliphatic amines that are ubiquitously distributed in living organisms. Polyamines have been shown to be closely implicated in plant biotic and abiotic stress response. In the last years we have carried out work to identify the role of polyamines in stress response of citrus or its closely related genus. The major work is following. First, analysis of polyamine levels in different tissues under stressful condition. Second, investigation of exogenously applied polyamines on the enhancement of dehydration tolerance. Third, molecular cloning and functional characterization of polyamine biosynthetic genes, such as *arginine decarboxylase* (*ADC*) and *S-adenosylmethionine decarboxylase* (*SAMDC*). Last, polyamine biosynthetic genes were used for genetic engineering in an effort to producing novel germplasms with enhanced stress tolerance. Our data showed that *PtADC* from *Poncirus trifoliata* was upregulated by different types of stresses, including salt, low temperature, dehydration. Ectopic expression of *PtADC* gene conferred enhanced tolerance to different stresses in transgenic plants. All these results show that polyamines are important players that can be manipulated to increase stress tolerance in citrus.

S08O02

'*Omics*' and chemical approaches used to monitor iron-deficiency in citrus rootstocks

Licciardello C.[1], Muccilli V.[2], Torrisi B.[1], Tononi P.[3], Fontanini D.[4], Allegra M.[1], Sciacca F.[1], Foti S.[2], Delledonne M.[3], Intrigliolo F.[1] and Reforgiato Recupero G.[1]

[1]Research Centre for Citriculture and Mediterranean crops (CRA-ACM), Italy; [2]University of Catania, Department of Chemical Science, Italy; [3]University of Verona, Department of Biotechnology, Italy; and [4]University of Pisa, Department of Biology, Italy. concetta.licciardello@entecra.it

Two different '*omics*' approaches were performed to a better comprehension of biological mechanisms involved in citrus iron deficiency. Tips roots from 'Swingle' citrumelo and 'Carrizo' citrange (sensitive and tolerant rootstocks, respectively), growing in pots with control and chlorotic soil, were used for transcriptomic and proteomic analysis. CombiMatrix array was performed to isolate differential genes, among which *glutathione peroxidase* and *SAUR* gene showed to be the most involved ones. They were switched on 'Swingle' citrumelo grown on calcareous conditions compared to 'Carrizo' citrange (in the same soil) and to the same stock in the control soil. The over-expression of peroxidase could be the effort of plants to neutralize the oxidative environment produced by stress. The involvement of auxin in the regulation of Fe deficiency responses is also well known. Both genes, together with peroxidase and ferric chelate reductase activities and iron and chlorophyll content, were used as markers to monitor the degree of suffering on seven tolerant and sensitive rootstocks growing on the field under natural chlorotic conditions. Among differentially expressed proteins, isolated using 2D-PAGE and RP-HPLC/nESI-MSMS, a strong down-regulation of cytosolic pyrophosphate-dependend phosphofructokinase beta subunit and NADPH-isocitrate dehydrogenase could produce plant inability to sustain the energetic request of cell roots.

S08O03

Root protein interatomic network obtained from citrus seedlings subjected to water deficit

Magalhães de Oliveira T.[1], Silva F.[2], Morillon R.[3], Coelho Filho M.A.[4], Neves D.M.[1], Costa M.G.C.[1], Pirovani C.P.[1] and Bonatto D.[2]

[1]Universidade Estadual de Santa Cruz (UESC), Brasil; [2]Universidade Federal do Rio Grande do Sul (UFRGS), Brasil; [3]CIRAD, France; and [4]Embrapa Mandioca e Fruticultura, Brasil. tmaga21b@gmail.com

Citrus Brazilian industry may be strongly affected by drought. Characterize rootstock behavior under different environmental conditions is crucial, since rootstocks may confer better adaption to environmental stresses. Plant responses to drought require the production of important functional and regulatory proteins. In the

present study, we identified proteins related to water deficit response in tolerant ('Rangpur' lime, *Citrus limonia*) and susceptible ('Sunki Maravilha' mandarin, *Citrus sunki*) genotypes. From 105 differentially expressed proteins, twenty-nine proteins from roots of both genotypes were identified and sequenced by mass spectrometry. Protein interaction analysis of orthologous proteins from *A. thaliana* protein expressed in the two citrus varieties was obtained by ontological and cluster analysis.Among the 16 amino acid sequences found in 'Rangpur' lime and 'Sunki Maravilha' mandarin, 13 proteins were found in *A. thaliana* via BLAST, of which, 12 showed protein-protein interaction. It is possible to group these 12 proteins into a single network interatomic, comprising 3302 proteins. Among the different biological processes found in the protein networks, regulation of response to water deficit and osmotic stress, signaling pathway of abscisic acid were highlighted. A point to take into account is the importance role of the DREB2, a protein hub/bottleneck which participate in the stimulus and the response to water stress.

S08O04

Hormone and metabolite traits related to abiotic stress tolerance in citrus

Arbona V., de Ollas C., Argamasilla R., López-Climent M.F. and Gómez-Cadenas A.

Universitat Jaume I (UJI), Dept. Ciències Agràries i del Medi Natural, Spain. arbona@uji.es

Plants of the citrus rootstocks 'Carrizo' citrange (CC, soil flooding tolerant) and 'Cleopatra' mandarin (CM, drought tolerant) were subjected to water stress and soil flooding conditions to study root responses associated to tolerance. Responses could be classified in groups which different involvement in plant tolerance. Some of them, such as the increase in proline levels, were common to the two species and the two stress conditions assayed. Other, as the abscisic acid and jasmonic acid signals or the phenylpropanoid profiles followed similar trends in both species varying with the stress imposed. Finally, other responses as the increase in salicylic acid were specific of each genotype and stress situation. Moreover, following a metabolomics approach, it was showed that the altered compounds in response to each adverse condition had a low degree of overlapping which accounts for the specificity of the plant response to the different environmental restraints. Finally, under control conditions, different metabolites had higher levels in CM than CC which suggested that pre-existent defenses are important as a stress tolerance trait.

S08O05

The adaptation of 'Sunki Maravilha' mandarin to drought depends on the ABA accumulation balance between roots and canopy

Neves D.M.[1], Coelho Filho M.A.[2], Bellete B.S.[3], Silva M.F.G.F.[3], Souza D.T.[4], Soares Filho W.S.[4], Costa M.G.C.[1] and Gesteira A.S.[4]

[1]Universidade Estadual de Santa Cruz (UESC), Departamento de Ciências Biológicas, Brasil; [2]Embrapa Mandioca e Fruticultura (CNPMF), Renai, Brasil; [3]Universidade Federal de São Carlos (UFSCar), Departamento de Química, Brasil; and [4]Embrapa Mandioca e Fruticultura (CNPMF), Nugene, Brasil. asgesteira@gmail.com

Abscisic acid (ABA) is an important plant hormone that participates in the adaptation to many abiotic stresses such as drought. The 9-*cis*-epoxycarotenoid dioxygenase (*NCED*) is the key enzyme of ABA biosynthesis in higher plants. The importance of ABA in two citrus varieties of rootstock, the 'Rangpur' lime and the 'Sunki Maravilha' mandarin, cultivated on hydric restriction by expression analysis of five *NCED* genes and correlation with the accumulation of ABA were evaluated. In the 'Rangpur' lime the profile of ABA accumulation ratifies that perception and induction of adaptation signals against water stress occurs from the roots and leads to a decrease in transpiration. However, this decrease is not sufficient to reduce the water flow from roots to canopy keeping its active growth even in conditions of low water availability. Instead, the adaptation of 'Sunki Maravilha' mandarin depends on the balance of ABA accumulation between root and canopy which increases with water restriction, inducing a decrease in transpiration rate and water flow from roots to canopy. Under drought, water requirement for 'Sunki Maravilha' mandarin decreases and it adapts to a lower availability of water in the soil. The use of this mandarin in combination with a scion variety with characteristics similar to its own, that responds to stress by water deficit with ABA accumulation in leaves, may have good drought tolerance under field conditions. In this study we correlated the *NCEDs* with ABA accumulation.

S08O06

Roots are necessary for the responses of *in vitro*-cultured citrus plants to high salinity but not to osmotic stress

Pérez Clemente R.M., Montoliu A., Vives-Peris V., Espinoza V., Zandalinas S.I. and Gómez-Cadenas A.

Universitat Jaume I (UJI), Dept. Ciències Agràries i del Medi Natural, Spain. rclement@uji.es

In the field, plants are exposed simultaneously to variable biological and environmental conditions that can make physiological studies very difficult. The *in vitro* tissue culture techniques can overcome some of these limitations. In the present work, this methodology was applied to the study of salt and osmotic stress conditions on 'Carrizo' citrange. The stress conditions were generated by adding either NaCl or polyethylene glycol, to the culture medium. Micropropagated shoots, growing under salt- or osmotic-stress, shown symptoms of leaf damage very similar to those found in intact plants, which confirmed the incidence of the imposed stress on plant physiology. In whole plants, it has been reported that physiological responses to water and salt stress are essentially identical; on the contrary, in shoots cultured *in vitro*, levels of stress markers such as malondialdehyde and proline incrased only under water deficiency but not under elevated salt conditions Differences were also observed in the hormonal regulation of the shoots subjected to each abiotic stress condition. Abscisic acid concentration increased in shoots grown under osmotic stress conditions whereas no differences with the controls were observed in salt-stressed ones. It can be concluded that, at least when culture *in vitro*, citrus roots are necessary for the perception and signaling of the salt stress conditions. On the contrary, the presence of this organ is not necessary to modulate the response of shoots to osmotic stress.

S08O07

Physiological analysis of salt stress behaviour of citrus species and genera: low chloride accumulation as an indicator of salt tolerance

Hussain S.[1], Luro F.[2], Costantino G.[2], Ollitrault P.[3] and Morillon R.[3]

[1]Bahauddin Zakariya University Multan, Department of Horticulture, Pakistan; [2]INRA Unité de Recherche 1103 Génétique et Ecophysiologie de la Qualité des Agrumes, France; [3]Unité Mixte de Recherche Amélioration génétique et Adaptation des Plantes, CIRAD - Instituto Valenciano de Investigaciones Agrarias (IVIA) Equipe Amélioration des Plantes à Multiplication Végétative, Spain. sajjad_h@yahoo.com

Tolerant citrus rootstocks are defined as Cl- excluders. However, little is known about the salt tolerance of cultivars used as scions, particularly the tolerance of monoembryonic citrus genotypes. To enhance the genetic resources for generating improved hybrid rootstocks, the evaluation of large samples of citrus species, including both monoembryonic and polyembryonic genotypes, is necessary. In this study, 12 citrus genotypes representing the major *Citrus* species and all the three genera of the *Rutaceae* family were subjected to moderate salt stress (75mM) for 12 weeks to characterise their physiological response to salt stress. Various symptoms and physiological parameters were evaluated to characterise their salt sensitivity. These included plant growth (stem diameter), leaf chlorophyll content, leaf flavonoid content, maximum quantum yield of PSII [*(Fm-F0)/Fm*)], net photosynthesis, stomatal conductance and leaf Na and Cl- contents. The results clearly demonstrated that the most salt sensitive genotypes accumulated high concentrations of Na and Cl- and maintained a fair growth and photosynthetic rate. By contrast, salt-tolerant genotypes accumulated less Na and Cl- and decreased their growth and gas exchange. 'Poncire commun' citron and 'Marumi' kumquat were the most sensitive species, while mandarins, pummelo and 'Australian' sour orange were the most tolerant species. Among the genotypes, 'Engedi' pummelo presented a specific trait for salt tolerance that has not been previously reported. Taken together, the results suggest that low leaf chloride content can be used as an indicator of salt stress tolerance in citrus genotypes. Exploitation of this indicator will enable the improved evaluation of citrus genetic resources and should lead to the identification of new sources of tolerance for rootstock breeding.

S08O08

Microsprinkler irrigation for frost protection of citrus in Florida

Parsons L.R.

University of Florida (UF CREC), Citrus Research & Education Center, USA. lparsons@ufl.edu

In 1979, it was not known if microsprinkler irrigation could provide any frost protection for citrus, and some assumed that this irrigation would not. Tests during freezes between 1980 and 2010 showed that microsprinklers were effective in protecting the lower 0.9 m of young citrus trees, thereby saving the bud union and allowing later regrowth. Microsprinklers also benefit mature trees. The amount of air temperature warming depends on several factors, including volume of water applied & wind speed. Microsprinkler irrigation is more effective for cold protection when higher volumes of water are applied. Irrigation rates of 18.7 m3 ha^{-1} hr^{-1} have been recommended. At these rates, average air temp warming at a 1.3 m height is 1 to 1.5°C. At tree heights of more than 2.6 m, warming is commonly less than 0.5°C. In windy, low humidity freezes, evaporative cooling can damage trees, particularly if the emitter is on the downwind side of the tree. Protection is better if the microsprinkler is placed on the upwind (northwest in Florida) side of the tree. Microsprinklers elevated to a height of 0.6 to 1 m protect more of the tree than those at 0.2 m. While it was initially thought that microsprinklers provided no frost protection to the fruit, recent research has shown that this irrigation can promote better production of kg of soluble solids than when no irrigation is used. Microsprinkler irrigation has become the most commonly used form of frost protection in Florida citrus.

S08P01

Response of 'Washington' navel orange plants to application of Helpstar and Saltrad as saline correctors

El-SHazly S.M.[1], Eisa A.M. [1] and Sarhan Z.H.[1]

[1]Faculty of Agriculture, Alexandria University, Pomology Department, Egypt. samyshazly55eg@yahoo.com

The aim of this study is to evaluate the effect of different salinity levels (zero, 1500, 3000 and 4500 ppm NaCl) and salinity correctors Saltrad (CaO) and Helpstar (K_2O) at (zero, 5 and 7.5 cm^3 / pot / month) on vegetative growth parameters, and leaf and root chemical composition of 'Washington Navel' orange plants. The results indicated that all vegetative growth parameters (growth rate, plant height, stem diameter, root length, volume index and trunk cross sectional area), leaf chlorophyll content, leaf peroxidase activity and leaf and root contents of carbohydrates, N, P, K, Ca and Mg have decreased, meanwhile leaf and root proline and Na and Cl contents have increased with salinity increments. Salinity correctors enhanced all vegetative growth parameters, chlorophyll, peroxidase activity, proline, carbohydrates and N, P, K, Ca and Mg contents, meanwhile Na and Cl were reduced. Generally, it can be concluded that using Saltrad at 7.5 cm^3 /pot / month gave the highest values of vegetative growth parameters and leaf and root chemical composition. Due to lack of significance – in most parameters- between Saltrad treatments at 5 and 7 cm^3 /pot, we recommend the use of Saltrad at 5 cm^3 /pot / month to reduce the negative impact of salinity on 'Washington' navel orange plants.

S08P02

Effects of salinity on some citrus rootstocks

Yeşiloğlu T.[1], İncesu M.[1], Yılmaz B.[1], Tuzcu Ö.[1], Uysal Kamiloğlu M.[2] and Çimen B.[1]

[1]University of Cukurova (CU), Department of Horticulture, Turkey; and [2]University of Mustafa Kemal (MKU), Department of Horticulture, Turkey. meralincesu@gmail.com

Citrus is a major horticultural crop worldwide and known as relatively susceptible to salinity. Salt damages are usually displayed as leaf burn and defoliation and are associated with accumulation of toxic levels of Na and/or Cl^- in leaf cells. Reactions against these toxicities depend on the rootstock used. In this study we have evaluated 'Tuzcu 891' sour orange, 'Swingle' citrumelo, 'Volkameriana', 'Rubidoux' trifoliate and

'Citremon' in terms of salinity. The experiment was carried out in a greenhouse. The treatments were control [nutrient solution with electrical conductivity [EC(w) of 0.41 dS m($^{-1}$)] and salinity [nutrient solution with EC(w) 6.0 dS m($^{-1}$)] in a randomized block design with -thirty replicants -during 90 days period. Thus shoot and root dry weights, leaf and root potassium (K) concentrations, leaf and root calsium (Ca) concentrations, leaf and root chloride (Cl) concentrations, leaf and root sodium (Na) concentrations and symptoms in leaves were observed. Although root dry weight did not differ among those rootstocks, significant differences were determined in K, Ca, Cl, Na concentrations and shoot dry weights.

S08P03

Salt stress tolerance in acidic and sweet mandarins

Ben Yahmed J.[1], Ben Mimoun M.[2], Talon M.[3], Ollitrault P.[1] and Morillon R.[1]

[1]Centre de coopération internationale en recherche agronomique pour le développement (CIRAD), Amélioration des Plantes à Multiplication Végétative, France; [2]Institut National Agronomique de Tunisie (INAT), Tunisie; and [3]Centro de Genomica, Instituto Valenciano de Investigaciones Agrarias (IVIA), Spain. jihene.benyahmed@gmail.com

Mandarin genotypes can be split in two main groups: the acidic and the sweet mandarins. Acidic are used as rootstocks whereas sweet mandarins are the varieties that are consumed for fruits. The acidic 'Cleopatra' mandarin (*Citrus reshni*) is considered to be the most salt stress tolerant rootstock since it is able to limit the absorption of chloride and sodium at root level. As a consequence, limited amount of toxic ions are translocated to leaves of the seedling or the variety grafted onto the rootstock. If salt stress tolerance of 'Cleopatra' mandarin and its hybrids has been quite documented, little data exist for sweet mandarins. We investigated 16 genotypes representing the large diversity of mandarins when subjected to salt stress. Physiological parameters such as gas exchanges, quantum yield of PSII electron transport, osmotic pressure and leaf chloride contents were analyzed. Samples were also harvested for molecular and biochemical analysis. Very different behaviors were observed. If most of the acidic varieties were considered to be tolerant, also some sweet varieties showed traits of tolerance. Taking account of the results of leaf ion contents, we observed that large leaf chloride contents were not always associated with sensitivity. Molecular analyses are going on to decipher the origin of the tolerance in these genotypes.

S08P04

Changes in transcriptional profiles of mature and immature citrus leaves acclimated to salinity

Pérez-Pérez J.G.[1], Talón M.[2], Brumós J.[2], Botía P.[1] and Colmenero J.M.[2]

[1]Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA), Department of Citriculture, Spain; and [2]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Genómica, Spain. juang.perez@carm.es

While the molecular response of salinized plants in the short-medium term (hours-days) has been broadly studied, the knowledge about the nature of the genes involved in maintaining homeostatic conditions the long term (months-years) has remained elusive. With this aim, we have analyzed the transcriptome of leaves from citrus plants acclimated to moderate salinity (2 years with NaCl 30 mM). Through functional genomics, using the 7K cDNA chip from "Consorcio Valenciano de Genómica Funcional de Cítricos", the transcriptome of citrus plants acclimatized to salinity was analyzed in mature leaves (8 months) and immature leaves (2 months). Although immature leaves accumulated low levels of chloride (0.51%±0.06), they exhibited high responsiveness to salinity (1,211 differential-responsive genes) compared with mature leaves, which cumulated higher chloride levels (1.05% ± 0.01), and showed a lower number of differentially-responsive genes (100 genes). Immature leaves induced functional categories that were not induced in mature leaves, like "cell wall biosynthesis", "metabolism", "defense", "secretion and membrane traffic", "water transport" and "antioxidant activity". On the other hand, the degree of coincidence was higher in the group of genes that were repressed by salinity. Both mature and immature leaves repressed genes mostly involved in "stress response". We highlight how the same plant organ in different developmental stages show huge differences in the amount and nature of genes which are responding to an abiotic stimulus.

S08P05

Role of ammonium nutrition on salt-induced oxidative stress in 'Carrizo' citrange plants

Fernández-Crespo E.[1], Gómez-Pastor R.[2], Matallana E.[2], Llorens E.[1], Lapeña L.[1], Scalschi L.[1], Camañes G.[1] and García-Agustín P.[1]

[1]Universitat Jaume I (UJI), Ciencias Agrarias y del Medio Natural, Spain; and [2]Universitat de Valencia (UV), Bioquímica y Biología Molecular, Spain. ellorens@uji.es

Salinity is amongst the most significant environmental factors responsible for substantial losses in agricultural production worldwide and it is a critical problem especially in citrus since they are one of the most globally important horticultural crops considered as salt sensitive. Salt stress has two effects on plants, an initial osmotic shock followed by a toxic phase due to ion accumulation. Moreover, a high cellular NaCl concentration enhances accumulation of reactive oxygen species (ROS). To avoid such salt stress-derived injuries, tight regulation of ROS homeostasis is necessary and is provided by a network through complex loops between oxidants and scavengers. NH4is a paradoxical nutrient ion because it is a main N source, but high concentrations of this ion in the soil may cause damaging effects. In this study, we have analyzed the influence of NH_4 nutrition on 'Carrizo' citrange plants undergoing 90 mM NaCl. Plants were grown with 1 mM NH_4NO_3 (control) and 5 mM N-NH_4. To clarify the relationship between the antioxidant mechanisms induced by ammonium nutrition and salt tolerance, we analyzed enzymatic antioxidant activities (SOD, CAT, GR), ascorbate and glutathione concentrations and antioxidant properties of proline and putrescine. 'Carrizo' plants showed optimal growth in both treatments and we confirmed that N-NH_4treatment enhance resistance to salt stress. Based on this evidence, we hypothesized that N-NH_4treatment triggers mild chronic stress in 'Carrizo' which could prime plant defenses by stress imprinting, thus conferring plant resistance. Moreover, subletal concentration of ammonium could act as a mild oxidative stressor triggering antioxidant cellular machinery against subsequent salt stress.

S08P06

Characterization of the *Arum*-type mycorrhiza in *Citrus macrophylla* rootstock under salinity stress

Rodríguez Morán M.[1], Navarro J.M.[1] and Morte A.[2]

[1]Departamento de Citricultura, IMIDA, Murcia 30150, Spain; and [2]Departamento Biología Vegetal y Botánica, Facultad de Biología, Universidad Murcia 30100, Spain. ringil_1@hotmail.com

Arum and *Paris* mycorrhizas are two major morphological classes of arbuscular mycorrhizal (AM) symbiosis which differ in fungal structures. AM symbiosis is thought to increase host resistance to salinity stress, a characteristic that could be interesting in areas where the scarcity of irrigation waters force to growers to use low-quality irrigation water. The type of AM colonization was analyzed in order to study if it is influenced by saline conditions. Seedlings of *Citrus macrophylla* (CM) were inoculated with a mixture of two AM fungi. Ninety-six days after inoculation, plants were irrigated with Hoagland modificated solution containing 0, 20, 40, 60 and 80 mM NaCl. CM showed an *Arum*-type mycorrhiza. Epidermical cells were never colonized and, the infection developed longitudinally and radially in the cortex of the root by many intercellular hyphae. Branches from intercellular longitudinal hyphae gave rise to arbuscules in the cells. These arbuscules were scattered in the cells of the medium and inner cortex, with the outer cortical cell layers relatively free of intracellular fungal structures. The vesicles were also located in the intercellular spaces of the same cortical cell layers. Most of the mycorrhizal roots under salt treatments showed an increasing number of vesicles respect to mycorrhizal roots without salt treatment. Under salinity stress, no differences were observed in the mycorrhizal colonization percentages. This could explain the observed increasing tolerance of mycorrhizal plants under high level of salinity.

S08P07

In vitro screening of four genotypes of citrus for salt tolerance

Chetto O.[1], Dambier D.[2], Fadli A.[1], Talha T.A.[3], Benkirane R.[1] and Benyahia B.H.[3]

[1]Ibn Tufail University (UIT), Morocco; [2]Center for International Cooperation in Agronomic Research for Development (CIRAD), Genetic Improvement of Vegetatively Propagated Species, France; and [3]National Institute of Agronomic Research (INRA), Laboratory of Citrus Improvement and Biotechnology, Morocco. ouiam_ch@hotmail.com

Soil and water salinity is an important abiotic stress that can affect both production and quality of citrus. The use of tolerant rootstocks in these conditions would be the solution the most used. The objective of this study

was to compare *in vitro* behavior of four citrus genotypes in terms of tolerance to salinity. The genotypes used were 'Cleopatra' mandarin, 'Chios' mandarin, 'Shamouti' orange and 'Star Ruby' grapefruit. Salt tolerance was evaluated by determination of callus and suspension cells growth, the fresh and dry weight, water content and chloride ion content after one month of culture in solid and liquid media supplemented with NaCl at different concentrations: 50, 100, 150 and 200 mM. At 0 mM NaCl, the results revealed that callus of the four genotypes tested have a similar behavior in term of tolerance to salinity as the suspension cells. However, a differential behavior was observed at high salt concentrations depending on genotypes and the nature of medium.

S08P08

Screening of ten citrus rootstocks for salt tolerance at seedling stage

Fadli A.[1], Chetto O.[1], Talha A.[2], Beniken L.[2], Benkirane R.[1] and Benyahia H.[2]

[1]Ibn Tofail University (UIT), Department of Plant Science, Kenitra, Morocco; and [2]Kenitra Regional Center for Agricultural Research (CRRAK), Department of Plant Breeding and Phytogenetic Ressources Conservation, Kenitra, Morocco. a.fadli.uit@gmail.com

Salinity affecting 35% of Moroccan irrigated soils represents a serious problem for citrus production in these areas. Furthermore, the wide spread of Tristeza disease in the mediterranean region will soon limit the use of sour orange which is a salt tolerant rootstock commonly used in Morocco. Since then, the selection of new salt tolerant rootstocks has become an important research area for citrus breeding programs. In this study, nine citrumelo (C.) rootstock lines i.e. 'C. 57-98-502', 'C. 57-98-506', 'C. 4475 BB6A9', 'C. 4475 AB6A4', 'C. 4475 B2.G3', 'C. sacaton 30057', 'C. Swingle 74.1', 'C. Swingle F.9.22.5 (80.1)' and 'C. Winter Haven B231431' were evaluated for salinity tolerance by applying a fast test of screening. The seedlings of these rootstocks were grown in greenhouse conditions and treated for two months with a saline solution containing NaCl at 0, 35 and 85 mM concentrations. 'Rangpur' lime was included in the experiment as control. The results showed a wide variation of response. At high level of salinity (85 mM), 'C. Winter Haven' maintained a higher growth, higher number of leaves and higher leaves water content than the other rootstocks studied and manifested less toxicity symptoms (necrosis, defoliation of leaves). However, in the same concentration, 'C. Swingle 74.1' and 'C. B2.G3' proved to be more performants respectively in regards to leaves chlorophyll and chloride contents. In general, the study suggested that the salt tolerance of the ten rootstocks can be classified in the following ascending order : 'C. B2.G3', 'C. 4475 BB6A9', 'C. sacaton 30057', 'C. 57-98-506', 'C. 57-98-502', 'C. Swingle 74.1', 'C. Swingle F.9.22.5', 'C. 4475 AB6A4', 'C. Winter Haven B231431', 'Rangpur' lime.

S08P09

Physiological response of *Citrus macrophylla* inoculated with arbuscular mycorrhizal fungi under salt stress

Navarro J.M.[1], Morte A.[2], Rodríguez Morán M.[1] and Pérez-Tornero O.[1]

[1]Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA), Citriculture, Spain; and [2]Universidad de Murcia. Facultad de Biología Vegetal y Botánica, Spain. josefam.navarro2@carm.es

Seedlings of *Citrus macrophylla* were inoculated with arbuscular mycorrhizal (AM) fungi, or left non-inoculated (-AM). Forty-five days after inoculation onwards, half of the AM and -AM plants were irrigated for three months with a nutrient solution containing 50 mM NaCl. Due to the high sensitivity of *C. macrophylla* to salinity, AM inoculation only partly compensates the growth limitations imposed by salinity. The high intercellular CO2 and the oxidative stress levels suffered by -AM plants decreased with AM inoculation. Although salinity strongly decreased photosynthesis in -AM plants, AM colonization alleviated this decrease in AM plants due to the higher total chlorophylls than in -AM. However, the chlorophyll and photosynthesis levels found in AM under salinity were still lower than in control treatments, so AM did not completely compensate for the negative effect of salinity on the photosynthetic response. Although AM significantly increased the water percentage in leaves, it did not modify the water potential in AM or -AM plants. Mycorrhizal inoculation increased the osmotic potential and consequently decreased turgor. However, this decrease did not affect the physiological processes that depend of leaf turgor such as photosynthesis, which even increased in AM plants. Given that, in the saline conditions used, AM plants had higher leaf Cl levels than -AM plants but showed better growth and a better physiological response, leaf Cl concentration was not related with salt tolerance in this experiment.

S08P10

Cloning and functional analysis of stress-responsive genes in *Poncirus trifoliata*

Liu J.H., Wang J., Huang X.S. and Sun P.P.

Huazhong Agricultural University, College of Horticulture and Forestry Science, China. liujihong@mail.hzau.edu.cn

Poncirus is a widely used rootstock for citrus because it has several desirable attributes, such as cold hardiness and Citrus Tristeza Virus tolerance. However, it is not drought and salt tolerant. On the other hand, most of citrus commercial cultivars are not cold tolerant. Therefore, it is necessary to obtain novel germplasms of either rootstock or scion cultivars with enhanced stress tolerance. As a complementation to the traditional breeding, genetic transformation has been proven as an effective approach to generate plants with enhanced stress tolerance. To make this new approach applicable to citrus cultivar improvement, it is obligatory to clone and functionally characterize genes involved in stress response. During the last years we have made efforts to isolate several functional or regulatory genes involved in stress response from trifoliate orange, including *PtADC*, *PtrABF* and *PtrMAPK*. Function of the genes in stress tolerance was characterized using *Arabidopsis* or tobacco transformation. For example, overexpression of *PtADC* in *Arabidopsis* led to enhanced tolerance to osmotic stress, drought and cold stress. Interestingly, transgenic plants exhibited longer roots and smaller stomatal density. Transformation of *PtrAFB* and *PtrMAPK* in tobacco resulted in elevated drought tolerance, accompanied by the induction of stress-responsive genes and enhanced antioxidant system for ROS scavenging. These genes hold great potential for genetic transformation in citrus or trifoliate orange.

S08P11

Characterization of water deficit tolerance of *Poncirus trifoliata* genotypes as related diversity

Ben Yahmed J.[1], Costantino G.[2], Ben Mimoun M.[3], Talon M.[4], Ollitrault P.[1], Morillon R.[1] and Luro F.[2]

[1]Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Amélioration des Plantes à Multiplication Végétative, France; [2]Institut National des Recherches Agronomiques (INRA), France; [3]Institut National Agronomique de Tunisie (INAT), Arboriculture Fruitiere, Tunisie; and [4]Centro de Genómica, Instituto Valenciano de Investigaciones Agrarias (IVIA), Spain. jihene.benyahmed@gmail.com

Rootstock provides better adaptation to biotic and abiotic constraints. *Poncirus* and its hybrids are widely used since they are tolerant to Citrus Tristeza Virus. However, when grafted, most of them are considered to be sensitive to salinity and water deficit. Diversity does exist within the *Poncirus trifoliata* specie but little is known regarding the behaviour variability of genotypes to abiotic constraints. The citrus diversity of 72 *P. trifoliata* genotypes was investigated using SSR markers. Two mayor genetic groups were clearly identified. Five genotypes, belonging to each genetic group, were then selected to investigate their properties of tolerance to water deficit. Water deficit was applied by withdrawing irrigation for 4 weeks. Physiological parameters such leaf stomatal conductance and quantum yield of PSII electron transport, soil water potential, leaf osmotic pressure and transpiration were estimated. Among genotypes, we observed that some genotypes such as' Rubidoux' were clearly more tolerant to water deficit than others such as 'Pomeroy'. Interestingly, the genotypes that were sensitive belonged to one genetic group and the tolerant ones belonged to the other group. Therefore, it sounds that among the *Poncirus* genus, it is possible to select genotypes as parent in breeding programs, which are more tolerant to water deficit.

S08P12

Screening nine citrumelo rootstocks for tolerance to drought conditions

Fadli A.[1], Beniken L.[2], Omari F.E.[2], Benkirane R.[1] and Benyahia H.[2]

[1]Ibn Tofail University (IUT), Department of Plant Science, Kenitra, Morocco; and [2]Kenitra Regional Center for Argricultural Research (CRRAK), Department of Plant Breeding and Phylogenetic Ressources Conservation, Kenitra, Morocco. a.fadli.uit@gmail.com

Two months old citrumelo rootstocks groups seedlings were grown in 0.5 L plastic pots in a greenhouse at El Menzeh (CRRA Kenitra), INRA Morocco, under three moisture regimes (100%, 75% and 50% Hfc) to investigate the effects of water stress on their morphological and physiological characteristics such as plant height, number of leaves per plant, fresh matter accumulation, transpiration ratio, chlorophyll content (SPAD), soluble solids and proline content, respectively. A split plot design with ten citrus rootstocks × three treatments and

three replications was used. The measurements were taken at the end of the experiment. Growth parameters (plant height, number of leaves per plant, fresh matter accumulation), stomata conductance and chlorophyll content (SPAD) decreased under water deficit (50% Hcc). Increase in water stress increased the soluble solids and proline content. It is concluded that water stress significantly ($p<0.05$) affects physiological and morphological characteristics of citrus rootstocks and under extreme water deficit (50% Hcc) the different responses are evident among the citrumelo rootstocks.

S08P13

The effect of water stress on ABA, JA and physiological characteristic of citrus

Xie S.X., Lu X.P., Nie Q. and Zhao X.L.

Hunan Argricultural University, College of Horticulture and Landscap, China. shenxixie@163.com

Citrus is one of the most popular fruit tree crop in China, the main production area is located in south China where rainfall is uneven distributed in different seasons; thus, the seasonal drought is the main environmental stress that affects yield and quality of citrus fruits. Two-year-old 'Newhall' orange (*Citrus sinensis*) and 'Yamasitaka' (*Citrus unshiu*) seedlings were transplanted into pots containing 2 Lof dried mix-soil. Thirty days after transplanting, plants were subjected to water-deficit stress by withholding water to four different levels: control (CK, well-watered plants) and plants watered to achieve soil water contents that were 21 %, 14% and 7% the CK content. Levels of abscisic acid (ABA) and jasmonic acid (JA) were significantly affected by water stress in 'Yamasitaka' and 'Newhall' orange plants. The content of ABA in 'Newhall' orange was 5.9 ng/g, 7.2 ng/g, 9.3 ng/g and 13.7 ng/g under CK, 21%, 14% and 7% soil water treatment respectively. Under CK conditions, the ABA content was 7.1 ng/g in 'Yamasitaka' and there were no significant differences among CK, 21% and 14% water-stress treatments; however, the ABA content increased to 10.4 ng/g under the 7% water-stress treatment. The content of JA in 'Newhall' orange was 2.2 ng/g, 2.3 ng/g, 3.5 ng/g and 6.7 ng/g under CK, 21%, 14% and 7% soil water content respectively. There was a similar trend of JA content in' Yamasitaka', JA content was 1.3 ng/g, 1.7 ng/g, 2.3 ng/g and 3.6 ng/g under CK, 21%, 14% and 7% soil water treatment respectively. The content of proline was 399.6 µg/g, 525.8 µg/g, 770.5 µg/g and 1004.0 µg/g, under CK, 21%, 14% and 7% soil water treatment respectively in 'Newhall' orange. While content of proline in 'Yamasitaka' was 714.1 µg/g, 1032 µg/g, 1215 µg/g and 1229 µg/g, under CK, 21%, 14% and 7% soil water treatment respectively. Water stress could induce reductive sugar accumulation in both two cultivars, the content of reductive sugar reach its maximum under 7% soil water treatment.

S08P14

Physiological responses of diploid and doubled diploid 'Rangpur' lime and 'Carrizo' citrange under water deficit

Magalhães de Oliveira T.[1], Micheli F.[1], Maserti E.[2], Navarro L.[3], Talon M.[4], Ollitrault P.[5], Gesteira A. S.[6] and Morillon R.[4]

[1]Universidade Estadual de Santa Cruz; CIRAD (UESC/CIRAD), Departamento de Ciências Biológicas, Brasil/França; [2]Istituto di BioFisica, Itália; [3]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, España; [4]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Genómica, España; [5]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, España; and [6]Embrapa Mandioca e Fruticultura, Brasil. tmaga21b@gmail.com

In citrus, the use of rootstocks promotes productivity, improves fruit quality and may confer resistance or tolerance to biotic and abiotic stress. 'Rangpur' lime (*Citrus limonia*) is one of the most tolerant rootstock to drought and is largely used by the Brazilian citrus industry. In contrast, 'Carrizo' citrange (*Citrus sinensis* × *Poncirus trifoliata*), which is one of the most popular rootstocks in the Mediterranean basin, is sensitive to drought and salt stress but confers tolerance to Citrus Tristeza Virus, and also promotes very good fruit quality. Previous studies have shown that citrus doubled diploid (4x) seedlings are more tolerant to salinity than their respective diploid (2x). In the present study, we characterized the water deficit tolerance in 2x and 4x 'Rangpur' lime and 'Carrizo' citrange seedlings. Water deficit was applied respectively for 25 and 35 days, followed by recovery irrigation. Several physiological and biochemical parameters were measured periodically during the experiment and samples were collected for molecular analysis. Doubled diploid seedlings were more drought tolerant than 2x in both genotypes. Water deficit caused a greater reduction in photosynthetic rates and stomatal conductance in 2x compared to 4x. Biochemical and genes expression analyzes are under way to decipher the mechanisms leading to the better tolerance in 4x seedlings.

S08P15

Flooding and soil temperature affect photosynthesis of citrus rootstock leaves.

Otero A.[1], Goñi Altuna C.T.[2] and Syvertsen J.P.[3]

[1]Instituto Nacional de Investigacion Agropecuaria (INIA), Ecophysiology Laboratory, Uruguay; [2]Instituto Nacional de Investigacion Agropecuaria (INIA), Soil and Plant Nutrition Laboratory, Uruguay; and [3]University of Florida. IFAS Citrus Research and Education Center (UF-IFAS), Citriculture, USA. aotero@sg.inia.org.uy

We evaluated the effects of the soil temperature (15ºC, 25ºC and 35ºC) and soil anoxia from flooding for 20 days on citrus rootstock seedlings of trifoliate ('CT33'), 'Carrizo' citrange and 'Troyer' citrange along with 'Tucuman', 'Pomeroy' and 'Rubidoux' trifoliates in a greenhouse. Flooding had no effect on shoot water potential (Ψs) and leaf relative water content (RWC) at 25ºC but at 15ºC and 35ºC, there were differences from non-flooded trees. 'Carrizo' and 'Troyer' had higher Ψs than 'CT33', and 'Tucuman' had the highest Ψs among the trifoliates. Soil temperature was more important than flooding in determining plant water status. In 'Troyer' at 25ºC, flooding decreased net assimilation of CO2 (ACO2) faster than in 'Carrizo' and 'CT33'. At 35ºC, ACO2 was reduced up to 14% of non-flooded plants in only 4 days. 'Tucuman' had higher ACO2 than 'Rubidoux' and 'Pomeroy'. Higher values of internal CO2 concentration in leaves were associated with a reduction in maximum quantum efficiency (Fv/Fm) indicating that non-stomatal factors were more limiting to ACO2 than stomatal conductance at 25ºC and 35ºC, but not at 15ºC. In flooded plants, the decrease of Fv-Fm was highest at 35ºC. 'Carrizo' and 'CT33' maintained higher ACO2 at different soil temperatures but 'Troyer' had the lowest. Based on leaf net gas exchange, chlorophyll fluorescence and water relations, 'Troyer' citrange was less tolerant to flooding than 'Carrizo' and' CT33' whereas 'Tucuman', 'Pomeroy' and 'Rubidoux' had similar tolerance to anoxia regardless of temperature.

S08P16

Flooding affects fruitlet abscission in satsuma mandarin

Goñi Altuna C.T.[1], Otero A.[2] and Syvertsen J.P.[3]

[1]Instituto Nacional de Investigacion Agropecuaria (INIA), Soil and Plant Nutrition Laboratory, Uruguay; [2]Instituto Nacional de Investigacion Agropecuaria (INIA), Ecophysiology Laboratory, Uruguay; and [3]University of Florida. IFAS Citrus Research and Education Center (UF-IFAS), Citriculture, USA. aotero@sg.inia.org.uy

A field trial tested the effect of soil anoxia and flooding on fruit drop in a mature orchard of satsuma mandarin (*Citrus unshiu*) on 'Pomeroy' (*Poncirus trifoliata*) rootstock. Flooded trees were maintained with 10 cm water above the soil surface using a 60 cm high aluminum barrier which was buried to depth a 45 cm around the trees. Flooding lasted for 30 days, from flower anthesis to early fruit set. Waterlogging reduced the soil oxygen levels from 5 to 2 µg O_2 cm^{-2} min^{-1}. Relative fruit drop rate was higher in flooded trees than in non-flooded trees. Flooding reduced the spring flush shoot numbers but not the shoot length of the fewer shoots. Flooding reduced shoot water potential was but leaf chlorophyll was not affected. Flooding reduced net CO_2 assimilation rate (ACO2) and stomatal conductance but there were only small changes in internal CO_2 concentration in leaves and no changes in maximum quantum efficiency (Fv/Fm) or basal chlorophyll florescence (Fo). Thus, stomatal factors were more important than non-stomatal factors in limiting ACO2 in leaves of flooded trees. Proline was high in roots of flooded plants but not in leaves. Leaf starch was high in flooded trees however total reducing carbohydrates were unchanged. Low soil oxygen reduced root function and stomatal conductance and caused an accumulation leaf starch and early fruit drop. Soil anoxia from anthesis to early fruit set induced fruitlet abscission in satsuma mandarin and caused changes in tree water relations

S08P17

Frost damage in lemon orchards in the region of Murcia

Conesa A.[1], Martinez-Nicolas J.[2], Manera J.[3] and Porras I.[2]

[1]Miguel Hernandez University (UMH), Department of Vegetable Production and Microbiology, Spain; [2]Murcian Institute of Agriculture and Food Research and Development (IMIDA), Department of Citriculture, Spain; and [3]Miguel Hernandez University (UMH), Department of Physics and Architecture of Computers, Spain. agustin.conesa@umh.es

Every few years frosts importantly affect the cultivation area surrounding the Region of Murcia (Spain). The most important frost period in recent years occurred at the winter of 1994-95 and the latest ones at 2004-

05, 2010-11 and 2011-2012. In this work we describe and compare the effects of the last two frost periods on fruit and vegetative damage in lemon trees varieties (*Citrus limon*) in the above mentioned area. During the 2010-2011 campaign, frost damage was more severe than in the current year 2011-2012. Temperatures in the citrus fields reached 6.63 °C with 10 hours of temperatures below 0 ºC. Frost-induced damage in fruits and vegetative tissue in orange and mandarin varieties were scarce whereas they were higher in grapefruit and severe in lemon trees, both in varieties grafted on *Citrus macrophylla* and on sour orange. Among lemon varieties studied, the most sensitive to frost turned out to be 'Messina'. The risk of frost in the citrus area of Murcia for a critical temperature of -3 °C was also studied. The probability of frost damage is 2% on the coast and 60% in the inland valleys of Murcia.

S08P18

Physiological investigation of tolerance to iron chlorosis of 'Navelina' orange budded on different citrus rootstocks

Çimen B., Yeşiloğlu T., İncesu M. and Yılmaz B.
University of Cukurova (CU), Department of Horticulture, Turkey. bcimen@cu.edu.tr

In this study, physiological responses of 'Navelina' orange grafted on eight different citrus rootstocks to iron deficiency were investigated under high pH conditions. 'Navelina' scion grafted on 'Tuzcu 31-31' sour orange, 'Gou Tou' sour orange, 'Volkameriana', 'Antalya Cleopatra' mandarin, 'Carrizo' citrange, 'Troyer' citrange, 'C-35' citrange and *Poncirus trifoliata* were grown in a plant growth chamber and irrigated with a modified Hoagland nutrient solution. In order to mimic an iron.deficient environment, 10 µM FeEDTA, 3 mM $NaHCO_3$ and 2g/l $CaCO_3$ (pH=7.8) were added to the nutrient solution. Control plants were irrigated with a nutrient solution containing 100 µM FeEDTA (pH=6) for five months. At the end of the experiment, iron chlorosis color scale, leaf total and active Fe concentrations, Photosystem II efficiency (Fv'/Fm'), peroxidase and catalase activities were determined. In Fe-deprived leaves of 'Navelina' grafted on 'C-35' citrange and *Poncirus trifoliata*, total and active Fe concentrations and efficiency of PSII significantly decreased. These plants exhibited more severe Fe chlorosis symptoms than plants grafted on the rest of rootstocks. Young 'Navelina' trees grafted on 'Tuzcu 31-31' sour orange and 'Gou Tou' were less affected in terms of all parameters. As a result, 'Navelina' scions grafted on 'Tuzcu 31-31' and 'Gou Tou' sour oranges were the most tolerant; the ones grafted on 'C-35' citrange and *Poncirus trifoliata* were the most susceptible to iron deficiency.

S08P19

Differential tolerance to iron deficiency of citrus rootstocks grown in calcareous soil

İncesu M., Yeşiloğlu T., Tuzcu Ö. and Çimen B.
University of Cukurova (CU), Department of Horticulture, Turkey. meralincesu@gmail.com

In this study, seedlings of 14 citrus genotypes used as rootstocks were tested in calcareous soil conditions (pH 7.8.) for their tolerance to lime-induced chlorosis in Adana-Turkey. The effect of iron deficiency on leaf active and total iron, leaf chlorophyll and leaf visual chlorosis were investigated. According to results of leaf chlorophyll and visual leaf chlorosis, 'Swingle' citrumelo, 'Pomeroy' trifoliata and 'Sarawak' bintangor showed severe chlorosis on their leaves but these genotypes had the highest leaf total iron concentration (Fe paradox determined on these genotypes). At the end of the experiment, genotypes were classified in six categories: a) very tolerant: 'Tuzcu 31-31' sour orange, 'Gou Tou' sour orange, 'Antalya Cleopatra' mandarin and 'Carrizo' citrange; b) tolerant: 'Tuzcu 891' sour orange, 'Volkameriana',' Nasnaran' mandarin and 'Marumi' kumquat; c) moderately tolerant: hybrid of 'Cleopatra' mandarin and 'Swingle' citrumelo and 'C-35' citrange d) susceptible: 'Swingle' citrumelo and 'Pomeroy' trifoliata e) moderately susceptible: 'Sarawak' bintangor; and f) very susceptible: local *Poncirus trifoliata*.

S08P20

Orange varieties as interstock in 'Verna' lemon trees increase the salt tolerance but not the drought or flooding tolerance

Gimeno V.[1], Simón I.[2], Martínez V.[1], Nieves M.[3], Balal R.M.[4] and García-Sánchez F.[1]

[1]Centro de Edafología y Biología Aplicada del Segura (CEBAS), Plant Nutrition, Spain; [2]Escuela Politécnica Superior de Orihuela (EPSO), Física y ATC, Spain; [3]Escuela Politécnica Superior de Orihuela EPSO (EPSO), Agroquímica y Medio Ambiente, Spain; and [4]University of Sargodha, Horticulture, Pakistan. fgs@cebas.csic.es

We investigated the ability of interstocks to increase in abiotic stress tolerance in lemon trees with-three-independent experiments. We compared 2-year-old 'Verna' lemon trees [*Citrus limon*; VL] grafted on sour orange (*Citrus aurantium*; SO) rootstock either without an interstock (VL/SO), or interstocked with 'Valencia' orange (*Citrus sinensis*; VL/V/SO), or with 'Castellano' orange (*C. sinensis*; VL/C/SO) in salinity, drought and flood conditions. In salinity experiment, trees were supplied with nutrient solutions containing 0, 30, or 60 mM NaCl for two months. In drought experiment, half the trees were reduced by 25% watering every 5 days for 20 days. In flood experiment, half the trees were flooded for 9 days. The experiments were performed under greenhouse conditions. Leaf water relations, leaf gas exchange, chlorophyll fluorescence parameters, mineral nutrition, organic solutes, and carbohydrate concentrations were measured. Interstocked citrus trees are more salt-tolerant than noninterstocked trees. Leaf concentrations of Cl^- and Na were higher in VL/SO trees than in VL/C/SO or L/V/SO trees, the mechanism involved in the reduction of leaf Cl^- ions by an interstock could be linked to its low Eleaf values during the day and/or to their high S:R DW ratios.' Valencia' imparted greater salt-tolerance to VL trees than 'Castellano', even though leaf Cl^- and Na levels were similar in the two interstocked trees. 'Verna' lemon trees interstocked with orange varieties did not improve the flooding and drought tolerance. Even, when 'Valencia' orange was used as the interstock, the flooding and drought tolerance was drastically reduced.

S08P21

Environmental effects on citrus growth and yield in the arid and semi-arid conditions of Kingdom of Saudi Arabia

Aljaleel A.[1], Ali-Dinar H.[2] and Noga G.[3]

[1]Najran Horticulture Research and Development Centre, Ministry of Agricuture, Kingdom of Saudi Arabia; [2]FAO-UN, Riyadh, Kingdom of Saudi Arabia; and [3]Institute of Horticultural Sciences, University of Bonn, Germany. bstanh@awalnet.net.sa

The Kingdom of Saudi Arabia is situated in south west Asia between latitude 16 32 North and longitude 35 65 East. The total area of the Kingdom is about 2.25 million square kms which represents about 80% of the area of the Arabian Peninsula. Most of the Kingdom's regions are dominated by the arid and semi-arid environmental conditions and irrigation is vital to cultivation and crop production. The Kingdom is characterized by a cropping system that is dominated over the years by date palm (*Phoenix dactylifera*) culture. However, in the early eighties, the cropping systems gradually changed to citrus cultivation in certain regions of the Kingdom, particularly Najran. These efforts were led by Najran Horticulture Research and Development Center (NHRDC) through which many citrus cultivars were introduced and evaluated. The performance of the different citrus cultivars was investigated over the years by (NHRDC). Differential observations were noticed on tree growth, flowering pattern and bloom intensity, fruit quality and yield and time of harvest. These differences were further substantiated by physiological parameters such as seasonal photosynthetic gas exchange. Grapefruit (*Citrus paradisi*), sweet orange (*Citrus sinensis*), lemon (*Citrus limon*), lime (*Citrus aurantifolia*) and tangerine (*Citrus reticulata*) genotypes have shown differential vegetative and reproductive growth due to seasonal environmental changes particularly, temperature and rain patterns. Vegetative and reproductive components of grapefruit and lime were less affected by these patterns. However, sweet orange and tangerine genotypes reflected noticeable differences, particularly on flowering patterns and bloom intensity and fruit juice nutritional aspects. The data are used to develop guidelines to citrus growers to adjust their cultural practices according to the environmental patterns to maximize yield and improve fruit quality.

S08P22

Micromorphological studies on the relation between fruit development and creasing in orange (*Citrus sinensis* cv. 'Moro' sanguine)

Rajaei H.

Shiraz University, Biology Department, Iran. hrajaei@susc.ac.ir

Creasing is a preharvest physiological disorder which causes cracks in the albedo, resulting in the appearance of irregular grooves on the surface of the fruit. Albedo breakdown has been reported from many citrus growing areas worldwide, but not from Iran so far. This two years study has been performed on *Citrus sinensis* cv. 'Moro' sanguine in Mazandaran province, Northern Iran. Incidence and evolution of creasing were followed in 40 trees, at successive stages of development, from anthesis to fruit maturity. Morphological symptoms of creasing appeared at color break, first on the NE quadrant of all the trees and on the shaded side of each fruit. The disorder progressed towards other sectors along with the fruit development. Small pieces of rind in mature healthy and affected fruits were fixed with glutaraldehyde and osmium tetroxide, dehydrated in ethanol series and air dried, coated with gold-palladium, and viewed under the scanning electron microscope. First microscopic evidence of the disorder was observed in apparently healthy fruits, as a small hole or a thinning area, predominantly under the oil glands. Four progressive stages of creasing were recognized, based on SEM observations. The whole albedo cracked at the final stage, at the border line of the oil glands, and the exocarp was not affected by the disorder. Creasing seems to result from separation of albedo cells, rather than cleavage of individual cells.

S08P23

Behavior of diploid and tetraploid genotypes of 'Carrizo' citrange under abiotic stresses

Ruiz M.[1], Pina J. A.[1], Alcayde E.[2], Morillon R.[3], Navarro L.[1] and Primo-Millo E.[2]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain; [2]Instituto Valenciano de Investigaciones Agrarias (IVIA), Citricultura y Protección Vegetal, Spain; and [3]Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), UMR AGAP, France. ruiz_marval@gva.es

Tetraploidy is a spontaneous phenomenon in citrus and some anatomical differences have been described between tetraploid (4x) citrus plants and their corresponding diploids (2x). The choice of a suitable rootstock is often very restricted by a combination of biotic and abiotic stresses. Recent data points out that tetraploidy in citrus induces interesting phenotypes like dwarfing and tolerance to abiotic constraints. In this work we investigated the behavior of 4x and 2x 'Carrizo' citrange (*Citrus sinensis* X *Poncirus trifoliata*) subjected to the main abiotic stresses that threat Spanish citrus industry. Seedlings were exposed to salinity, drought and iron deficiency and physiological parameters were measured. Results suggest that 4x plants stressed by drought were able to maintain high leaf water potential more efficiently than 2x, thus gas exchange parameters (transpiration, stomatal conductance, and photosynthesis) were less affected. Under salt stress no difference was found in leaf chloride content neither in gas exchange parameters, but leaf burn was less severe in 4x than in 2x. Concerning iron deficiency, a better tolerance of 4x compared to 2x was supported by higher ferric-chelate reductase activity, SPAD index and chlorophyll fluorescence. These preliminary results suggest that 4x 'Carrizo' citrange could be a promising rootstock, having the same general performance than the original 2x, but producing smaller trees more tolerant to abiotic stresses.

S08P24

Comparison of photosynthesis and antioxidant performance of several *Citrus* and *Fortunella* species under natural chilling stress

Santini J.[1], Giannettini J.[2], Pailly O.[1], Herbette S.[3], Ollitrault P.[4], Berti L.[2] and Luro F.[1]

[1]Institut National de Recherche Agronomique (INRA), GEQA, France; [2]CNRS UMR 6134 (SPE), Laboratoire Biochimie et Biologie Moléculaire du Végétal, Corsica, France; [3]Institut National de Recherche Agronomique (INRA), UMR Physiologie Intégrée de l'Arbre Fruitier, France; and [4]Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), AGAP, France. santini4@univ-corse.fr

Citrus plants originate from southeastern Asia, in a large area with various climates characterized by a broad range of temperatures. Temperature is assumed to be a key factor in *Citrus* species adaptation and diversification.

In a field experiment, the tolerance of the three fundamental *Citrus* species (*C. medica* –citron–, *C. reticulata* –mandarin– and *C. maxima* –pummelo–) and *Fortunella japonica* –kumquat– to photooxidative stress caused by seasonal climatic changes was evaluated on adult trees by measuring net photosynthesis (*P*net), stomatal conductance (*G*s), maximum photosynthesis (*P*max) and chlorophyll fluorescence (*F*v/*F*m). In addition, seasonal changes in oxidative status, antioxidant enzymes and antioxidant metabolites were monitored. Mandarin and pummelo appeared to be the most tolerant, showing the lowest down-regulation of photosynthetic parameters, and the lowest accumulation of oxidized compounds associated with efficiency of their antioxidant system. Kumquat showed intermediate behaviour, with a large diminution of photosynthetic parameters and marked accumulation of hydrogen peroxide, whereas the malondialdehyde content remained low, with a strong induction of glutathione synthesis. Finally, citron appeared to be the most sensitive genotype with a marked decrease in photosynthetic performance, the largest accumulation of oxidative parameters, insufficient induction of antioxidant enzymes and down-regulation of ascorbate and glutathione synthesis.

S08P25

'Flying Dragon' as a very cold hardy citrus rootstock in Northern Iran

Ebrahimi Y.[1], Joshari H.[2], Tavakoli A.[2], Ghasemi S.[2], and Rajabi A.[2]

[1]Younes Citrus and Kiwi fruit Research and Propagation Station, Iran; and [2]Guilan Emdad Economical Complex, Iran.
U_ebrahimi2000@yahoo.com

Because of freeze damage to citrus orchards in Northern Iran, different citrus rootstocks were evaluated since 1964 at the Ramsar Citrus Experimental Station. On request of Y. Ebrahimi, sixteen 'Flying Dragon' seeds were sent by Dr. Roose from Univ. of California Riverside to Ramsar, Iran in 1994. These sixteen seeds created the mother trees for 'Flying Dragon' seed production in Iran. Taking into consideration to grow citrus in Lakan area in Guilan province in 2006, the Agricultural Deputy of the Economical Group of Relief Committee (EGRE) of Iran established an experimental citrus pilot with 867 'Thompson' navel oranges on 'Flying Dragon' rootstock in Lakan, Rasht (Guilan province). In January 2007, a very heavy snow fall followed by a destructive freeze (-14ºC) was occurred and confirmed the cold hardness of 'Flying Dragon' rootstock. This paper describes this very destructive frost and the results which were obtained.

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POSTHARVEST PHYSIOLOGY AND PATHOLOGY

S09O01

Genome sequence of the necrotrophic fungus *Penicillium digitatum*, the main postharvest pathogen of citrus

Ballester A.R.[1], Marcet-Houben M.[2], de la Fuente B.[1], Harries E.[1], Marcos J.F.[1], Gabaldón T.[2], and González-Candelas L.[1]

[1]Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Food Science, Spain; and [2]Center for Genomic Regulation (CRG-UPF), Comparative Genomics, Spain. lgonzalez@iata.csic.es

Penicillium digitatum is a fungal necrotroph causing Green Mold in citrus fruit during postharvest storage. Despite its economic importance, the genetic bases of its virulence mechanisms and its high host-specificity remain unknown. Here we present the genomes of two *P. digitatum* strains that differ in their antifungal resistance traits, and their comparison with 28 other Pezizomycotina. The two sequenced genomes are highly similar, yet important differences include the presence, in the resistant strain, of a unique gene cluster, and mutations previously shown to confer fungicide resistance. The two strains and one isolated in China have identical mitochondrial genome sequences, suggesting a recent worldwide expansion of the species. Comparison with the closely-related but non-phytopathogenic *P. chrysogenum*, reveals a much smaller gene content in *P. digitatum*, consistent with a more specialized lifestyle. Large regions in *P. chrysogenum* genome, including entire super-contigs, are absent in *P. digitatum*, probably as the result of large gene family expansions, rather than acquisition through horizontal gene transfer. Our analysis of *P. digitatum* genome suggests a heterothallic sexual reproduction and reveals the molecular bases behind the inability of this species to assimilate nitrate or to produce the metabolites patulin and penicillin. Finally, we identify the predicted secretome, which provides a first approximation to the protein repertoire used during invasive growth.

S09O02

RNA-Seq analysis of yeast antagonist *Metschnikowia fructicola* during interactions with *Penicillium digitatum* and grapefruit peel reveals specific transcriptional responses

Hershkovitz V.[1], Sela N.[2], Taha L.[3], Rafael G.[1], BenDayan C.[1], Feygenberg O.[1], Aly R.[3], Wisniewski M.[4], and Droby S.[1]

[1]The Volcani Center (ARO), Institute of Postharvest and Food Science, Israel; [2]The Volcani Center (ARO), Department of Plant pathology and weed research, Israel; [3]The Volcani Center, Newe-Yaar Research Center, Department of Plant Pathology and Weed Research, ; and [4]U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS), Appalachian Fruit Research Station, USA.vhershko@agri.gov.il

The yeast *Metschnikowia fructicola* is a biological control agent with broad activity against various postharvest diseases of several fruits. Information about the yeast antagonist mode of action still lacking and largely rely on non-direct evidence. In this study, we performed transcriptome analysis on *M. fructicola* following its interaction with the host (grapefruit) and the pathogen (*Penicillium digitatum*) using next-generation sequencing technology for RNA (RNA-Seq). We obtained more than 26 million sequencing reads that were then assembled into 9674 unigenes and half of these genes had an annotation with matches in the NCBI database. Assembled sequences were annotated with gene description, gene ontology and clusters of functional group terms. Differential expression analysis revealed more than 250 genes with specific expression responses. In the antagonist-pathogen, genes related to transmembrane multidrug transport and to amino acid metabolism were induced. In antagonist-fruit interaction, expression of genes involved in oxidative stress, iron homeostasis, zinc homeostasis and lipid metabolism were enhanced. We investigated the gene expression patterns at the transcript levels in the two types of interactions by quantitative real-time PCR analysis. The data revealed that genes encoding hydrolytic enzymes glucanase (*GLU*) and chitinase (*CHI*), superoxide dismutase (*SOD1*) and signal transducing MEK kinase (*MEKK*) had specific expression pattern. Our results provide new insight into the biology of interaction between host-antagonist-pathogen interactions.

S09O03

The mitogen-activated protein kinase *PdSLT2* of *Penicillium digitatum* is required for fungal pathogenesis/virulence during citrus infection.

de Ramón-Carbonell M., and Sánchez-Torres P.

Instituto Valenciano de Investigaciones Agrarias (IVIA), Departamento de Protección Vegetal y Biotecnología, Spain. palomas@ivia.es

Postharvest decay accounts for the most significant losses of postharvest citrus worldwide. Green Mold caused by *Penicillium digitatum* is the major responsible of the postharvest decay of citrus in Mediterranean regions.

Nevertheless our knowledge on its pathogenicity/virulence mechanisms is still very limited. The mitogen-activated protein kinase (*MAPK*) cascade is involved in the transduction of a variety of extracellular signals and the regulation of several developmental processes. In most filamentous fungi, *SLT2 MAPK* family is involved in the cell integrity pathway and in many cases is essential for full virulence since it is implicated in sporulation, surface sensing of germ tubes, host penetration and lesion formation. In this work *Penicillium digitatum SLT2* homologous gene *PdSLT2* have been identified and characterized. Analysis of its role in pathogenesis/virulence was carried out through the generation of gene knock-out mutants with *Agrobaterium tumefaciens* mediated transformation (ATMT) and evaluating infection capacity. Expression analysis was conducted by qRT-PCR. Gene expression was determined in orange fruits during *P. digitatum* infection, and during growth of *P. digitatum* in culture medium PDB of both wild type and null mutants. To our knowledge this is the first MAPK identified in *P. digitatum* and in this work we confirm that *PdSLT2* is involved in fungal virulence and the expression patterns of several genes in null mutants revealed which genes are transcriptional regulated by *PdSLT2*.

S09O04

Function of *PdCrz1*, a calcineurin-responsive transcription factor, in *Penicillium digitatum* in conidiation, virulence and responses to abiotic stresses

Zhang T., and Li H.Y.

Institute of Biotechnology, Zhejiang University, China. hyli@zju.edu.cn

Calcineurin-responsive zinc finger transcription factor *Crz1* regulates many genes involved in fungus growth, morphogenesis, responses to stresses, resistance to drugs and pathogenicity in their hosts. In the present study, we characterized an ortholog of *Crz1*, *PdCrz1*, in *P. digitatum*, the most important pathogen of postharvest citrus. *PdCrz1* encodes a protein that shares a high amino acid sequence similarity with *Crz1* in *Aspergillus spp*. To determine the function(s) of *PdCrz1*, a gene-disruption mutant was generated using an *Agrobacterium tumefaciens*-mediated transformation procedure (ATMT). The *PdCrz1* disruption (Δ*PdCrz1*) mutant was defective in conidiation, cell wall integrity, and virulence, and is hypersensitivity to stresses caused by Ca^2, H_2O_2 and azole fungicides. Expression of cell wall synthase genes (*CHS2*, *CHS3* and *FKS1*), P-type ATPases genes (*PMR1* and *PMC1*) was significantly reduced in the *PdCrz1* mutant compared with the wild type *P. digitatum*. Calcineurin inhibitors were shown to enhance the effect of imazalil on *P. digitatum*. In contrast, activation of the Ca^2 /calmodulin-dependent pathway by Ca^2 alleviated the antifungal activity by imazalil. These results indicate that *PdCrz1* plays important roles in *P. digitatum* conidation, pathogenicity and membrane lipid homeostasis. In addition, the study also indicates a synergistic fungicidal activity on *P. digitatum* between calcineurin inhibitors and DMI fungicides, implying that specific inhibitors of calcium signalling pathway could be potentially developed as useful adjuvants of conventional DMIs for the control of fungal diseases.

S09O05

The Protein O-Mannosyltransferase PMT2 of the citrus-specific postharvest pathogen *Penicillium digitatum* is involved in conidiogenesis, virulence and sensitivity to the antifungal peptide PAF26

Harries E., Gandía M., Carmona L., and Marcos J.F.

Instituto de Agroquímica y Tecnología de Alimentos (IATA), Food Science, Spain. jmarcos@iata.csic.es

Our group has demonstrated that genes encoding Protein O-Mannosyl Transferases (PMT) determine the sensitivity of the yeast *Saccharomyces cerevisiae* to the short antifungal hexapeptide PAF26. In previous studies, we had identified the family of *PMT* genes in the citrus fruit postharvest pathogen *Penicillium digitatum* and showed their expression during axenic growth and fruit infection. To study functional significance, *Agrobacterium*-mediated transformation (ATMT) was used to: (i) disrupt *PdigPMT2*, and (ii) overexpress it under control of the *A. nidulans gpdA* promoter. The mutant Δ*pmt2* displayed slow rate of growth and a strong reduction of conidia production. Interestingly, the growth of Δ*pmt2* was recovered with osmotic stabilization, while conidiation was only partially restored. Microscopy observation showed balloon-like swollen cells and defects in conidiophore formation in the Δ*pmt2* mycelium. The mutant also showed increased sensitivity to

calcofluor white (CFW) or congo red (CR), but remarkably resistance to reactive oxygen species (ROS). Conidia of the mutant were also resistant to the fungicidal activity of PAF26, confirming that protein glycosylation also determines the sensitivity to antimicrobial peptides in filamentous fungi. None of the overexpressors displayed variations in susceptibility against the antifungal compounds analyzed. In bioassays of controlled inoculation, we confirmed that *PMT2* is required for full virulence of *P. digitatum* to citrus fruit.

S09O06

PdCYP51B, a new sterol 14α-demethylase gene of *Penicillium digitatum* involved in resistance to imazalil and other fungicides inhibiting ergosterol synthesis

Sun X.P., Wang J.Y., Feng D., and Li H.Y.

Institute of Biotechnology, Zhejiang University, China. hyli@zju.edu.cn

The phenotypes and genotypes of 403 isolates of *Penicillium digitatum*, collected from packing houses and supermarkets in Zhejiang, China, during 2000 to 2010, were characterized in terms of their imazalil sensitivity. The frequency of detected imazalil-resistant (IMZ-R) isolates increased from 2.1% in 2000 to 60–84% during 2005–2010. Only 6.5% and 4.5% of the collected IMZ-R isolates belong to the previously described IMZ-R1 and IMZ-R2 genotypes, respectively. A homolog (*PdCYP51B*) of *PdCYP51*, which encodes the target of imazalil, was revealed from genomic database of *P. digitatum*. Sequence analysis of *PdCYP51B* from six IMZ-R3 and eight imazalil-sensitive (IMZ-S) isolates indicated that a unique 199-bp insertion was presented in the promoter region of *PdCYP51B* in all IMZ-R3 isolates but absent in all IMZ-S isolates. Transcription levels of *PdCYP51B* in IMZ-R3 isolates were 7.5- to 13.6-fold higher than that in two IMZ-S isolates. Introduction of another copy of *PdCYP51Bs* (from IMZ-S) into an IMZ-S isolate increased the resistance of *P. digitatum* to demethylase inhibitors (DMIs) only to a small extent, but introduction of a copy of *PdCYP51BR* (from IMZ-R3) dramatically increased the resistance level of *P. digitatum* to DMIs. Based on these results, we concluded that (1) the *CYP51* family of *P. digitatum* contains another member *PdCYP51B*, in addition to the known gene *PdCYP51A* (previously *PdCYP51*); (2) *PdCYP51B* is involved in DMI fungicide resistance; and (3) overexpression of *PdCYP51B* resulting from a 199-bp insertion mutation in the promoter region of *PdCYP51B* is responsible for the IMZ-R3 type of DMI resistance in *P. digitatum*.

S09O07

Current and emerging strategies for Sour Rot management of citrus in Australia

Taverner P.D., and Cunningham N.M.

South Australian Research and Development Institute (SARDI), Sustainable Systems, Australia. Peter.Taverner@sa.gov.au

In Australia, Green Mould (*Penicillium digitatum*) and Blue Mould (*P. italicum*) are serious diseases affecting citrus fruits. Sour Rot (*Geotrichum citri-aurantii*) is sporadic but control options are more limited. Sour Rot, or rather a complex of Sour Rot with Mould, is an intractable problem for Australian citrus exports. Researchers work closely with citrus packers to integrate control measures for sour rot into existing treatments for mould control. This usually involves combining chemical fungicides with sanitisers &/or generally regarded as safe compounds (GRAS). Careful evaluation is necessary because chemical mixtures can be neutral, additive, synergistic or antagonistic. Generally, research adheres to two main aims; sour rot spore control in water suspensions, and sour rot control on fruit. The first approach uses sanitisers in water washes and fungicide applications. Sanitisers added to fungicide dips can reduce sour rot spore loads but chemical incompatibility is common. The response varies with sanitiser active, fungicide active and fungicide formulation. The second approach aims to enhance sour rot control on fruit by combining fungicides and GRAS compounds. Various mineral and organic salts have demonstrated enhance sour rot control. Sanitising these mixed solutions is problematic, with the choice of fungicide, salt and sanitiser influencing compatibility and efficacy. Increased worldwide concern in Sour Rot has lead to productive research, which may lead to new approaches for control of this disease. The studies used to develop current strategies and possible future research directions are discussed.

S09O08

A new prespective in controlling postharvest citrus rots

Fallanaj F., Sanzani S.M., and Ippolito A.

Department of Soil, Plant and Food Science, Univeristy of Bari 'Aldo Moro', Italy. fridafallani@yahoo.it

The need for alternative control means to reduce postharvest decay of fruits is becoming increasingly important. Frequently, contaminations take place in the postharvest environment e.g. in the wash water. Several sanitizers have been studied for their effectiveness in the inactivation of this pathogenic microflora, however their limited efficacy or drawbacks prevented their practical use. The electrolyzed water represents a major step forward, as an effective and economical alternative. EW has been shown to have broad-spectrum antimicrobial activity in inactivating a variety of bacteria of public health significance, which might affect minimally-processed vegetables. Recent studies have also shown a certain efficacy of EW against postharvest rots on peaches, pears, grapes, and citrus fruits. However, they rely on the electrolysis of NaCl solutions, thus not avoiding the formation of chlorine by-products which may represent a limitation. The present study was carried out to evaluate the effectiveness of EW in controlling citrus rots during processing in the packinghouse environment. We focused on developing an effective protocol for practical application during postharvest handling of citrus, which does not require the use of chlorine salts. Our results showed that once the machine parameters and water composition are carefully analyzed and set up, EW may be effective in prolonging the shelf life of citrus fruit during storage, although its potential needs to be further investigated.

S09O09

Global solution for preserving citrus fruit using natural treatments

Conesa E.

Fomesa Fruitech (FOMESA), Technical Department, Spain. econesa.fruitech@fomesa.net

Different tests were carried out in conjunction with the IVIA's Post-Harvest Technology Centre to assess the efficiency of natural treatments replacing standard phytosanitary treatments. First, the efficiency of an aqueous treatment containing a food preservative was assessed. This system significantly reduced the appearance of decay in 'Marisol' mandarins inoculated with 10^5 spores/mL of *Penicillium digitatum* and *P. italicum*. The same food preservative was used in conjunction with a detergent on 'Washington Navel' sweet orange fruit previously inoculated with the same mix of *P. digitatum* and *P. italicum*. The reduction in decay was similar to that obtained using guazatine as reference. Finally, two different natural products were tested. One was combined with a natural coating and the food additive, whilst the other was applied as a fumigant. The results of the these two assays showed a significant reduction of decay in 'Clemenules' mandarins and 'Valencia-Late' sweet orange fruit, respectively, which were both previously inoculated with *P. digitatum* and *P. italicum*. In summary, different treatments have been assessed which significantly prevented decay in citrus fruit inoculated with *Penicillium* spp. These treatments can be considered as a viable alternative to the use of phytosanitary products.

S09O10

Could ethylene influence Peteca Spot incidence of lemon fruit?

Cronjé P.J.R.

Citrus Research International (CRI), South Africa. paulcronje@sun.ac.za

Peteca Spot (PS) of lemon is a postharvest physiological disorder resulting in the collapse of the oil gland. Subsequently, the oil leaks into the adjacent tissue and causes a darkened depression. The occurrence can be severe, without any specific pre- or postharvest practices to avoid reduce the incidence. PS occurs in all citrus production areas of South Africa and is thought to be the result of the immature rind being subjected to postharvest stress associated with high CO_2 concentration in degreening, as well as the physical impact of the packing line and wax application. Over several seasons the incidence of PS was investigated to identify factors that influence the incidence of the disorder. The first observation was the highly erratic incidence between seasons as well as within an orchard in a season, with the early fruit being highly susceptible. In experiments to identify postharvest factors which influence the PS incidence, 3 ppm ethylene and 1% CO_2 were applied in

a continuous flow-through system (20°C for 3 d). The CO_2 treatment resulted in significantly higher incidence compared to the rest. Following on from these results, postharvest ethephon (2-chloroethyl phosphoric acid) (200 mg/L and 400mg/L) and AVG (aminoethoxy-vinylglycine) (400 mg/L and 800 mg/L) applications to fruit resulted in a significant reduction in PS. In the subsequent season, the same treatments were applied in an orchard one week before harvest and a similar reduction in PS was recorded. Ethylene production from these fruit after harvest was measured and showed a transient spike in fruit from the Ethephon and AVG treatments. The results collected over several seasons could indicate a protective action of ethylene in reducing rind sensitivity to PS. It is hypothesised that if the internal ethylene synthesis is increased prior to harvest in sensitive fruit, i.e. immature fruit, a reduction in PS can occur.

S09O11

Tahiti lime post-harvest evaluation and non-destructive assessment of essential oils by NIR spectroscopy

Diaz-Candelas C.C.[1], Morales-Payán J.P.[1], and Romañach R.[2]

[1]Universidad de Puerto Rico, Recinto Universitario de Mayaguez (UPR-Mayaguez), Crops and Agroenvironmental Sciences, Puerto Rico; and [2]Universidad de Puerto Rico, Recinto Universitario de Mayaguez (UPR-Mayaguez), Chemistry, Puerto Rico.
cecilia.diaz1@upr.edu

Near Infrared spectroscopy (NIRS) has been used as a non-destructive, real time analysis method to evaluate the terpene content in the essential oils in citrus fruits. The C-H stretch bands confirmed the presence of terpenes in Tahiti lime flavedos analyzed directly without solvent extractions. This method was utilized to evaluate whether biorregulators affect the content of terpenes in the flavedo. A Tahiti lime orchard located at Lajas, Puerto Rico was submitted to seven independent treatments of biorregulators to evaluate their effect in fruit abscission, post-harvest life and terpene content. The solutions consisted of aminoethoxyvinylglycine (AVG), a sea weed emulsion with a 0.01% kinetin content (Kn), a shark emulsion (H), a fertilizer containing tocopherol and brassinolide (T Br), GA3, GA4&7, and a mixed solution of these GA's. NIR spectrums were taken within three days of harvest. Post-harvest quality trait changes were observed during eight weeks to fruits stored at 10 ºC and 85% RH. The GA3 treatment yielded smaller fruits that took longer to change their green color. AVG treatments yielded fruits with higher juice content followed by GA4&7 and the mixed GA's. AVG and Kn treated trees had less abscission and yielded the most fruits for six weeks after which Kn continued this low abscission rate. With NIRS it was possible to differentiate between flavedo, albedo and pulp and to correlate intense peaks of the flavedo spectra with three terpenes. Results strongly suggest a directly proportional relation between terpene content and fruit maturity. A calibration model is in development.

S09O12

Postharvest blue light treatments affect citrus fruit susceptibility to disease by altering oxylipin biosynthesis

Alférez F.[1], Liao H.L.[2], and Burns J.K.[2]

[1]Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Laboratorio de Fisiología Y Biotecnología Postcosecha, Spain; [2]Citrus Research and Education Center-University of Florida (CREC-UF), Horticultural Sciences, USA. alferez@iata.csic.es

The effect of blue light on infection by different postharvest pathogens was investigated in tangerines and sweet oranges during postharvest. Exposure of fruit to 410-540 nm blue light at a fluency of 40 µmol m-2s-1 reduced symptom development of Blue Mold (*Penicillium italicum*), Green Mold (*P. digitatum*), and Stem End Rot (*Diaporthe citri*) postharvest decays. However, blue light also reduced in vitro fungal growth of *P. italicum* and *D. citri* and activity of fungal polygalacturonase. Blue light treatment induced phospholipase A2 (PLA2) gene expression in 'Fallglo' tangerines. Inhibition of PLA2 activity allowed infection, whereas inhibition of phospholipase D (PLD) expression by 580-670 nm red light was correlated with infection. These data suggested induction by blue light of the oxylipin pathway. Gas chromatography-mass spectrometry analysis of flavedo oil showed that blue light induced accumulation of the C8 oxylipin octanal. Application of octanal suppressed growth of *P. italicum*, *P. digitatum*, and *D. citri in vitro*. Treatment of fruit with octanal suppressed symptom development caused by *Penicillium* and *D. citri*. The effect of blue light on citrus decay could be due to its impact on fungal growth and development, and the induction of fruit response via oxylipin synthesis in the peel.

S09O13

Potassium sorbate increases citrus weight loss in postharvest treatments but it does not provide good decay control in wax

Parra J.P.

Postharvest Technology, Spain. encarna@citrosol.com

Potassium sorbate (PS) is a well-known and widely used food preservative. Among other applications, it is used as a GRAS fungistatic postharvest treatment for citrus. We studied the efficacy of wax containing increasing concentrations of PS on decay control of *Penicillium digitatum*, and its effect on fruit weight loss. Decay control and weight loss increased with the concentration of PS in wax. Wax with typical amount of 2–5% PS showed poor decay reduction indices (around 20%), whereas fruit weight loss increased compared with non-waxed control. Waxing the fruit reduced weight loss by 25%, but the addition of just 2% PS to the wax caused an increase in fruit weight loss of almost 40% compared with waxed fruit. The hygroscopic effects of PS are more damaging for citrus fruits with leaves. Leaves weight loss was very rapid when PS was added to the wax, becoming desiccated in 24 hours, old and brittle. This issue is very important for the fresh packer because these fruits are sold with leaves as proof of their freshness. Results of a similar study where PS was applied to citrus as an aqueous treatment will be also presented. When applied in water, PS was more effective for decay control than when applied in wax, but there was a considerable increase in fruit weight loss. Combining aqueous PS with our Fortisol® Ca Plus phytofortifier completely solved the problem of weight loss and achieved outstanding decay control, making these mixtures as promising treatments.

S09P01

Transcriptional regulation of the *Citrus sinensis* ABA-signalosome during fruit dehydration

Romero P., Rodrigo M.J., and Lafuente M.T.

Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Food Science. Postharvest Physiology and Biotechnology, Spain. mtlafuente@iata.csic.es

Water stress is an important factor affecting fruit quality. Since abscisic acid (ABA) is a key regulator of the dehydration response, understanding how the hormone is perceived will help to identify targets to improve drought hardiness in horticultural crops. To study the involvement of the ABA perception system in the dehydration response of citrus fruit, we have performed a comparative transcriptional analysis of the ABA-signalosome components in water-stressed fruit of 'Navelate' orange (*Citrus sinensis*) and its fruit-specific ABA-deficient mutant 'Pinalate', which is more prone to dehydration. No significant differences in the absolute expression levels of ABA receptors (*CsPYR/PYL/RCAR*) were found between wild-type and mutant fruit, while those of the negative regulators (*CsPP2CA*) were slightly lower in the mutant. Low differences in ABA receptors and downstream protein kinases (*CsSnRK2*) transcripts levels were found in response to dehydration. Contrary, *CsPP2CA*s gene expression was highly regulated by ABA content and showed differences between cultivars, which suggest that *CsPP2CA*s play a key role regulating ABA response in citrus fruit to water stress. Results obtained by ABA application indicate that although the mutant may sense ABA, the hormone signal could be impaired because of reduced *CsPP2CA*s levels. Overall results highlight these components as potential targets to improve drought hardiness in citrus fruits.

S09P02

Delayed color break in 'Tardivo', a late ripening mandarin mutant, is related to a defective ethylene response

Alos E.[1], Distefano G.[2], Rodrigo M.J.[1], Gentile A.[2], and Zacarias L.[1]

[1]Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Food Science, Spain; and [2]University of Catania, Dipartamento di Scienze delle Produzioni Agrarie e Alimentari, Italy. lzacarias@iata.csic.es

Late ripening mutants, with delayed peel color changes and senescence, are of paramount importance for the citrus industry because they allow an extended harvesting and marketing period. Hence, the characterization of these mutants constitutes a useful system to decipher the molecular bases that regulate the timing and rate of color development in citrus fruits. Although the causes for these phenotypes may be diverse, recent

experimental evidences in mandarin fruit link delayed degreening with differential ethylene sensitivity. 'Tardivo' is a late ripening mutant of clementine mandarin that displays a certain inability to ethylene induced-degreening. In the present work, we have explored if this phenotype is associated with differential responses to ethylene-regulated gene expression. To this end we have analyzed the expression of key genes involved in carotenoid, ABA and phenylpropanoids biosynthesis and also in the ethylene biosynthetic and signaling pathways. Results indicate an altered ethylene-induced gene expression in 'Tardivo' fruits, displaying a transient response to ethylene that is unable to sustain it subsequently.

S09P03

Comparative proteome and metabolome profiling analysis heat treatment induced fruit resistibility during postharvest storage

Yun Z., Liu P., Liu S.Z., Luo T., Jin S., Gao H.J., Xu Q., Xu J., Cheng Y.J., and Deng X.X.

Key Laboratory of Horticultural Plant Biology of Ministry of Education, Huazhong Agricultural University, China.
yjcheng@mail.hzau.edu.cn

From harvest to human consumption, the commercial production of fresh fruits requires time for shipping, storage and marketing. During these processes, physiological disorders and pathological diseases are the main causes of fruit loss. Heat treatment (HT) has been widely used to maintain fruit quality during postharvest storage. However, little information is available related to the underlying mechanisms occurring during storage at a systemic biological level. 'Kamei' Satsuma mandarin (*Citrus unshiu*) fruits were selected for use in exploring the mechanisms induced by HT which allow fruit to resist such damage during postharvest storage. Proteome and metabolome studies were conducted based on two-dimensional gel electrophoresis (2-DE), gas chromatography coupled to mass spectrometry (GC-MS), and liquid chromatography/electrospray ionisation time-of-flight mass spectrometry (LC/QTOF-MS). The results showed resistance associated proteins were up-regulated, such as beta-1, 3-glucanase, Class III chitinase, 17.7 kDa heat shock protein and low molecular weight heat-shock protein. This study provides new insights into HT induced fruit resistance to pathogens and physiological disorders. Interpretation of the data for the proteins and metabolites revealed reactive oxygen species (ROS) play an important role in HT induced fruit disease and stress resistance. Redox metabolism proteins were down-regulated in HT fruits while the intracellular H2O2 content decreased in HT fruits, which might increase fruit resistibility in response to external stress. Also, flavonoids were up-regulated in HT fruits, a process which was directly involved in the response to external stress. Moreover, the H2O2 in the fruit was used to synthesize lignin, which leads to increased lignin content in HT fruits. The increased lignin-induced thickening of cell walls was involved in isolating external pathogens from fruits.

S09P04

Postharvest water stress leading to peel disorders in citrus fruit involves regulation of phospholipases by ABA

Romero P., Gandia M., and Alférez F.

Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Laboratorio De Fisiología y Biotecnología Postcosecha, Spain.
alferez@iata.csic.es

The interplay between abscisic acid (ABA) and phospholipases A2 and D (PLA2 and PLD) in the response of citrus fruit to water stress was investigated during postharvest by using an ABA-deficient mutant from 'Navelate' sweet orange. Fruit from both varieties were subjected to prolonged water stress conditions inducing non-chilling peel pitting in the rind. Treatment with phospholipase inhibitors reduced the disorder in both varieties suggesting that phospholipid metabolism is involved in citrus peel quality. Expression of genes encoding PLA2 and PLD was studied by real time RT-PCR during water stress and in response to ABA. Expression of *CsPLA2α* was dependant on ABA in fruit from both varieties, since ABA application restored expression in the mutant, showing for the first time the involvement of this hormone in the activation of PLA2. *CsPLDα* expression increased with dehydration in mature-green fruit from 'Navelate' but not in the mutant and ABA did not counteract this effect, suggesting that ABA deficiency in the mutant impairs sensitivity. Our results show interplay between PLA2 and PLD substrates and products, and suggest that ABA action is upstream phospholipase activation in response to water stress.

S09P05

Quality attributes of Valencia orange (*Citrus sinensis*) fruit coated with different edible covers

Mohammadi-Shamloo M.[1], Sharifani M.M.[2], Daraei-Garmakhany A.[2], and Seifi E.[3]

[1]Gorgan University of Agricultural sciences, Horticulture, Iran; [2]Gorgan University of Agricultural Sciences and Natural Resources, Horticulture, Iran; and [3]Gorgan University of Agricultural Sciences and Natural Resources, Food Science and Technology, Iran. mmsharif2@gmail.com

This study was aimed to determine the best treatment to control fruit rot, quality preservation and increase in storage life of 'Valencia' sweet orange. Fruits were treated with clove oil using (1%), edible wax, mixture of clove oil with wax and control. Treated fruits were stored (7°C and 85% RH) and several quality attributes such as fruit peel firmness, weight losses, rate of infection by rot agent, TSS, pH, TA, vitamin C and all related sugars were measured every three weeks. The data were analyzed using a factorial statistical design in a frame of RCD using SAS software. According to the results, none of the treatments had significant effect on pH, TA and TSS (%). Vitamin C content (mg/100g) indicated an increasing trend until 42 days of storage and then a decreasing trend was appeared. The cover types had no significant effect on vitamin C content. The highest water loss was observed in the control, even though different treatments could diminish the water loss. However, none of these treatments caused significant differences. Fruit firmness and rot value differences were significant. Clove oil could inhibit growth of any rot agent. The cover types had significant impact on the available glucose in the orange juice. The mixture of wax and clove oil provided highest glucose (mg/100g) remaining in the edible tissue ($P < 0.01$).

S09P06

Influence of maturity stage and intermittent warming on storage of 'Nagpur' mandarin fruit

Ladaniya M.

National Research Centre for Citrus, Nagpur (NRCC), ICAR, INDIA. msladaniya@gmail.com

Influence of fruit maturity stages ('green' and 'orange' rind colour) and intermittent warming (IW) 2 weeks at 3.5oC followed by 1 week at 12.5oC, on fruit composition, decay, rind colour and respiration of 'Nagpur' mandarin was investigated for 75 days. Fruit were not treated with any fungicide. Effect of post-storage holding (7 days at 22 ±2oC and 70±5% RH) after 30, 60 and 75 days of chilling storage was also studied. Chilling injury did not develop until 75 days of storage and or after 1-week holding at each interval. Weight loss was 4.8- 4.9% in fruit at the two maturity stages. Alternaria Rot was detected as stem-end rot and core rot. Up to 30 days, decay was negligible but after 60 and 75 days followed by one week self-life, it increased to 19.83% and 22.26% in coloured orange fruit and 11.97% and 16.27% in green fruit, respectively. Juice content, fruit firmness and titratable acidity were significantly higher in green fruit while TSS and ascorbic acid contents were higher in orange fruit. After prolonged storage, fruit colour increased, with an increase in chroma and a decrease in hue values. Colour and appearance score was almost similar after 60 days and 75 1 week self-life in fruit at both maturity stages. Chlorophyll content declined and total carotenoids increased during storage. Fruit respiration rate was 5 mgCO2 kg-1 hr-1 at chilling temperature and increased by nearly 4-times during IW and 5-6-times when transferred to ambient conditions. Flavour score was initially higher in orange fruit than in green fruit, but was similar and without off-flavour in both maturity stages after 75days 1 week self-life.

S09P07

Effect of postharvest temperature on accumulation of primary metabolites in juice sacs of satsuma mandarin (*Citrus unshiu*) fruit

Matsumoto H.M., and Ikoma Y.

NARO Institute of Fruit Tree Science (NIFTS), Citrus Research Division, Japan. hikaruoo@affrc.go.jp

Sugars, organic acids, and amino acids are major metabolites in the juice sacs of citrus fruit and are important for fruit quality. To elucidate the effect of postharvest temperature on the accumulation of these metabolites and to determine the optimal temperature to minimize postharvest changes, the content of 3 sugars, 4 organic acids, and 20 amino acids in juice sacs of satsuma mandarin (*Citrus unshiu*) fruit stored at 5, 10, 20, and 30°C

for 14 days was investigated. Changes in sugar content were negligible at the different temperatures. Organic acids decreased slightly at all temperatures, with the exception of malic acid at 30°C, which increased slightly. For 13 amino acids, postharvest changes differed between low and high temperatures. Ornithine and glutamine increased at 5°C but not at other temperatures. The concentration of 11 amino acids (isoleucine, leucine, valine, threonine, lysine, methionine, phenylalanine, tryptophan, tyrosine, histidine, and γ-amino butyric acid) was higher at 20 and 30°C than at other temperatures. Thus, the content of amino acids was more variable in response to the storage temperature than that of sugars and organic acids. Moreover, changes in amino acids by temperature differed among species, 2 cold-responsive and 11 heat-responsive. Results indicated that 10°C was the optimal temperature to minimize postharvest changes in metabolite profiling in the juice sacs of Satsuma mandarin fruit.

S09P08

Stem End Rind Breakdown of citrus fruit a new postharvest physiological disorder of lemon fruit in tucuman (Argentina)

Torres-Leal G.J., Velazquez P.D., Carbajo M.S., Farias M.F., and Pedraza J.R.

Instituto Nacional de Tecnología Agropecuaria (INTA-Famaillá), Frutihorticulture, Argentina. gtleal@correo.inta.gov.ar

Lemons from packinghouses destined for exportation of the season 2008 to 2012 were surveyed. Fruits of lemon from Santa Lucia and Yacuchina (Monteros–Tucuman) exhibited unusual symptoms consisting in sunken brown spot around the stem of rind and later became dark during the packing process. The objective of this work was to determine the causal factor of this disorder. Affected tissues were collected and streaked onto PDA to identify possible causal fungal pathogens. Meteorological data were analyzed from 2008 to 2012 of study area. No pathogens were isolated. Rain and relative humidity during the months previous to harvest (March to April) were lower and temperature higher than the average values over the last 45 years. Based on symptoms and information from other citrus-growing areas of the world, a physiological disorder known as Stem End Rind Breakdown of citrus (SERB) was determined. This disorder was found in thin-skinned fruit from humid, high rainfall areas and water stressed trees. Collapse of rind tissues not discolored around the button (thick layer of natural wax of cuticle) was the first symptom observed. Subsequently, the oil glands collapsed followed by discoloration of the collapsed area. Postharvest management practices that decrease fruit desiccation also reduce SERB incidence and severity.

S09P09

The effects of some post-harvest applications on fruit quality of 'Valencia-Late' oranges

Dal B.

Bati Akdeniz Agricultural Research Institute Antalya (Batem), Fruit Department, Turkey. banudal@yahoo.com

During this research, the post harvest quality of 'Valencia-Late' sweet orange fruit was investigated. The study was carried out in the pomology laboratory and storage rooms of Batı Akdeniz Agricultural Research Institute in 2012. 'Valencia-Late' fruits were divided into six lots. The control group was left untreated during the trials. One lot of fruit was waxed immediately after harvest and other was dipped in ethanol (70%) solution. The other lots were dipped in propolis solutions (70% ethanol extracted propolis solution) of 3%, 5%, 7%, respectively. Fruits were kept for 10 days at 20°C±1 and U-60 relative humidity and calculations and pomological analysis were conducted. During the trial, weight loss (%), fruit juice amount (g citric acid/100 mL fruit juice), total soluble solid amount (%), titratable acid amount (%), total soluble solids/titratable acidity ratio, colour measurement and change in rind thickness (mm) were examined with three repetitions.

S09P10

Influence of light on carotenoid accumulation in 'Star Ruby' grapefruit

Lado-Lindner J.[1], Lado-Lindner J.[2], Cronje P.J.R.[3], Rodrigo M.J.[1], and Zacarías L.[1]

[1]Instituto de Agroquímica y Tecnología de Alimentos, Consejo Superior de Investigaciones Científicas (IATA-CSIC), Ciencia de los Alimentos, Spain; [2]Instituto Nacional de Investigación Agropecuaria (INIA), Postcosecha de cítricos, Uruguay; and [3]Citrus Research International (CRI), Horticultural Science, South Africa. jlado@iata.csic.es

Accumulation of the red carotene pigment, lycopene, in citrus fruit is an unusual feature and restricted to grapefruit, pummelo and a few sweet orange mutants. Recent studies indicated that differential mechanisms

may be operating in the different lycopene-accumulating varieties, highlighting the importance to understand these processes. It is well characterized in citrus fruit that light positively affects color development and consequently carotenoid content and composition. However, it is paradoxical that by shading red grapefruit, degreening is accelerated and external color is enhanced (higher red coloration). To further understand the regulatory mechanisms of light on carotenoids biosynthesis in lycopene-accumulating grapefruit, carotenoid composition, and expression of carotenoid biosynthetic genes were analyzed in the peel of shaded and light-exposed 'Star Ruby' grapefruit grown in South Africa and Spain. The stage of fruit development at the time of shading appears to be influential in color development. In the peel of shaded fruits, total carotenoids (x4) and particularly lycopene experienced a dramatic increased (x10). These changes were associated with a lower expression of early genes in the pathway as well as β-*lycopene cyclase* and β-*carotene hydroxylase* genes, envisaging their involvement in lycopene accumulation. In addition, the effect of light or shade exposure on other nutritional and health-related metabolites will be presented and discussed.

S09P11

Assessment of antioxidant compounds during storage period of 'Valencia' orange fruit

Sharifani M.M.[1], Mohamadi-Shamlou M.[1], Daraei A.[2], and Seifie E.[1]

[1]Gorgan University of Agricultural Sciences and Natural Resources, Horticulture, Iran; and [2]Gorgan University of Agricultural Sciences and Natural Resources, Food Science and Technology, Iran. mmsharif2@gmail.com

Citrus fruits have an important role to prevent from cardiovascular diseases. This effect is contributed to their antioxidants compounds which are able to absorb free radicals and active oxygen species. This compound could eliminate free radicals and decline the activity of effective enzymes in oxygen reduction pathway. In this study variation of antioxidant activity, total phenol and vitamin C content were determined during three months storage at temperature about 7° C and 85 % relative humidity (RH). Orange samples were treated with clove oil with 1% concentration, wax, wax-clove oil mixture and control. The amount of antioxidant activity was determined by DPPH method, and the total phenol compound and vitamin C were measured with folin-ciocalteu and 2,6 -diclrophenol indophenols titration methods respectively. The data used in a factorial statistical design in a frame of RCD using SAS software program. Results showed during storage period the total phenol had a decreasing trend while vitamin C contents had an increasing trend (42 days after storage) and then antioxidant activity was decreased. More discussion comes up on health status of fruits during storage.

S09P12

Postharvest ethylene treatment reduces quality loss of stored mature sweet orange 'Navelate'

Lafuente M.T., Alférez F., and Romero P.

Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Food Science. Postharvest Physiology and Biotechnology, Spain. mtlafuente@iata.csic.es

Ethylene is usually associated with senescence but it may also play a role protecting fruits from stress conditions causing tissue damage. Its effect may be influenced by the concentration applied, the fruit physiological stage or the susceptibility of a specific cultivar to develop physiological disorders The industry only applies this hormone to citrus fruit for degreening purposes in spite of ethylene may reduce physiological disorders in mature fruits harvested after colour break. The effect of postharvest degreening on the quality of citrus fruit fruits has been documented. Nevertheless, there is no information on the effect of ethylene on the quality of fruits harvested after colour break and stored under commercial conditions required to extend its postharvest life. To address this question, we have examined the effect of treating mature 'Navelate' sweet orange (*Citrus sinensis*) for 4 days with 2 µL/L ethylene at 90-95% RH on the quality of fruit stored at 2 or 12°C and 90-95% RH. Exposure to ethylene had little effect on increasing fruit colour but considerably reduced the incidence of calyx abscission, the incidence of non-chilling peel pitting at 12°C and of chilling injury after 40 days storage at 2°C. Ethylene had no deleterious effect on fruit firmness, weight loss, calyx senescence or acidity and reduced soluble solids by only 4%. Furthermore, ethylene did not enhance off-flavour perception and increased the content of bioactive flavonoids by about 18% in fruit stored for up to 40 days at 2 or 12°C. Therefore, treating the fruits with 2 µL/L ethylene may constitute a useful tool to extend postharvest life of mature citrus fruit since it reduces postharvest physiological disorders and has no negative effect on external or internal fruit quality

S09P13

Organoleptic quality and preservation of fruits tangerines cold storage in relation to application of waxes for postharvest

Nascimento L.M., Machado M.A., Brito M.C.R., Moreira J., Brito P.C.N., and Sanches J.M.

Instituto Agronômico, Brasil. lenice@centrodecitricultura.br

This study aimed to develop new wax formulations with the requirements of the integrated citrus fruit production, in order to maintain the quality of citrus fruit with high commercial potential. These new wax formulations were suited to citrus varieties under study and the local conditions of production in the State of Sao Paulo and other Brazilian regions, allowing the production of fruits with good appearance and minimal change in flavor. The development of new formulations requires a detailed and comprehensive physiological study to allow the availability of reliable products that do not interfere with the physicochemical properties of the fruit during marketing. For that reason, it was necessary to evaluate some physical and chemical parameters that may somehow interfere with the flavor and appearance of the fruit. We evaluated two cultivars of mandarin: 'Ponkan' and 'Decopon'. The main parameters analyzed to evaluate the effectiveness of new wax formulations were: texture, skin color, total acidity, soluble solids, °Brix, ratio, brightness, alcohols, weight loss and internal fruit quality by NMR. We evaluated the genetic code by the production region of the cultivars. The results showed that some formulations led to the change of taste, when subjected to sensory test by a panel of trained tasters.

S09P14

Effect of storage temperature on pitting development in satsuma 'Okitsu'

Nuñez F.N.[1], del Campo R.d.C.[2], Pascale D.P.[3], Díaz L.D.[2], Solari J.S.[3], Larrechart L.L.[1], and Mara H.M.[2]

[1]Empresa Citrícola (NORIDEL S.A.), Salto, Uruguay; [2]Instituto de Investigaciones Biológicas 'Clemente Estable' (IIBCE), Montevideo, Uruguay; and [3]Empresa Citrícola (MODISUR S.A), Salto, Uruguay. raqueldc60@gmail.com

Rind disorders on satsuma mandarin originate serious postharvest losses in Uruguay. Causes of these disorders are not well understood and their incidence is erratic, with high variability from year to year and among orchards. Satsuma fruit suffered chilling injury during cold storage, and pitting are common postharvest symptoms. The objective of this study was to analyze fruit responses to different storage temperatures on pitting development and the incidence of different drench treatments on both fruit rot and pitting development. Satsuma 'Okitsu' fruit were harvested from two different farms, one with high pitting history (Q102) and the other with low pitting history (Q100). Fruit from each farm were drenched with five different treatments and compared to a non-treated control. After the drench treatment, fruit were degreened, waxed and stored at three different temperatures: 3°C, 6°C and room temperature, during 30 days. Fruit stored at room temperature did not show pitting. Fruit stored at 3°C showed the greatest pitting incidence. Farm Q102 showed higher incidence of pitting than farm Q100. The different drench treatments affected rot incidence but not pitting level. Fruit stored at 3°C and 6°C had less incidence of fruit decay than that stored at room temperature. Rind samples from fruit with high pitting of Q102 and from fruit without pitting from both orchards Q100 and Q102 were analyzed for their nutrient composition.

S09P15

RNA-seq and phenylpropanoid metabolism of orange-*Penicillium digitatum* interaction

Ballester A.R.[1], Marcet-Houben M.[2], Gabaldón T.[2], Lafuente M.T.[1], and González-Candelas L.[1]

[1]Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Food Science Department, Valencia, Spain; and [2]Centre for Genomic Regulation (CRG-UPF), Comparative Genomics, Barcelona, Spain. ballesterar@iata.csic.es

Penicillium digitatum is the causal agent of the Green Mold Rot of citrus fruit, being the major postharvest pathogen of these fruits in the Mediterranean-climate regions. The application of fungicides constitutes the most common method to control postharvest diseases. However, due to the development of resistance to fungicides among fungal pathogens and the growing public concern on their effects on human health and environment, there is a need to develop alternative methods to control postharvest diseases. In this study,

with the aim of better understanding the infection process on citrus, we used RNA-Seq transcriptomic analysis of infected oranges at 0, 24, 48 and 72 hours post-inoculation (hpi). Massive parallel pyrosequening with 454 FLX Titanium technology was used for sequencing the libraries. To identify the putative origin of the reads, two reference genomes were used: (i) the *Citrus sinensis* Genome Assembly v1.0 (http://www.citrusgenomedb.org/), and (ii) the *P. digitatum* genome, elaborated in house (submitted). Quantitative reverse transcription PCR profiling of selected fruit and fungal genes revealed dynamic expression patterns during infection of oranges by *P. digitatum*. To further investigate the putative involvement of the phenylpropanoid pathway in the defense of citrus fruit, changes in the metabolic profile of the flavedo (outer colored part of the peel) and albedo (inner white part) infected with *P. digitatum* was studied by means of HPLC-PDA-FD. Metabolite accumulation levels along the time course suggest that flavanones, flavones, polymethoxylated flavones and scoparone are induced in citrus fruit in response to *P. digitatum* infection, although with different trends depending on the tissue.

S09P16

Disruption of the chitin synthase gene *PdigChsVII* in the citrus postharvest pathogen *Penicillium digitatum*

Gandia M., Harries E., and Marcos J.F.

instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Food Science, Spain. mgandia@iata.csic.es

One of the main citrus postharvest pathogens is *Penicillium digitatum*, a citrus specific necrotrophic fungus that penetrates fruit through injured peel and is responsible for important losses. The development of new control methods as alternative to the current use of fungicides is urgently needed. Fungal cell wall (CW) is composed of chitin, glucans, mannans and glycoproteins, and is considered an excellent potential target for novel antifungals. In filamentous fungi, chitin is synthesized by a complex family of chitin synthase genes (*Chs*) grouped into seven classes. We have characterized *P. digitatum Chs* gene expression during different axenic growth conditions and fruit infection. Previous studies suggest a critical involvement in pathogenesis for *Chs* genes of Class V and Class VII. To determine functional relevance, we have obtained deletion mutants of *PdigChsVII* using *Agrobacterium tumefaciens* mediated transformation (ATMT). The resulting deletion strains (Δ*PdigChsVII*) showed reduced growth in axenic culture, higher sensitivity to CW-interfering compounds such as Calcofluor White (CFW) or SDS, and alterations of hyphal morphology. It also showed increase sensitivity to reactive oxygen species (ROS) such as H2O2. Infection assays of citrus fruits with Δ*PdigChsVII* showed virulence similar to the parental strain but defects in mycelium development and, importantly, also in conidia production.

S09P17

Proteins contributing to the pathogenicity of *Penicillium digitatum* towards citrus fruit

Yamashita Y.K.[1], Arimoto Y.A.[1], Makino M.H.[2], Annaka A.H.[1], and Iida I.A.[1]

[1]Riken (Riken), Arimoto Lab., Japan; and [3]Agriculture Optical Laboratory (AOL), Plant Disease, Japan. arimoto@riken.jp

Penicillium digitatum, the causal agent of Citrus Green Mold, infects citrus fruit through injuries in the epicarp causing damage and decay. *P. digitatum* is widely distributed in citrus growing areas around the world and it is considered one of the most destructive pathogens of the citrus industry, resulting in losses of more than half of the harvested fruits. In environments in which pH is around 5.5, *P. digitatum* is saprophytic and does not invade living cells even if there is a wound. However, the fungus is pathogenic at pH lower than 3.5 and infects not only citrus fruit, but all plant cells regardless of the species. In an effort to identify proteins contributing to pathogenicity towards citrus fruit, we performed a 2D-DIGE analysis of *P. digitatum* cultured in citrus epicarp exudate adjusted to pH 5.5 and pH 3.5. At pH 3.5, we identified SOD1, AHA1, CYPA, CYPB, and CFL as highly expressed proteins and TPIA as a low abundant protein. To investigate the contribution of each protein to citrus fruit infectivity, we generated a series of mutants in which the gene coding for each of these proteins was disrupted. The rate of infection of mature fruit was the same as the wild type for all the mutants but the rate of infection in immature fruit was dramatically reduced in the *Δsod1* mutant. At the side of epicarp wounding, generation of O2 was 1.5 to 9 times greater in immature than in mature lemon fruit.

SOD activity in the *Δsod1* mutant was approximately 50% of the wild type. Furthermore, since cell invasion by *P. digitatum* is pH-dependent, we also investigated the pH-dependent transcription factor *PacC*. The *ΔPacC* mutant almost completely lost its pathogenicity towards mature fruit, suggesting that PacC also contributes to the *P. digitatum* pathogenicity.

S09P18

Identification and expression analysis of *Penicillium digitatum* genes involved in fungal virulence during citrus fruit infection

de Ramón-Carbonell M.[1], Ballester A.R.[2], González-Candelas L.[2], and Sánchez-Torres P.[1]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología PVyB, Spain; and [2]Instituto de Agroquímica y Tecnología de Alimentos (IATA), Fisiología y Biotecnologíam Postcosecha, Spain. palomas@ivia.es

Penicillium digitatum, the causal agent of Citrus Green Mold Rot, is the major pathogen of citrus fruit during postharvest. The control of this pathogen relies on the use of fungicides. However, due to the appearance of resistant strains and the harmful effects of synthetic fungicides on human health and the environment, there is a need to adopt new and safer control methods. Our knowledge of the mechanisms underlying pathogen's virulence in postharvest pathogenic fungi, especially in *Penicillium*-fruit interactions, is still very poor. The aim of this study was to identify genes expressed in a virulent *P. digitatum* strain (Pd1) during the early stages of citrus fruit infection through suppression subtractive hybridization (SSH), using a low virulence *P. digitatum* isolate (CECT2954) as a driver. The subtracted cDNA library was sequenced using 454-FLX Titanium technology to perform a global transcriptomic analysis of putative fungal virulence genes. Gene expression analysis of selected *P. digitatum* genes putatively involved in fungal virulence was carried out by qRT-PCR. The expression of these genes was analyzed in both the high and the low virulent *P. digitatum* strains in three different situations: i) in orange disks after 24 h of *P. digitatum* interaction, ii) during growth of *P. digitatum* strains in PDB medium from 24 to 72 h and iii) in orange fruits during *P. digitatum* strains infection from 24 to 72 h. qRT-PCR profiling of selected fungal genes revealed different expression patterns during infection of orange fruits.

S09P19

Metabolism of flavonoids and mycotoxins in citrus fruits infected by *Alternaria alternata*

Del Río J.A.[1], Díaz L.[1], Ortuño A.[1], García-Lidón A.[2], and Porras I.[2]

[1]Faculty of Biology. University of Murcia. (UMU), Plant Biology, Murcia (Spain); and [2]Murcian Institute of Agriculture and Food Research and Development (IMIDA), Citriculture, Murcia (Spain). ignacio.porras@carm.es

Fungi of the genus *Alternaria* are responsible for substantial pre-harvest losses in citrus. The susceptibility to *A. alternata* depends on the citrus species. In this communication, the metabolism of *Alternaria* mycotoxins and citrus flavonoids are analyzed in leaves and fruits of different citrus species and cultivars. HPLC-MS study revealed the presence of two *A. alternata* mycotoxins involved in the evolution of brown spot in leaves and fruits. These were identified as 3,7,9- trihydroxy-1-methyl-6H-dibenzo [b,d] piran-6-ona, known as alternariol (AOH), and 3,7-dihydroxy-9-methoxy-1-methyl-6H-dibenzo[b,d] piran-6-ona, known as alternariol monomethyl ether (AME). Biological assays involving the inoculation of leaves and fruits with the above mentioned toxins showed that both molecules caused necrosis in the inoculated citrus tissues, suggesting that the mycotoxins may be involved in the evolution of necrotic spot caused by *A. alternata*. The increased expression of the above mycotoxins was associated with the end of mycelial growth, high sporulation and an increase in hyphal melanisation. Citrus flavanones and flavones such as hesperidin, naringin, diosmin and sinensetin increased the biosynthesis of these micotoxins. On the other hand, a degradation of flavonoids was observed when leaves and fruits were infected with *A. alternata*. A decrease in the concentration of the flavanones hesperidin, naringin and their respective aglicons was observed in leaves and fruits of susceptible citrus. An increase of extracellular proteins with oxidant activity was detected in leaves and fruits inoculated with *Alternaria*. The study of substrate specificity, molecular weight and different inhibitors confirmed that *Alternaria* expressed an extracellular laccase, and that the citrus flavonoids are substrates of this enzyme. The involvement of mycotoxins, laccase enzyme and degradation of flavonoids in the pathogenesis of *A. alternata* in citrus is discussed.

S09P20

Modulation of host responses by the biocontrol yeast *Metschnikowia fructicola*

Hershkovitz V.[1], Sela N.[2], Rafael G.[1], BenDayan C.[1], Wisniewski M.[3], and Droby S.[1]

[1]The Volcani Center (ARO), Institute of Postharvest and Food Science, Israel; [2]The Volcani Center (ARO), Department of Plant Pathology and Weed Research, Israel; and [3]U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS), Appalachian Fruit Research Station, U.S.A.. vhershko@agri.gov.il

To gain a better understanding of the molecular changes taking place in citrus fruit tissue following the application of the yeast biocontrol agent, *Metschnikowia fructicola*, microarray analysis was performed on grapefruit surface wounds using an Affymetrix Citrus GeneChip. Using a cut off of $p<0.05$ and 1.5 fold change difference as biologically significant, the data indicated that 1007 putative unigenes showed significant expression changes following wounding and yeast application compared to wounded controls. Microarray results of select genes were validated by reverse transcription-quantitative real-time PCR (RT-qPCR). The data indicated that yeast application induced expression of *Rbo*, *MAPK* and *MAPKK*, *G-proteins, CHI*, *PAL*, *CHS*, *4CL*. In contrast, three genes *POD*, *SOD* and *CAT* were down regulated in grapefruit peel tissue treated with the yeast cells. Moreover, suppression was correlated with significantly higher levels of hydrogen peroxide, superoxide anion and hydroxyl radical production in yeast-treated surface wounds. Interestingly, high amounts of hydrogen peroxide were detected inside yeast cells recovered from wounded fruit tissue, indicating the ability of the yeast to actively ROS when it is in contact with plant tissue. This study provides the first global picture of gene expression changes in grapefruit in response to the yeast antagonist *M. fructicola*.

S09P21

Protein hydrolysates as resistance inducers for controlling Green Mould of citrus fruits

Lachheb N.[1], Sanzani S.M.[1], Nigro F.[1], Boselli M.[2], and Ippolito A.[1]

[1]Department of Soil, Plant and Food Science, University of Bari Aldo Moro, Italy; and [2] Department of Biotechnology, University of Verona, Italy. antonio.ippolito@uniba.it

Green Mould caused by *Penicillium digitatum* is responsible for considerable economic losses of harvested citrus fruit. This disease is traditionally controlled by synthetic fungicides. However, because of the appearance of resistant strains and the public concern over pesticide residues in food and environment, safer alternative strategies are needed. In this context, natural elicitors of vegetal, animal, and micro-organic origins might represent promising control means, which could replace or be combined with fungicides. In the present investigation the effectiveness of different products (soybean, lupin, pea, yeast, casein, and malt hydrolysate) was tested *in vitro* and *in vivo* against *P. digitatum*. In *in vitro* experiments, using the tested products at different concentrations (1.6, 3.2, and 6.4 mg/mL), no direct effect was observed on radial growth of *P. digitatum*. Thus, in order to determine the ability to induce resistance, orange fruit were treated and inoculated 24 hours later in different wounds. In *in vivo* application the substances (1.6 mg/mL) effectively reduce diseased incidence (37 to 80%) and lesion diameter (24 to 65%) with best activity recorded by legume-seed-mix and casein hydrolysates. Although further studies are needed, actual data are encouraging and foresee their possible application against postharvest rot caused by *P. digitatum* on citrus fruit.

S09P22

Biochemical and transcriptomic changes associated with induced resistance in citrus fruit treated with sodium salts

Youssef K.[1], Sanzani S.M.[2], Ligorio A.[2], Fallanaj F.[2], Nigro F.[2], and Ippolito A.[2]

[1]Agricultural Research Center, Plant Pathology Research Institute, 9 Gamaa St., 12619 Giza, Egypt; and [2]Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi 'Aldo Moro', Via Amendola 165/A, 70126 Bari, Italy, Italy. fridafallani@yahoo.it

Sodium salts are promising alternative treatments to substitute/integrate the use of synthetic fungicides for controlling postharvest decay of citrus. In vitro studies indicate a direct activity of these salts against *Penicillium* attacking-citrus, inhibiting spore germination, germ tube elongation, and production of pectinolytic enzymes. However, *in vivo* trials differences of salts effectiveness on various citrus species

were observed. Thus, other possible mechanisms of action can be involved. In this study, changes in enzymatic activity and gene expression level of chitinase, ß-1,3-glucanase, peroxidase, and phenylalanine ammonia lyase (PAL) in wounded and unwounded 'Valencia late' sweet orange treated with Na2CO3 and NaHCO3 (3% w/v), were investigated. Both salts increased ß-1,3-glucanase, peroxidase, and PAL activities in orange tissues, whereas chitinase activity decreased. Transcriptomic analyses confirmed PAL up-regulation, particularly at 12 h after treatment. Thus, although other pathways cannot be excluded, the defense response induced by Na2CO3 and NaHCO3 in citrus fruit seems to be correlated with the up-regulation of phenylpropanoid pathway, which has a role in the adaptation process to various stresses. This response could sum up to citrus natural reaction to wounding and pathogen attack, enhancing its protective effect. The results suggest that induced resistance should be considered as one of the possible mechanisms of salts in controlling postharvest citrus decay.

S09P23

In vitro and *in vivo* antifungal activity of eight medicinal plants against Citrus Sour Rot agent *Geotrichum candidum*

Talibi I., Askarne L., Boubaker H., Boudyach E.H., and Ait Ben Aoumar A.
Laboratoire des Biotechnologies et Valorisation des Ressources Naturelles (LBVRN), Biologie, Maroc. drissdesa@gmail.com

The aim of this work was to find an alternative to the chemical fungicides currently used in the control of *Geotrichum candidum*, the cause of Citrus Sour Rot. Hexane, chloroform, ethyl acetate and methanol extracts of eight medicinal plants were evaluated for their *in vitro* and *in vivo* antifungal activity against *G. candidum*. *Cistus villosus, Ceratonia siliqua* and *Halimium umbellatum* methanol extracts proved to be the most effective inhibitors, totally inhibiting arthrospore germination of *G. candidum* at concentrations of 2.5 mg/mL and lower. The methanol extracts of *H. umbellatum, C. villosus* and *C. siliqua* exhibited strong antifungal activity with minimum inhibitory concentrations values ranged between 0.156 and 1.25 mg/mL, and minimum fungicidal concentrations values ranged between 2.5 and 5 mg/mL. Incidence of Sour Rot was lowered to 0, 3.33 and 11.66% when mandarin fruit were treated with *C. villosus, C. siliqua* and *H. umbellatum* methanol extracts at 50 mg/mL, respectively, compared with 95% in the control. These findings suggest that *C. villosus, H. umbellatum* and *C. siliqua* plants may be useful and effective agents for control of Citrus Sour Rot. Such natural products therefore represent a sustainable alternative to the use of synthetic fungicides.

S09P24

In vitro and *in vivo* antifungal activity of several Moroccan medicinal plants against *Penicillium italicum,* the causal agent of Citrus Blue Mold

Askarne L., Talibi I., Boubaker H., Boudyach E.H., Msanda F., Saadi B., Serghini M.A., and Ait Ben Aoumar A.
Laboratoire de Biotechnologies et Valorisation des Ressources Naturelles (LBVRN), Biology, Morocco. as.latifa@gmail.com

Penicillium italicum is the most important pathogen causing Blue Mold in stored citrus fruit. Current control treatments of the disease involve application of fungicides, which may have adverse effects on the environment and promotes the development of strains resistant to the active compound. To overcome these problems, and the increasing concern of consumers towards the use of fungicides, alternative methods for decay control are being investigated. The use of natural substances is a promising alternative that has been explored to manage postharvest decay of fruits. The aim of this study is to evaluate the antifungal activity of 50 plant species, harvested in south of Morocco, against *P. italicum* infection. The *in vitro* antifungal activity of plant powders was determined using the agar plates method. Results showed that among the 50 plants tested, the powder of *Anvillea radiata* and *Thymus leptobotrys* at a concentration of 10% (w/v) completely inhibited mycelial growth of *P. italicum*. The effect of plant aqueous extracts on spore germination and germ tube elongation varied significantly among the different plants. Aquous extracts with high *in vitro* activity were also tested *in vivo* on sweet orange fruit. Incidence of Blue Mold was significantly lowered to 5 and 25% when fruits were treated with *Halimium umbellatum* and *Inula viscosa* aqueous extracts, compared with 98% in control fruits.

S09P25

Effects of fluorescent *Pseudomonas* and *Bacillus sp*. on Blue Mold of citrus caused by *Penicillium italicum*

Askarne L., Talibi I., Boubaker H., Boudyach E.H., Msanda F., Saadi B., Serghini M.A., and Ait Ben Aoumar A.
Laboratoire de Biotechnologies et Valorisation des Ressources Naturelles (LBVRN), Biology, Morocco. as.latifa@gmail.com

Blue Mold of citrus caused by *Penicillium italicum* is an important and devastating disease during storage of citrus fruits. Currently control treatments to manage this disease involve the application of synthetic fungicides, which have adverse effects on the environment and health. To overcome these problems and current concern of consumers towards the use of pesticides, alternative methods for decay control are needed. The use of antagonistic microorganisms is becoming popular throughout the world. A total of 466 bacteria isolated from the surface of citrus fruits, leaves and rhizospheric soil were *in vitro* screened for their antifungal activity against *P. italicum*. Results showed that among 110 active isolates, 69 were fluorescent *Pseudomonas* and 41 belong to the *Bacillus* genus. Isolates which presented the widest inhibition zone (25 mm<Ø< 45 mm) in the *in vitro* study, were *in vivo* screened against Blue Mold on 'Valencia-Late' sweet orange fruit harvested in the Souss-Massa-Draa valley, Agadir, Morocco. The results showed that treatment of fruit by antagonistic bacteria, 2 h before inoculation with the pathogen, induced significant protection. Indeed, more than 46% of tested isolates reduced the incidence of Blue Mold by 0 and 16% after 5 days of incubation at 20°C

S09P26

Practical impact of imazalil resistance on control of postharvest Citrus Green and Blue Mould

Erasmus A.[1], Rikhotso V.[1], Lesar K.H.[2], Lennox C.L.[3], and Fourie P.H.[4]

[1]Citrus Research International (CRI), Postharvet Plant Pathology, South Africa; [2]Citrus Research International (CRI), Extension, South Africa; [3]University Stellenbosch (U.S.), Plant Pathology, South Africa; and [4]Citrus Research International (CRI), Disease Management, South Africa. arno@cri.co.za

Imazalil (IMZ) is highly effective in controlling Green and Blue Mould caused by *Penicillium digitatum* (PD) and *P. italicum* (PI), respectively. The aim of this study was to elucidate the practical relevance of reduced IMZ sensitivity in PD and PI. Nine isolates of PD and 5 of PI with various levels of IMZ sensitivity were selected to conduct *in vitro* sensitivity trials, while fruit inoculation trials were conducted to determine the IMZ residue benchmark levels for curative (24-h-old infections) and protective control. IMZ residues were loaded by dipping orange fruit in 5, 10, 20, 40, 80, 160, 320, 640, 1280 or 2560 µg/mL IMZ sulphate solution. Residue levels ranged from 0.08 to 5.28 µg/g. Mean EC50 values for sensitive (S) PD isolates were 0.03 µg/g, 0.82 µg/g for moderately resistant (MR) and 1.74 µg/g for resistant (R) PD isolates. Benchmark residue levels indicating 75% curative control were 0.31 µg/g for S, 0.91 µg/g for MR, and 2.54 µg/g for R PD isolates; for 75% protective control benchmarks were 0.14 µg/g for S, 1.95 µg/g for MR, and 4.02 µg/g for R PD isolates. All PI isolates were sensitive (0.02 µg/g EC50) with 0.264 and 0.238 µg/g as 75% inhibition benchmarks for curative and protective control, respectively. From these findings, it is clear that reduced IMZ sensitivity in PD and PI will lead to loss of control, which highlights the need for optimised fungicide application and resistance management in citrus packhouses.

S09P27

Effectiveness of the fungicide application system in the control of *Penicillium digitatum* in orange in Tucuman, Argentina

Torres-Leal G.J.[1], Kamiya N.[2], Carbajo M.S.[1], Lacina M.[2], and Farías M.F.[1]

[1]Instituto Nacional de Tecnología Agropecuaria (INTA-Famaillá), Frutihorticulture, Argentina; and [2]Estación Experimental Agroindustrial Obispo Colombres (EEAOC), Chemistry, Argentina. gtleal@correo.inta.gov.ar

The Citrus Green Mold caused by *Penicillium digitatum* produces the main postharvest decay and its control depends on fungicide application. A rotating spray system is currently used in packing-houses for fungicide application. The aim of this work was to compare the effectiveness of the rotating spray (RSS) and immersion systems (IS) for disease control, measuring the residual active ingredients (RAI) in fruits. Sweet orange fruit were inoculated using a punch previously dipped in a spore suspension and stored for 24 h at 20ºC and 90% RH. Then,

fruits received the following treatments: T1 water (control), T2 0.16% IMZ PRM applied with RSS at the packing line, T3 wax 0.2% IMZ with RSS at the packing line, T4 dipping in 0.16% IMZ PRM for 30 seconds, T5 dipping in wax 0.2% IMZl for 30 seconds. Fruits were stored at 4ºC and 90% RH during 15 days and then at 20ºC and 90% HR during 7 days. Fruit damage were evaluated as % of control (incidence). The RAI levels were measured in fruit, flesh and essential oils. Results indicated that the best treatment was T4 with 100% disease control. The RAI in fruit were: 2 mg/kg of PRM and 3.8 mg/kg IMZ, and in essential oils: 104 mg/kg of PRMl and 182 mg/kg of IMZ. T2 treatment yielded 30 % of disease control and the RAI in fruit were: 1.2 mg/kg of PRM and 0.69 mg/kg of IMZ and in essential oils: 89 mg/kg of PRM and 37 mg/kg of IMZ. No residue was detected in the flesh. The effectiveness of the application system was directly related to the RAI levels found in fruits and in essential oils.

S09P28

Resistant strains to postharvest fungicides in Algarve citrus companies. Packinghouse sampling and *in vivo* assays

Salazar M.[1], Mendes S.[2], and Nunes C.[1]

[1]Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM), Universidade do Algarve. FCT, Portugal; and [2]Instituto Universitário D. Afonso III (INUAF), CICAE., Portugal. miguel.salazar@agro-on.pt

Use of fungicides is the main way to control postharvest diseases of citrus fruits. Tiabendazole (TBZ) and imazalil (IZ) are the most used fungicides to control *Penicillium digitatum* and *P. italicum*. However, prolonged use of TBZ and IMZ leads to the selection of resistant strains. Therefore, to identify resistant strains and quantify the level of the resistant population in a packinghouse may be a useful management tool. Sampling methods are based on *in vitro* assays, but *in vivo* responses may differ, since disease development depends on many factors. The aim of this work was to quantify TBZ and IZ *P. digitatum* and *P. italicum* resistant strains in representative packinghouses in Algarve region, Portugal, and to evaluate its potential commercial impact by performing *in vivo* tests. Surveys were conducted during 7 seasons (2004-05 to 2010-11) using a gravimetric method with Petri dishes of PDA, PDA amended with 10 ppm of TBZ, and PDA amended with 1 ppm of IZ. Resistant strains were found in all 14 packinghouses surveyed. Resistance to TBZ was around 24% and to IZ 11%, increasing over the years. Resistant strains were counted in samples from drenching treatments. *In vivo* experiments were conducted to evaluate the outcome obtained from *in vitro* assays. In spite of variability observed, the incidence of resistant strains in commercialized fruits is of paramount importance and should be the subject of further work.

S09P29

Influence of paraben concentration on the development of Green and Blue Molds on 'Valencia' orange fruit

Moscoso-Ramírez P.A.[1], Montesinos-Herrero C.[1], and Palou L.[2]

[1]Colegio de Postgraduados (COLPOS), Campus Tabasco, H. Cárdenas, Tabasco, México; and [2]Institut Valencià d'Investigacions Agràries (IVIA), Centre de Tecnologia Postcollita (CTP), Spain. palou_llu@gva.es

Three paraben sodium salts (p-hydroxy-benzoate) were evaluated as postharvest treatments to control Citrus Green and Blue Molds, caused by *Penicillium digitatum* and *P. italicum*, respectively. The curative activity of sodium methyl (SMP, at 0.1, 1, 10, 40, 70, 100, 150, 200 and 250 mM), ethyl (SEP, at 0.1, 1, 10, 20, 30, 40, 70, and 100 mM) and propyl (SPP, at 0.1, 1, 4, 7, 10, and 100 mM) parabens was evaluated on 'Valencia' sweet orange fruit by inoculating *P. digitatum* and *P. italicum* in rind wounds and, about 24 h later, placing a drop of the solutions in the same rind wound. Disease development was assessed on fruit incubated at 20ºC for 6 days. All three paraben salts reduced mold development. Concentrations of 150, 200 and 250 mM of SMP were the most effective and completely controlled both diseases. Concentrations of 20, 30, 40, 70 and 100mM of SEP reduced the incidence of Green and Blue Mold by 100, 94, 100, 100 and 73% and 87, 62, 93, 100 and 80%, respectively. Concentrations of 7, 10 and 100 mM of SPP reduced the incidence of Green and Blue Mold by 94, 89 and 94% and 70, 88 and 100%, respectively. Most of these concentrations of SMP, SEP and SPP also significantly reduced the severity of both diseases. SMP at 200 mM, SEP at 80 mM and SPP at 100 mM were, among the range of concentrations tested, selected as the best to be used in further research.

S09P30

Selection and *in vitro* evaluation of yeasts for control of G*eotrichum candidum*

Cunha T.[1], Tosin E.S.[2], and Kupper K.C.[3]

[1]Universidade Estadual Paulista 'Júlio Mesquita Filho' - Campus de Jaboticabal (UNESP), Microbiologia Agropecuária, Brazil; [2]Universidade Federal de São Carlos - Campus Araras (UFSCar), Ciências Biológicas, Brazil; and [3]Centro APTA Citros Sylvio Moreira (IAC), Fitopatologia e Controle Biológico, Brazil. taticunha1989@hotmail.com

This paper aimed to test different yeast isolates obtained from soils, leaves, fruits and flowers of citrus from different places in the State of São Paulo/Brazil for the antagonism to G*eotrichum candidum*, the causal agent of Sour Rot of citrus. The decimal dilution technique, plating in YPD, YM and WLN medium, was used for the isolation of potential antagonists. The colonies obtained were incubated at 25° C for 7 days in BOD chamber and the isolated showing a distinctive morphology were purified in YEPD medium. These isolates were evaluated for the antagonism by means of dual cultures in Petri dishes. The experiment was arranged in a completely randomized design, with six replicates and the evaluation was performed by measuring the average diameter of colony growth. The effect of yeast strains on the spore germination of *G. candidum* was assessed using the technique of water-agar on glass slides, evaluating the 15 isolates with higher inhibitory effect *in vitro*. A total of 87 yeast isolates were obtained, and the best results were obtained with the yeast isolates ACBL-77, ACBL-23, and ACBL-52, with a inhibitory effect ranging from 28% to 35%. The germination test showed that, except ACBL-21, all isolates significantly inhibited the germination of *G. candidum* spores. The isolates ACBL-68 and ACBL-23 showed the highest inhibitory rates, with values of 87.7% and 83.4%, respectively.

S09P31

In vitro and *in vivo* evaluation of yeast isolates for biocontrol *of Penicillium digitatum,* causal agent of Green Mold

Kupper K.C.[1], Cunha T.[2], and Tosin E.S.[3]

[1]Centro APTA Citros Sylvio Moreira (IAC), Fitopatologia e Controle Biológico, Brazil; [2]Universidade Estadual Paulista Júlio Mesquita Filho - Campus de Jaboticabal (UNESP), Microbiologia Agropecuária, Brazil; and [3]Universidade Federal de São Carlos - Campus de Araras (UFSCar), Ciências Biolólicas, Brasil. katia@centrodecitricultura.br

This work aimed to test different yeast isolates obtained from soils, leaves, fruits and flowers of citrus from different places in the State of São Paulo/Brazil for *in vitro* and *in vivo* antagonism to *Penicillium digitatum*, the causal agent of Green Mold. The *in vitro* evaluation was performed by means of dual cultures in Petri dishes. The experiment was arranged in a completely randomized design, with six replicates and the evaluation was performed by measuring the average diameter of colony growth. 'Valencia' sweet orange fruit (*Citrus sinensis*) were washed and surface disinfected with a 0.7% sodium hypochlorite solution Fruit were injured in two equidistant points with a 3 mm depth needle and inoculated with a conidial suspension of *P. digitatum* (10^5 spores/mL) 24 hours before and after the treatments. Treatments were applied by dipping the fruits into the antagonist suspensions (10^7 cfu/mL) for 2 minutes, or a fungicide solution of imazalil (0.2%). Inoculated non-treated fruit were considered as control. All fruits were maintained at 20ºC and 99% RU for 7 days. A completely randomized design with three replicates of 20 fruits was used. Control efficacy was evaluated based on the percentage of diseased fruits. The best results *in vitro* were obtained with the isolates ACBL-07, ACBL-14, ACBL-58 and ACBL-67, but they were ineffective in the assays *in vivo*.

S09P32

Optimal concentration of inoculum, types of injuries and control of *Penicillium digitatum* in orange fruits

González-Fierro P.[1], Nieto-Ángel D.[1], Téliz-Ortiz D.[1], Lara-Viveros F.M.[1], and Orozco-Santos M.[2]

[1]Colegio de Postgraduados (CP), Instituto de Fitosanidad, México; and [2]Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Campo Experimental Tecomán, México. orozco.mario@inifap.gob.mx

Green Mold, caused by *Penicillium digitatum*, is the most important postharvest disease of citrus in Mexico. The objectives of this study were: 1) to evaluate the effect of nine different spore concentrations of the fungus in orange fruits, 2) to determine the effect of three types of injuries to allow the disease after inoculation, and

3) to evaluate different treatments for disease control. Disease was observed in 100% of the inoculated fruits when the inoculum concentration was equal or higher than $6x10^5$ spores/mL. Symptoms did not appear with concentrations of $1x10^4$ and $3x10^4$ spores/mL. When injuries were superficial (bruises and deformation) and did not penetrate into the albedo, the infection did not occur. Wounds caused by punctures and cuts induced symptoms after the inoculation. All treatments with *Trichoderma viride* and *T. harzianum*, as well as the extract of *Melaleuca aternifolia*, failed to reduce disease incidence and severity in fruits. The best treatments to control Green Mold were the fungicides pyrimethanil and thiabendazole.

S09P33

In vitro comparison of the effectiveness of disinfectants and a fungicide on the viability of spores of *Geotrichum citri-aurantii*.

Diaz L.D.[1], del Campo R.d.C.[2], Peyrou M.P.[2], and Mara H.M.[2]

[1]Dirección General de Sevicios Agrícolas (MGAP), Montevideo, Uruguay; and [2]Instituto de Investigaciones Biológicas 'Clemente Estable' (IIBCE), Montevideo, Uruguay. raqueldc60@gmail.com

Sour Rot (*Geotrichum citri-aurantii)* is an important postharvest disease of citrus in Uruguay. The disease has been managed so far by using sodium orthophenylphenate (SOPP) and 9-Aza-1,17-diguanidinoetadecane (guazatine). Both fungicides leave residue levels in fruit that are generally not acceptable for export. Therefore, the development of alternative control treatments, such as disinfection, is required. The objective of this work was to compare the effect of various disinfectants and one fungicide on the viability of *Geotrichum* spores *in vitro*. The products evaluated were: 200 ppm sodium hypochlorite, 100 ppm stabilized chlorine dioxide (Tecsaclor®), pH 6 and 6.5; 5000 ppm chloramine-T (Bac-T®); 1600 ppm didecyl dimethyl ammonium chloride/ alkyl amido propyl chloride dimethyl benzyl ammonium chloride (Tefor®); 120 ppm dimethil didecyl ammonium chloride (Sporekill®). The efficacy of 20000 ppm SOPP was also evaluated. For all treatments, a suspension containing 106 spores/mL was prepared and exposed directly to each product solution for 1, 2 or 4 min. The results obtained were compared with those of untreated control fruit. The highest efficiency was obtained for ammonium and chlorine dioxide (pH 6) compounds, which reduced 100% spore viability for the three exposure times evaluated. Sodium hypochlorite during 2 and 4 min killed 97% and 100% of the spores, respectively. Tecsaclor (pH 6.5) reduced 99.8% and 100% of the spores after 2 and 4 min of exposure, respectively. SOPP did not produce a satisfactory effect.

S09P34

Alternative treatments for postharvest decay control on 'Ponkan' tangerines

Montero C.R.S.[1], dos Santos L.C.[2], Andreazza C.S.[2], Schwarz L.L.[2], and Bender R.J.[2]

[1](Fepagro), Research Station Serra do Nordeste, Caxias do Sul; and [2](UFRGS), Horticultural Sciences, Agronomy, Brazil. rjbe@ufrgs.br

Penicillium spp. are the most frequent pathogens associated with postharvest decay in citrus fruit. Pre- and postharvest chemical sprays are the most widespread methods to reduce incidence, though, in the scientific literature there is plenty of evidence on promising alternative postharvest treatments. Therefore, in the present work the objective was to evaluate the effects of alternative treatments on the control of Green and Blue Mold and on the postharvest quality of 'Ponkan' tangerines. During two seasons, fruit harvested in commercial orchards were treated with different combinations of sodium bicarbonate, potassium sorbate, and heat treatments, in single or combined applications. Two control treatments, chloride dioxide and imazalil were also included. The tangerines were evaluated after 15 or 30 days of cold storage at 5°C plus 5 more days at room temperature for internal quality and decay incidence. Quality traits were not affected by any of the treatments. Sodium bicarbonate at a concentration of 2% (w/v) and potassium sorbate at 0,5% (w/v) significantly reduced decay on 'Ponkan' fruit after 30 days of storage. However, peel brightness was negatively affected by an immersion treatment of 30 sec at 58°C with both salts at concentrations higher than 3% and 1%, respectively. Further research on salt concentration and temperature combinations should be conducted to conclude about the feasibility of alternative postharvest treatments for citrus fruit.

S09P35

Curative and protective control of *Penicillium digitatum* following imazalil application in aqueous dip and wax coating

Njombolwana N.S., Erasmus A., and Fourie P.H.

Stellenbosch University, Citrus Research International (US, CRI), Plant Pathology, South Africa. shellie@sun.ac.za

Imazalil (IMZ) is widely used in citrus packhouses to manage Green Mould, caused by *Penicillium digitatum*. The aim of this study was to investigate curative and protective green mould control by IMZ when applied in a postharvest wax coating, and double IMZ application in aqueous dip followed by coating. Trials were conducted on clementine, satsuma, 'Navel' and 'Valencia' sweet orange fruit and treatments were dip only (IMZ sulphate at 500 µg/mL for 45 s and 90 s), wax coating only (imazalil at 3,000 µg/mL at 1.8 L wax/ton), and a double application comprising of dip (45 s in IMZ sulphate at 500 µg/mL) followed by 2,000 µg/mL IMZ in wax coating at 0.6, 1.2 and 1.8 L wax/ton. Double application generally improved IMZ residue loading, more so with increasing coating load, while the wax coating only resulted in the highest residue level. Poor curative and protective control of the resistant isolate was observed (<46% and <55%, respectively). For the sensitive isolate, dip only treatments resulted in better curative control (≈ 77%) than protective control (≈ 38%). Double application increased protective control with increasing coating load (≈ 69%) as well as curative control (≈ 83%). Wax coating only treatment resulted in poor curative control (≈ 26%), but good protective control (≈ 80%) and improved sporulation inhibition. Double application of IMZ demonstrated superior Green Mould control given its good curative and protective control and sporulation inhibition.

S09P36

Fruit quaility and storage potential of 'Kinnow' mandarin (*Citrus reticulata*) in relation to different geographical locations of Pakistan.

Khalid M.S., Malik A.U., Amin M., Khalid S., Asad H.U., Raza S.A., and Malik O.H.

Postharvest Research and Training Centre (PRTC), Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan. shafiquemalik81@yahoo.com

The objective of this study was to evaluate the quality of 'Kinnow' mandarin and its storage potential on fruit produced in four different districts of Pakistan: Punjab, Sargodha, Toba Tek Singh, Vehari and Khanewal. Six orchards in each district were selected for comparison and fruit were analyzed for external and internal quality. Wind, mites and thrips were identified as the major causal agents of skin blemishes in all districts. Regarding the extent of blemishes, A and B grade fruit were found significantly higher in district Toba Tek Singh. Other physico-chemical quality attributes, such as fruit firmness and fruit diameter, were higher in Vehari district. However, the percentage of juice was higher in Sargodha district. Among other parameters, total soluble solids and acidity were higher in fruit from Vehari, while TSS/TA ratio and vitamin C content were higher in fruit from Sargodha. Total sugar content was higher in fruit from Toba Tek Singh. Significantly higher TSS and TSS/TA values were recorded in fruit from Toba Tek Singh kept at ambient temperature. Under cold storage, TSS was significantly higher in fruit from Toba Tek Singh after 60 days, whereas the TTS/TA ratio was higher in fruits from Sargodha stored for the same period. The environmental and specific climatic conditions were important factors affecting external and internal quality of 'Kinnow' mandarin fruit.

S09P37

Effect of organic and inorganic salts as alternative strategy for the control of the postharvest Citrus Sour Rot agent *Geotrichum candidum*

Talibi I., Askarne L., Boubaker H., Boudyach E.H., and Ait Ben Aoumar A.

Laboratoire des Biotechnologies et Valorisation des Ressources Naturelles (LBVRN), Biologie, Maroc. drissdesa@gmail.com

The aim of this study was to find an alternative to the fungicides currently used in the control of postharvest Citrus Sour Rot, caused by *Geotrichum candidum*. We screened thirty-four salt compounds, considered as common food additives, for their activity against *G. candidum*. The lowest Minimum Inhibitory Concentrations (MICs) values were obtained by ammonium carbonate and EDTA at a concentration of 0.1% (w/v) and boric

acid, sodium carbonate and sodium metabisulfite at 0.25% (w/v). Medium-pH in the range of 4.0 to 12.0 did not influence mycelial growth of the pathogen. The ten best salt compounds were tested for their ability to reduce arthrospore germination. The effects of salts varied significantly ($P<0.05$) among tested compounds, and were highly dependent on their concentrations. Arthrospore germination was completely inhibited by EDTA, boric acid, sodium metabisulfite, sodium carbonate, sodium sulfate and sodium thiosulfate, at 75 and 100mM. The most active salts *in vitro* were tested *in vivo* against Sour Rot on citrus fruit. Disease incidence was reduced to 26% and 39%, when mandarin fruit where treated by sodium salicylate, boric acid and EDTA, compared with 100% in the non-treated control. However, only the application of boric acid at 3% (w/v) reduced disease severity by more than 70%. These results suggested that sodium salicylate, boric acid and EDTA may be useful and effective compounds for control of Citrus Sour Rot in organic production, and provide a sustainable alternative to the use of guazatine.

S09P38

Lemon fruits from endochitinase transgenic plants exhibit resistance against postharvest fungal pathogens

Oliveri C.[1], Distefano G.[1], La Malfa S.[1], La Rosa R.[1], Deng Z.[2], and Gentile A.[1]

[1]Dipartimento di Scienze delle Produzioni Agrarie e Alimentari (DISPA), Italy; and [2]Horticulture and Landscape College, China.
larosar@unict.it

Constitutive over-expression of antifungal genes from microorganisms involved in plant defence mechanisms represents an efficient strategy to confer genetic resistance against a broad range of phytopathogenic fungi. In lemon (*Citrus limon*), fungal diseases cause economic losses in the field as well as in postharvest management. In this study, fruits collected from two different transgenic lemon clones (SR23 and SR24) expressing a chitinase gene (*chit42*) from *Trichoderma harzianum* were tested *in vivo* to investigate the level of resistance against *Penicillium digitatum*, *P. italicum, Colletotrichum gloesporioides* and *Botrytis cinerea*. Fruits were artificially inoculated with conidial suspensions of the above mentioned fungi. Experiments were carried out in duplicated in climatic chamber and symptoms development was evaluated 6, 14 and 21 days after inoculation. As compared to control fruit, transgenic fruits did not show mould symptoms when inoculated with *C. gloesporioides* and *B. cinerea* during the whole evaluation period. A reduction in the lesion area of 50% was recorded after 6 days from inoculation with *P. digitatum* and *P. italicum* in transgenic fruits compared to the control. However, differences among the analyzed genotypes were not evident after the moulds. The analyzed transgenic lemon clones were obtained from a widespread appreciated lemon cultivar and did not show any difference in morphological and qualitative fruit traits compared to the control. Therefore, the results of this study suggested that their use could represent an alternative for sustainable management of lemon postharvest diseases.

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WATERING AND NUTRITION

S10O01

Open hydroponics of citrus compared to conventional drip irrigation best practice: first three years of trialling and Australian experience

Falivene S[1], Navarro Acosta J.M.[2], and Connolly K[1].

[1]NSW Department of Primary Industries (NSW DPI), Intensive Industries, Australia; and [2]Dpto. Citricultura Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA), Spain. steven.falivene@dpi.nsw.gov.au

Hydroponics is a system of growing plants in an inert media (e.g. rockwool), supplying the plants' nutrition using mineral salts dissolved in the irrigation water and irrigating frequently throughout the day. "Open Hydroponics" (OH) is a system where trees are grown in soil, but nutrient needs are managed similarly to hydroponics and trees are irrigated throughout the day. OH is a relatively recent production innovation, and is being adopted around the world, and there are anecdotal reports of superior yields attributed to OH. But, OH programs do not necessarily consider the soil as a critical part of the production program. This has raised concerns regarding soil acidification, waterlogging and nitrate leaching. Few scientific data are available on OH, and there is an industry-wide knowledge gap. A replicated extension trial was conducted to compare citrus OH to conventional practice in young citrus. In the first three years, data indicates no differences in yield, tree growth, soil and/or leaf nutrient dry matter content. Soil solution data is highly variable but indicates that adequate levels of nitrogen are present in the soil for both programs. Anecdotal evidence suggests acidifying irrigation water has no benefit and water use in pulse irrigation is higher than best practice conventional. The uptake of OH in Australia has been mixed, some growers have benefited and others have found no benefit and reverted to conventional practice.

S10O02

Deficit irrigation strategies: preliminary assessment on a Sicilian young orange orchard

Stagno F.[1], Parisi R.[2], Cirelli G.[2], Consoli S.[2], Roccuzzo G.[1], Barbagallo S.[2], and Intrigliolo F.[1]

[1]Citrus and Mediterranean Crops research centre - CRA (CRA-ACM), Italy; and [2]Università di Catania (DiGeSA), Dipartimento di Gestione dei Sistemi Agroalimentari e Ambientali, Italy. giancarlo.roccuzzo@entecra.it

The economical sustainability of citrus orchards has to cope with the availability of water resources. One way to optimise water resources is to employ deficit irrigation (DI) strategies. DI effects on some physiological characters of young orange orchards were analysed. The orchard includes 300, 4-years old, plants located at the CRA-ACM experimental farm in Lentini, Sicily (37° 20'N; 14° 53'E). Four irrigation strategies were replicated 4 times: (i) Sub-surface drip irrigation, supplying 75% IR (irrigation requirement), (ii) Partial root-zone drying, supplying 50% IR, (iii) Regulated deficit irrigation, supplying 50-100% IR, depending on crop phenological phases and (iv) Surface drip irrigation, supplying 100% IR. The IR was calculated using the Penman-Monteith approach, by assuming a crop coefficient of 0.45, and correction coefficients accounting for the localized irrigation. On selected plants, measurements of LAI, PAR, xylematic potential, canopy temperature, gas exchanges, trunk diameter and shoot number were carried out. Soil water content (SWC) was monitored by using Time (and Frequency) Domain Reflectometry. Preliminary results, obtained during the 2011 season, showed that: (i) xylematic potential and stomatal conductance were slightly variable between the investigated treatments with values quite below the stress condition, (ii) LAI and PAR were slightly variable between each plant and treatments, (iii) SWC was below the field capacity, (iv) trunk diameters were quite similar among the analysed plants.

S10O03

Citrus water use in South Africa

Vahrmeijer J.T.[1], Annandale J.G.[2], Gush M.B.[3], and Taylor N.J.[4]

[1]Citrus Research International (CRI), Fruit Production and Quality, South Africa; [2]University of Pretoria (UP), Plant Production and Soil Science, South Africa; [3]Counsel for Scientific and Industrial Research (CSIR), Natural Resources and the Environment, South Africa; and [4]University of Pretoria (UP), Plant Production and Soil Science, South Africa. jtv@up.ac.za

Agriculture, as the largest user of fresh water worldwide, is under close scrutiny to justify its use of water, due to the increase in competition from a number of end-users. Apart from on-farm measures to increase

water use efficiency, government intervenes through policy instruments to influence water use behaviour. Citrus growers need to take heed of these changes in policy as the majority of citrus orchards are irrigated and more detailed information on seasonal water requirements and irrigation scheduling is needed to justify the quantity of water needed for the production of citrus. However, measurements of citrus orchard water use are not possible for all environmental and management combinations, primarily due to the expense and time it takes to make the measurements. Models, which are formidable tools to predict water use and crop performance, are therefore vital to provide accurate estimates of citrus water use across different climatic regions. In order to model citrus water use, a thorough understanding of the factors governing citrus water use is required. Results from measurements performed in a number of citrus orchards across climatic zones in South Africa indicated that citrus water use, under conditions of unlimited soil water supply, is not solely governed by atmospheric demand, but also by internal resistances to water movement within the plant, which limits the amount of water that a citrus tree can transpire on hot dry days. Increasing irrigation volumes during days with high evaporative demands will therefore not necessarily reduce tree stress but may exacerbate the problem, by increasing root resistance, as a result of poor aeration. We will report on the institutional arrangements regarding water use and results from current and previous research on the water use in citrus in South Africa.

S10O04

Evapotranspiration over an irrigated orange orchard using micrometeorological techniques and sap flow measurements

Consoli S.[1], Continella A.[2], Motisi A.[3], Papa R.[1], and Pasotti L.[4]

[1]University of Catania (DIGESA), Department of Agri-food and Environmental Systems Management, Italy; [2]University of Catania (DISPA), Department of Agricultural and Food Science, Italy; [3]University of Palermo (DEMETRA), Italy; and [4]Regione Siciliana (SIAS), Servizio Informativo Agrometeorologico Siciliano, Italy. acontine@unict.it

During recent years, a substantial progress in understanding the evolution of evapotranspiration processes in cropping systems has produced detailed models and innovative measurement techniques for describing the mass (water, CO_2) and energy (solar radiation) exchanges in the soil-plant-atmosphere continuum. Within this context, the aim of the study was to investigate, develop and validate integrated approaches to better understand and quantify mass and energy exchanges processes in an orange orchard in the Mediterranean environment. Different micrometeorological techniques, (i.e. eddy covariance, surface renewal analysis of the sensible heat fluxes, surface energy balance), were used to estimate the evapotranspiration fluxes of the irrigated orange grove in eastern Sicily (Italy). Transpiration was determined by the sap flow method. The results are referred to a 2-year period and showed satisfactory comparisons between the investigated methods. The findings of this study will allow for the definition of suitable techniques and methodology for the rational management of water resources in the tree crop environment under water scarcity conditions.

S10O05

Partial root-zone drying effects under different rootstocks and irrigation systems in Valencia, Spain

Ortega J.[1], Forner-Giner M.A.[2], Quiñones A.[2], Legaz F.[2], and Intrigliolo D.S.[1]

[1]Insituto Valenciano Investigaciones Agrarias (IVIA), Sustainable Agriculture, Spain; and [2]Insituto Valenciano Investigaciones Agrarias (IVIA), Citriculture, Spain. intrigliolo_die@ivia.gva.es

Citrus trees culture in Spain is mainly located in areas of water scarcity. It is then important to improve irrigation efficiency. With this aim, a research has been conducted in order to explore if partial root-zone drying (PRD), an irrigation technique aimed at alternating water applications along the two sides of a tree, could be used to increase water use efficiency (WUE). The research was conducted in Valencia in field and pot conditions under superficial and subterraneous drip irrigation systems and under different rootstocks (Carrizo citrange and the Forner-Alcaide dwarfing hybrids). In a first set of pot experiments, plantlets were submitted to either full water application, or deficit irrigation either, under PRD or conventional watering. Deficit irrigation allowed increasing WUE (total dry mass produced/water transpired), but PRD did not lead to any significant gain in WUE when compared to conventional DI regardless the rootstock employed. In field conditions, under a given watering regime, sub-surface drip irrigation allowed improving tree water status,

gas exchange, foliar SPAD index and yield when compared with superficial irrigation. On the other hand, deficit irrigation increased P, K and Na foliar concentrations but reduced yield and affected fruit quality (increasing fruit acidity). Overall the results indicate that deficit irrigation and the sub-surface irrigation system can be both used as measures for increasing plant WUE. However, PRD, contrarily to previous evidence obtained in more arid environmental conditions, did not allow any gain in plant performance when compared with a similar watering regime. Our results question the applicability of the more expensive PRD irrigation system in the commercial citrus orchards of the Valencia region.

S10O06

A critical evaluation of citrus leaf mineral status guidelines for optimal yield in Israel

Raveh E.

Agricultural Research Organization (ARO), Fruit trees, Israel. eran@agri.gov.il

Maintaining orchards at optimal leaf mineral concentration is one of the key issues for maximal yield. Experiments for verifying and updating guidelines are very rare since they require several years of field experiments in mature bearing trees which are expensive. The evaluations of such guidelines at the grower level are more complicated processes and therefore usually not done. -In the present paper we first evaluate the Israeli guidelines for citrus by comparing them to the Israeli orchards leaf mineral status using a 10 year leaf mineral database (results from 20244 leaf analysis that were sampled from commercial orchards all over Israel). Then we tried to create an updated guideline using a second database that summarized yield and leaf mineral concentration collected from 122 orchards all over Israel over 7 years (the Israeli National Wastewater Effluent Irrigation Surveys database). There was a disagreement between the local recommendations and the orchards leaf mineral status (e.g local recommendation were higher than orchard median values), which demonstrates the needs for guidelines updating. Based on the Israeli National Wastewater Effluent Irrigation Surveys database a new guideline was set. It was found that the optimal leaf mineral concentration for grapefruit is 1.7-2.1% DW of N, 0.08-0.010% DW of P, 0.37-0.48% DW of K, and 0.33-0.45% DW of Mg. For orange trees the optimal leaf mineral concentration is 1.9-2.3% DW of N, 0.11-0.14% DW of P, 0.80-1.00% DW of K, and 0.19-0.26% DW of Mg. For easy peelers trees the optimal leaf mineral concentration is 2.0-2.4% DW of N, 0.09-0.12% DW of P, 0.55-0.69% DW of K, and 0.19-0.26% DW of Mg. Maintaining the leaf within this rages can allow maximal yield of 110-120 t/ha for grapefruits, 65-70 t/ha for oranges, and 60-70 t/ha for easy peelers.

S10O07

What limits nitrogen fertilization responses of fertigated citrus orchards under tropical conditions?

Mattos Jr. D.[1], Quaggio J.A.[2], Boaretto R.M.[1], Souza T.R.[2], and Zambrosi F.C.B.[2]

[1]Instituto Agronômico (IAC), Centro de Citricultura Sylvio Moreira, Brazil; and [2]Instituto Agronômico (IAC), Centro de Solos e Recursos Ambientais, Brazil. ddm@centrodecitricultura.br

Fertigation of citrus orchards was recently adopted by growers in Brazil. The inherent low fertility and acid soils under the tropics implies that efficient nutrient management practices need to be conducted to ensure high fruit quality and yield. A field experiment with Valencia sweet oranges on Swingle citrumelo was established in 2003 when trees were 3-4-year old and conducted during a six-year period. Trees received 60, 120, 180 and 240 kg/ha per year of N, as ammonium nitrate (AN) or calciun nitrate (CaN), via fertigation using two dripper lines per row. Fruit yield for pooled data reached 70 t/ha with 180 kg/ha of N as AN, what agrees with reported fertilization curves, in which this maximum is attained with N rates of approximately 200 kg/ha. On the other hand, fruit yield increased linearly up to 85 t/ha with the highest N rate as CaN. Soil analysis demonstrated that acidification was greater after AN applications. In this case, soil pH was below 4.6 in the surface and 3.8 at deeper soil layers despite frequent liming applications to the soil. This resulted from increased ammonium uptake by roots, what correlated with nutrient concentrations in soil solution extracts. Leaf Ca was <30 g/kg with AN and 40 g/kg with CaN. Similarly, Ca content of flowers and fruitlets was greater for the CaN treatments. Therefore, unbalanced absorption of cations and anions caused by excess ammonium limited nitrogen fertigation responses in the orchard.

S10O08

Uptake of ^{44}Ca and ^{15}N by young citrus trees

Quiñones A., Martínez-Alcántara B., Alcayde E., and Legaz F.

Instituto Valenciano de Investigaciones Agrarias (IVIA), Citriculture and Vegetal Production, Spain. legaz_fra@gva.es

Calcium is a macroelement involved in numerous physiological processes in plants, such as the division and growth of cells. Calcium is part of the structure of cell walls and membranes and together with the magnesium participates in the activation of metabolic enzymes of proteins and carbohydrates. There are numerous studies on Ca conducted in the plant-soil system, however no information is available on their absorption and distribution among different organs. In this sense, the use of ^{44}Ca and ^{15}N as tracers is a useful tool since allows to know exhaustively the fate of the element applied in the plant-growing medium system. Ca absorption by plant roots depends on several factors, among which are the availability of this nutrient in the culture medium. Therefore, the main objective of this study is to evaluate the response of 7 different ^{44}Ca and ^{15}N concentrations on their uptake and their distribution among different organs in young plants of citrus grown in sand substrate in a growth chamber. At the end of the labelling period, plants were fractionated into young developing organs, old leaves, stem, coarse and fibrous roots. Increasing Ca concentration did not affect significantly the biomass of the different organs. However, significant differences were obtained in ^{44}Ca and ^{15}N uptake. Ca uptake efficiency decreased from 28% to 15% when increasing Ca supplied (0.5 mM to 32 mM), while N uptake efficiency decreased from 49% to 17% (2.5 mM to 70 mM). Whereas Ca was mainly partitioned to coarse roots, N accumulated in new flush leaves.

S10O09

A new approach to front citrus iron chlorosis: organo-mineral fertilizers from glass-matrix and organic biomasses

Torrisi B.[1], Trinchera A.[2], Allegra M.[3], Epifani R.[2], Marcucci A.[2], Roccuzzo G.[1], Intrigliolo F.[1], and Rea E.[2]

[1]Centro di ricerca per l'Agrumicoltura e le Colture Mediterranee (CRA-ACM), ITALY; [2]Centro di ricerca per lo studio delle Relazioni tra Pianta e Suolo (CRA-RPS), ITALY; and [3]Centro di ricerca per l'Agrumicoltura e le Colture Mediterranee (CRA-ACM), ITALY.
francesco.intrigliolo@fastwebnet.it

The aim of the present paper was to deepen the agronomical responses of glass-matrix based fertilizers (GMF), a typology of fertilizer able to release nutrients on the basis of plant-demand, whose nutrient release increases thanks to the mixing with organic biomasses, such as digested vine vinasse (DVV) and meat-meal (MM). Laboratory tests and short-term pots trials provided concurring results about the attitude of the mixture GMF DVV to increase nutrient availability and plant development. In the following stage these mixtures were tested for their ability to contrast citrus iron chlorosis in calcareous soils, the most complex phenomenon in citrus orchard and one of major abiotic stresses affecting fruit tree crops in the Mediterranean area. Three years studies were realized: i) in pots, on 'Tarocco Scirè' orange trees [*Citrus sinensis*] grafted on two different rootstocks (Carrizo citrange and Swingle citrumelo) and ii) in field, on 'Tarocco Scirè' on sour orange rootstock [*C. aurantium*], by comparing GMF and its mixtures with DVV and MM application to a synthetic Fe chelate one. The GMF DVV mixture was able to supply adequately micronutrients, particularly iron, reducing the chlorosis symptoms, increasing the leaf SPAD index, Fe concentration and decreasing Fe index.

S10O10

Nitrogen and calcium equilibrium on citrus nutrition

Quaggio J.A.[1], Souza T.R.[1], Boaretto R.M.[2], Zambrosi F.C.B.[1], and Mattos Jr. D.[2]

[1]Instituto Agronômico (IAC), Center of Soil, Brazil; and [2]Instituto Agronômico (IAC), Center of Citriculture, Brazil.
quaggio@iac.sp.gov.br

Nitrogen (N) and calcium (Ca) are major nutrients in citrus trees and responsible for the ionic equilibrium of plant cells. Additionally, Ca absorption is directly affected by the form of N supply; *e.g.* ammonium or nitrate. Therefore, high yield and fruit quality of citrus depend on balanced application of those, especially under intensive nutritional program as fertigation. The objective of this study was to evaluate the mineral N and Ca2 absorption

by citrus and consequent effects on nutrient concentrations in young mature leaves, stem sap extracts and wet bulb soil solution, and fruit yield. Data from two long-term field trials were evaluated in fertigated orchards with 10 to 12 –year old Valencia on Swingle citrumelo rootstock, with N rates varying from 0 to 240 kg $ha^{-1}yr^{-1}$ applied either as ammonium or calcium nitrates. The trials were monthly monitored by nutrient analyses. The results along two seasons showed: *(i)* an interdependence of N and Ca absorption, *(ii)* decreased $N\text{-}NO_3\text{-}/N\text{-}NH_4$ratio in the soil solution and in the sap extracts with increased ammonium nitrate rates, which negatively affected Ca absorption and its concentration in the sap extract and leaves, *(iii)* maximum fruit yield was attainted with $N\text{-}NO_3\text{-}/N\text{-}NH_4$ - ratios of 25 and 7, respectively, in the wet bulb solution and the sap extract, and *(iv)* unbalance absorption of cations and anions caused by excessive $N\text{-}NH_4$ absorption and, consequently, decreased soil pH solution, what compromises the fertigation sustainability of citrus groves in tropical soils.

S10P01

Physiological and nutritional responses of Navel orange trees to different irrigation and fertigation practices

Navarro J.M.[1], and Falivene S.[2]

[1]Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA), Citricultura, Spain; and [2]NSW Department of Primary Industries (NSW DPI), Australia. josefam.navarro2@carm.es

A trial was established on drip irrigated Atwood Navel orange trees growing in Australia to compare daily irrigation with conventional practices. Trees were subjected to five treatments of combined irrigation and fertigation treatments: conventional irrigation application of conventional nutrients; daily pulse irrigation application of conventional nutrients; conventional irrigation proportional injected nutrition; daily pulse irrigation proportional injected nutrition and continuous irrigation proportional injected nutrition. Since irrigation methods could modify the tree water status and fertigation could alter trees nutrition, we studied the physiological response during the summer-autumn period and the nutritional state after three years of application of the treatments. Trees were well-irrigated in all treatments, even at the most critical moments, and no clear differences in gaseous exchange parameters were found with different irrigation treatments. Conventionally irrigated trees had similar water potential values to daily irrigated trees and these values were always far away from the threshold for well-irrigated trees. When nutrients were applied in a conventional way, irrigation practices did not modify foliar N, P, and K levels; with proportional injected nutrition, pulse irrigation increased K and P foliar levels. As conventionally irrigated trees consumed lower amounts of water and had a similar yield to daily irrigated trees, they presented the highest water use efficiency.

S10P02

Influence of precipitation in the fruit growing season on sugar accumulation in Satsuma mandarin fruit juice

Takishita F.[1], Uchida M.[2], Hiraoka K.[1], and Nesumi H.[2]

[1]National Institute of Fruit Tree Science (NIFTS), Kuchinotsu, Japan; and [2]National Agricultural Research Center for Western Region (WeNARC), Citrus innovative production team, Japan. takisita@affrc.go.jp

The influence of precipitation during the fruit growing season on the fruit quality and fruit juice sugar concentration was investigated over 7 years in Satsuma mandarin trees cultivated with the conventional thinning method and the intentional alternate bearing system. In this experiment, there was no difference between the two thinning methods in fruit quality and fruit sugar concentration. On the other hand, there were significant differences among years, and a negative correlation was observed between the amount of precipitation in August and the total sugar concentration at harvest time. Precipitation from July to November(x) decreased the glucose, fructose, and total sugar concentration(y) at harvest time. The correlation was shown by the equation y=-0.0046x 11.39. The sucrose concentration increased successively throughout the fruit growing season. On the other hand, the glucose and fructose concentration increased due to little precipitation in July and August but decreased with significant precipitation in September. Thus, the concentration of monosaccharides were influenced by the precipitation in the fruit growing season and greatly affected the total sugar concentration at harvest time.

S10P03

Recover of plugged drippers in orange grove irrigation system using mechanical and chemical techniques

Fachini E.[1], Junqueira Franco A.C.B.[2], and Galbiatti J.A.[3]

[1]Centro Universitário de Araraquara (UNIARA), Agronomia, Brazil; [2]Aporte Técnico para o Desenvolviment (ATDA), Brazil; and [3]Universidade Estadual Paulista (UNESP - FCAV), Engenharia Rural, Brazil. emfachini@terra.com.br

A study was carried out to evaluate the effects of the combination of two techniques, chemical and mechanical on the dripper unclogged and uniformity distribution coefficient (UDC). The experiment was set up in Monte Azul Paulista city, in a commercial orange grove that was drip irrigated since 2002. The dripper were RAM type, with 3.5 L/h and the space between drippers was 0.85 m. The flowing evaluation from Keller and Karmeli (1975), modified by Deniculi et al. (1980) was used to evaluate 4 lateral lines (the first line, the lines located at 1/3, 2/3 of beginning of the block and the last line). In each line, 8 drippers were evaluated (the first, those located a 1/7, 2/7, 3/7, 4/7, 5/7, 6/7 from the beginning of the lateral line and the last one). Despite the UDC improved after all treatments applications, the drippers average flow increased when compared with the initial flow rate. The percentage of the drip plugged decreased to 0. The results showed that the treatments used were very effective in eliminating drip plugging but they modified the dripper flow rate.

S10P04

Effect of Sunred metabolic promoter and deficit irrigation on fruit quality of 'Valencia' oranges

Massenti R., Barone F., Farina V., and Lo Bianco R.

Università degli Studi di Palermo, Demetra, ITALY. roberto.massenti@unipa.it

This study evaluated the effects of deficit irrigation and Sunred foliar metabolic promoter on fruit quality of adult 'Valencia' orange trees. Three irrigation strategies were imposed in spring 2011: i) irrigation with volumes corresponding to 100% of crop evapotranspiration applied to the entire root-zone (CI), ii) partial root-zone drying (PRD) with 50% of CI water applied to one alternated side of the root-zone, and iii) continuous deficit irrigation (DI) with 50% of CI water applied to both sides of the root-zone. Four trees in each irrigation treatment were sprayed 30 and 20 days before harvest with Sunred metabolic promoter containing phenylalanine, methionine, mono-saccharides and oxylipins from plant extracts. Climate and soil parameters were monitored continuously in the field; crop load and fruit quality were measured at harvest in May 2012. Fruit peel color was determined by digital image analysis. After adjusting for crop load, fruits of PRD and DI trees tended to be smaller but sweeter than those of CI trees. Regardless of irrigation, Sunred increased mainly TSS:acid ratio by lowering acidity. Average fruit peel color was similar in sprayed and control trees, but a smaller coefficient of variation indicated greater uniformity in fruit peel color. These preliminary results suggest that, regardless of the irrigation strategy, pre-harvest Sunred foliar sprays may improve internal fruit quality of 'Valencia' oranges by advancing and making more uniform fruit maturity.

S10P05

Foliar and root application of potassium nitrate and calcium nitrate to *Citrus macrophylla* seedlings under NaCl stress

Navarro J.M., Andujar S., and Rodríguez Morán M.

Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA), Citricultura, Spain ringil_1@hotmail.com

A growth chamber experiment was conducted to determine whether KNO_3 or $Ca(NO_3)_2$ could mitigate the adverse effects of salt stress on *Citrus macrophylla* seedlings. Six treatments were studied: C (control), S (30 mM NaCl), KR (30 mM NaCl 10 mM KNO_3, applied to the root system), KL (30 mM NaCl 10 mM KNO3, applied to the leaves), CaR [30 mM NaCl 10 mM $Ca(NO_3)_2$, applied to root system] and CaL [30 mM NaCl 10 mM $Ca(NO_3)_2$, applied to the leaves]. Although plant growth was unaffected by the saline treatment, salt injury in the form of leaf burn occurred. Salinity also affected other parameters, such as leaf gas exchange, which

strongly decreased with the application of 30 mM NaCl. The potassium and calcium applications did not lead to increase photosynthesis rates. Neither the Ca nor the K treatment reduced the high Cl levels found in leaves, and leaf Na was only reduced by the CaR treatment. The calcium levels in leaves following the CaR treatment were higher than in C plants, whereas the CaL, KR and KL treatments led to lower Ca levels than in C plants. This was also the case with membrane stability, which increased with CaR but not with the foliar application of Ca or either of the K treatments. Cellular damage produced by salinity decreased with KL and both Ca treatments. These results suggest that, in general, the adverse effects of salinity on the nutritional and physiological response of *C. macrophylla* are not alleviated by the foliar treatments of Ca and K applied in this experiment.

S10P06

Effect of mulching and drip irrigation on Satsuma mandarin fruit quality in a volcanic ash soil

Han S.G., Kim Y.H., Moon Y.E., and Kang S.B.

Citrus Research Station, National Institute of Horticultural & Herbal Science, Rural Development administration, Korea.
skhan@korea.kr

The study aimed at improving water management in Satsuma mandarin (*Citrus unshiu* Cv. Okitsu Wase) cultivated with macro-porous film (MPF) mulching system in an open field environment under high precipitation regime. In those humid conditions, fruit often struggle to reach the desired total soluble solids (TSS). The MPF mulching systems was treated to repel water when it rains and water vapor releases from the soil. The mulching system was set up every early June before the onset of the rainy season and lasted up to the harvest period (November) from 2009 to 2011. Some observations indicated that TSS concentration increases from September to November, with the highest increasing rate observed in October. On the other hand, fruit titratable acidity (TA) decreased largely in September and October, with hardly decrease after November. Results show that fruit from trees cultivated under dry soil conditions had higher TSS and TA. In order to identify the optimal water supply after mulching, Satsuma mandarins were subjected to different watering treatments i) 0 (no irrigation), ii) irrigation application of 1.500 m^3/ha, and iii) watering at 2.400 m^3/ha. Irrigation started when fruit reached TSS 8.0 °Brix, and lasted until harvest. The control (no irrigation treatment) showed the highest TSS and also TA with 1.0% higher than the high quality standard (HQS; TSS≥12°Brix, TA≤1.0%). The 2.400 m^3/ha treatment lead to fruit with TSS values lower than the HQS of 12.0 °Brix. The 1.500 m^3/ha treatment showed higher TSS than 12.0 °Brix and TA lower than 1.0%. In summary, the high quality Satsuma mandarin could be produced by drip-irrigation with water supply at 1.500 m^3/ha.

S10P07

Tree-to-tree variation in plant-based measurements as indicators of orange water status

Motisi A.[1], Continella A.[2], Massenti R.[1], and Romolo F.[1]

[1]University of Palermo (DEMETRA), Italy; and [2]University of Catania (DISPA), Department of Agriculture and Food Science, Italy.
acontine@unict.it

A study was conducted in order to determine the sensitivity of measured indicators of the plant water status in a citrus orchard. In ten adult orange [*Citrus sinensis*] trees, grafted on sour orange (*C. aurantium*) rootstocks and drip irrigated every second day, sap flow by Granier TDP probes, leaf and xylem water potential, gas exchange and leaf and canopy thermal imaging were hourly monitored all over the day. The evaluations were performed during two separate clear-sky days with different environmental conditions, respectively in the summer and autumn seasons. Variations of environmental components (reference evapotranspiration, solar radiation, vapour pressure deficit and temperature) were recorded. Each measurement technique resulted effective to perceive continuous plant water status at the proper scale (tree and orchard level). In particular, transpiration rate, water deficit stress indicators, stomatal conductance and closure were related to diurnal changes in ET0 and net radiation. Tree-to-tree variations in the diurnal dynamics of tree gas exchange were evaluated to develop up-scaling rules of tree-level water consumption measurements.

S10P08

Predicting the impact of the climatic variability in the necessities of watering of the grapefruit in the Island of the Youth in Cuba

Cueto J.C., Pardo A.P., and Durán G.D.

Instituto de Investigaciones en Fruticultura Tropical (IIFT), Grupo Difusión Tecnológica Isla de la Juventud, Cuba. despacho@iift.cu

The expected increase in air temperature and the uncertainty related with the future predicted rainfall imply that irrigation might become a more important cultural practice for citrus trees located in humid areas. The objective of this work was to determine the present and future irrigation water needs for grapefruit citrus trees. The investigation was carried out in the conditions of the Island of the Youth (Cuba). The irrigation requirements for grapefruit culture during the period 1991- 2009 was calculated, and the predictions for 2050 were also performed. The analysis of the potential evapotranspiration/rainfall (ETo/P) ratio showed that this value decreases near one from mid-June until the end of August. However, if the current climatic trends will continue during the decade 2020-2030, the ETo/P ratio might decrease to values well below one. In that case, irrigation will have to be supplied to the plantations. It is predicted an annual increment of the irrigation needs of 60 m^3/ha.

S10P09

Effect of regulated deficit irrigation on vegetative growth, fruiting, stomatal conductance, leaf proline content and water use efficiency for Nules clementine grown in the Souss valley of Morocco

Bagayogo S., El-Otmani M., El-Fadl A, and Benismail M. C.

Institut Agronomique et Vétérinaire Hassan II, Complexe Horticole d'Agadir (IAV-CHA), Horticulture, Morocco. elotmani.mohamed@gmail.com

Water scarcity is becoming a major problem for agricultural development in the Mediterranean area including Morocco. This research aims at developing irrigation strategies on citrus with the goal of reducing water supply without significant negative effects on yield or fruit parameters. The effect of four treatments on 5-year old Clementine trees (*Citrus reticulata*) grafted on *Citrus macrophylla* was assessed using drip irrigation and supplying different water amounts according to tree phenological stage for a total of 470 mm of water [treatment T0 = crop water needs (ETc)], 580 mm [treatment T1 = 123% T0], 350 mm [T2 = 77% T0] or 304 mm [T3 = 67% T0] applied during the 1 Feb. - 30 Oct. cropping period. Irrigation water was applied daily using emitters with different water flows and quantities supplied were based on local evapotranspiration and crop water needs (ETc). The duration of irrigation varied from 1 h in the winter time (supplying 0.9 mm/day to the least watered trees to 1.8 mm/day to the well irrigated ones) to 2 h 40 min during hot summer days (supplying 1.8 to 3.7 mm/day, respectively). Results show that stressed trees have reduced vegetative growth but more flower buds and flowers. Stomatal conductance was positively correlated with water dosage whereas leaf proline content followed the inverse trend. These results and those on yield, final fruit size and quality (juice content, acidity, sugar content), and on water use efficiency (as kg fruit produced/m^3 of water used) will be presented and discussed in relation to the water deficit applied and its stage of application.

S10P10

Response of 'Star Ruby' grapefruit trees to deficit irrigation during different stages of fruit growth: effects on water relations, yield and fruit quality

Pérez-Pérez J.G., Robles J.M., García-Oller M.I., and Botía P.

Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA), Department of Citriculture, Spain. juang.perez@carm.es

Grapefruit is an emergent crop in the southeast of Spain in recent years. In spite of the fact that grapefruit is well adapted in semiarid regions, the irrigation necessities for fresh fruit production continue to be very high. The scarcity of water resources forces citrus growers to optimise water use by using deficit irrigation

strategies. The aim of this work was to evaluate the sensitivity to deficit irrigation (DI) of different fruit growth stages of 14-year-old 'Star Ruby' grapefruit (*Citrus paradisi* Mac.) grafted on 'Cleopatra' mandarin (*Citrus reshni* hort. ex Tan.), regarding water relations, yield and fruit quality. The experiment was carried out in an experimental orchard located in Torre Pacheco (Murcia, southeastern Spain) during two years. There were four irrigation treatments: Control (100% ETc) and three DI treatments (50% ETc) applied only during different fruit growth stages: DI_{Ph-I} (cell division), DI_{Ph-II} (cell elongation) and DI_{Ph-III} (final fruit-growth period, ripening, and harvest). The midday stem water potential (Ψ_{md}) of DI_{Ph-I} and DI_{Ph-III} were influenced by the rainfall regime in both years, whereas the Ψ_{md} of DI_{Ph-III} was decreased and remained lower during throughout the period. The main effects of both the DI_{Ph-I} and DI_{Ph-III} treatments were related with changes in fruit quality parameters, where DI_{Ph-I} reduced the percentage of juice and DI_{Ph-III} affected negatively the peel color when the water stress was moderate. However, the effects of DI_{Ph-II} were more drastic, decreasing yield due to smaller fruits, altering fruit composition, increasing much more the titratable acidity than the total soluble solids, and reducing peel color, therefore delaying fruit maturation processes. Based on these results, the periods less sensitive to DI were Ph-I and Ph-III, which should be considered for future studies of regulated deficit irrigation under Mediterranean climates.

S10P11

Water relations and productivity of Natal orange plants under controlled deficit irrigation in São Paulo State, Brazil

Pires R.C.M.[1], Silva A.L.B.O.[1], Ribeiro R.V.[1], Quaggio J.A.[2], Lima M.A.F.[1], and Zani M.L.[3]

[1]Agronomic Institute (IAC), Ecophysiology and Biophysic - Irrigation, Brazil; [2]Agronomic Institute (IAC), Soil nutrition, Brazil; and [3]Agricultural Engineering (FEAGRI - UNICAMP), Brazil. rcmpires@iac.sp.gov.br

Agriculture is responsible for a large consumption of water in the world when compared to other economic activities. Deficit irrigation is one of the methods that in some cases can promote water savings, without reducing significantly the crop yield. The aim of this work was to evaluate the plant water relations and productivity of "Natal" orange trees grafted onto Swingle citrumelo under controlled deficit irrigation. The experiment was carried out in randomized blocks with five treatments and four replications from 2009 to 2010. The treatments consisted of different irrigation regimes: T1 – optimal irrigation dose (100% of crop evapotranspiration - ETc), T2 - 80% of ETc, T3 - 60% of ETc, T4 - 40% of ETc and T5 - 20 % of ETc. The irrigation was applied by drip system. The effects of deficit irrigation on plant water relations were evaluated by measuring leaf transpiration and stomatal conductance. Both physiological variables were favoured in T1, T2 and T3. The yield and number of fruits were higher when applied 100%, 80% and 60% of ETc, in T1, T2 and T3, respectively. Our data revealed that a significant amount of water (~40%) can be saved with deficit irrigation without negative impacts on citrus yield and leaf water relations.

S10P12

Responses of Clementina de Nules citrus trees to deficit irrigation under different rootstocks. Tree performance and fruit quality

Ortega J.[1], Molina M.D.[2], Intrigliolo D.S.[1], de Miguel A.[2], Bonafé C.[2], Valero J.L.[2], Peiró E.[2], and Forner-Giner M.A.[3]

[1]Instituto Valenciano Investigaciones Agrarias (IVIA), Sustainable Agriculture Center, Spain. [2]Instituto Valenciano Investigaciones Agrarias (IVIA), Technology Transfer Center, Spain. [3]Instituto Valenciano Investigaciones Agrarias (IVIA), Citriculture, Spain. intrigliolo_die@ivia.gva.es

Rootstock tolerance to soil water deficit has been evaluated in a field trial located in Valencia (Spain). Clementina de Nules (*C. clementina* hort. ex Tan.) were grafted either on Carrizo citrange [*Citrus sinensis* (L.) Osb. x *Poncirus trifoliata* (L.) Raf.], the most common citrus rootstock in Spain, or Forner-Alcaide 5 (a hybrid of *C. reshni* hort. ex Tan. x *P. trifoliata*) recently released by the citrus breeding program of the Instituto Valenciano de Investigaciones Agrarias. Trees were planted in 1998 and the irrigation regime trial started in 2008 and lasted until 2011. Trees were submitted to either full irrigation (Control) or deficit irrigation (DI, watering at 60% of the Control regime). During the summer period, plant water status and leaf gas exchange

were evaluated. Yield, fruit quality and tree size were determined every year. Citrus fruit disorders were evaluated only in the last experimental season. There was a cumulative effect over years of deficit irrigation on tree growth. In both rootstocks, after five years, DI reduced tree canopy growth by 50%. Yield was only reduced by DI in the last experimental season by 12-20%. Juice content, acidity and total soluble sugar concentration were affected by both the rootstock type and the irrigation regime. Puffing of Clementine fruits was not affected by the irrigation regime. Creasing was only increased by DI in the Carrizo citrange rootstock while in the Forner-Alcaide 5 the irrigation regime did not affect this fruit disorder.

S10P13

Root distribution of orange trees under two different drip irrigation systems

Fachini E.[1], and Galbiatti J.A.[2]

[1]Centro Universitário de Araraquara (Uniara), Agronomia, Brazil; and [2]Universidade Estadual Paulista (UNESP), Engenharia Rural, Brazil. emfachini@terra.com.br

A study was carried out in two orange groves of irrigation and fertirrigation with a drip irrigation system to compare root distribution of trees under different management after four years. The experiment was set up on the commercial grove in Palestina and Altair, State of São Paulo, Brazil. Trees of Natal sweet oranges on Cleopatra mandarin were 21-year-old. The Altair grove was established with two drip lines per row of trees, and the Palestina grove with one drip line. The irrigation management in both places used the class pan evapotranspiration. Soil core samples were collected 1.0, 1.5 and 2.0 m from the canopy edge, either along the tree row or between rows at the 0-15, 15-30, 30-45, and 45-60 cm soil depth layers. The method of weight of root dry mass was used to estimate distribution in the soil. The distribution of roots along the tree row was 3.8 times bigger in the Palestina grove compared to the Altair one. The root distribution in between rows was 1.4 times bigger in Palestina than in Altair. In this study, the irrigation with one drip line per row of orange trees limits the root system growth. The distribution of roots within a smaller volume of soil could be important to increase efficiency of absorption of water and nutrients by trees.

S10P14

The effect of herbicide treatments on soil nutrient availability and plant nutritional status in a citrus garden

Wu C.Q.[1], Liao C.X.[2], Ou Y.[1], and Xie Y.H.[1]

[1]Chongqing Academy of Agricultural Sciences (CAAS), Fruit Research Institute, China; and [2]Chongqing agriculture commission (CAC), China.. cqxieyh@hotmail.com

This paper reports the effects of weed control with herbicides on the nutritional status of Licheng [*Citrus sinensis*]. Tested treatments were: control (CK) #1 (41% Nong pan glyphosate IPA salt, 200x diluted) #2 (41% Chun duoduo glyphosate IPA salt 200x diluted) #3 (10% Hai ning glyphosate 30x diluted) # 4 (30% Feida glyphosate 150x diluted) #5 (Paraquat 200x diluted). Results showed the content of total nitrogen in the soil did not vary for herbicides compared with CK. On the contrary, the content of available phosphorus in soil increased 2.0%~15.3% compared to the CK while the effect of treatments #1 and #2 were similar. Herbicides could decrease the content of available iron in soil, since effects of treatments #2 and #3 showed a remarkable difference between each other. At the late stage of herbicide use, the manganese content of soil became slightly higher than CK with no noticeable difference except for treatment #3. Otherwise, the content of zinc decreased in all herbicide treatments. We also found that total leaf iron decreased 1.9% to 3.7% in the CK compared to treatments # 2, #3 and #4. Herbicide treatments #1, #2 and #4 decreased zinc content of leaves by 2.3% to 3.7% compared with the CHK. The manganese leaf content could be increased to an extent of 2.3% to 5.7% in response to herbicide treatments. The nitrogen leaf content behaved was different in treatments #3 and #4. The content of phosphorus did not vary in all treatments. Therefore, herbicide use can decrease the content of available iron and zinc to plants in spite of its effectiveness in weed control.

S10P15

Efficiency of zinc (68Zn) fertilizers applied to the soil with citrus

Boaretto R.M.[1], Hippler F.W.R.[1], Quaggio J.A.[2], Boaretto A.E.[3], Abreu Jr. C.H.[3], and Mattos Jr. D.[1]

[1]Instituto Agronômico (IAC), Centro de Citricultura Sylvio Moreira, Brazil; [2]Instituto Agronômico (IAC), Centro de Solo e Recursos Ambientais, Brazil; and [3]Universidade de São Paulo (USP/ CENA), Centro de Energia Nuclear na Agricultura, Brazil.
boaretto@iac.sp.gov.br

The supply of zinc (Zn) affects citrus production in Brazil, because of inherently low nutrient availability and high predicted interaction with soil colloids. Therefore, the form of Zn application and fertilizer sources are important for nutrient management of orchards. The aim of this study was to evaluate the effect of Zn fertilizers applied to the soil and the nutrient absorption by citrus trees. The experiment was carried out in a greenhouse using one-year-old potted orange trees. The plants were grown in two different textured soils (181 and 644 g kg^{-1} of clay) that received 1.8 g $tree^{-1}$ of Zn either as ZnO or $ZnSO_4$. The Zn fertilizers were enriched with the stable 68Zn isotope, which allowed estimation of Zn absorption by roots and its partitioning into the orange trees. The Zn fertilization increased nutrient availability in the soil and content in the orange trees. The $ZnSO_4$ was the most efficient fertilizer source providing highest accumulation of ^{68}Zn in the tree parts, with 70% of the total accumulated found in the roots. The Zn in the plant derived from the labelled fertilizer was higher in the stem and branches than in the leaves. The application of Zn in the sandy loam soil provided greater dry matter production, accumulation of Zn and enzymatic activity of the superoxide dismutases in the trees. The application of Zn in the clayey soil was less effective to increase nutrient availability to the orange trees, especially when the ZnO was applied.

S10P16

Interaction of magnesium in calcium uptake in young citrus trees

Martínez-Alcántara B., Quiñones A., Alcayde E., and Legaz F.

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Citriculture and Vegetal Production, Spain. legaz_fra@gva.es

Calcium is a macroelement involved in numerous physiological processes in plants, such as cell division and growth, it is part of the structure of cell walls and membranes, and together with the magnesium participates in the activation of metabolic enzymes of proteins and carbohydrates. There are numerous studies on Ca conducted in the plant-soil system, however, no information is available on its absorption and distribution among different plant organs. In this sense, the use of the stable isotope ^{44}Ca as a tracer becomes a valuable tool since it allows to accurately determine the fate of the element applied in the plant-growing medium. Ca absorption by plant roots depends on several factors, among which are the availability of this nutrient in the culture medium and the presence of antagonists such as magnesium. The main objective of this study was to evaluate the effect of different Mg concentrations (0.5, 1, 2, 4, 8 mM) in the growing medium on Ca uptake and its distribution among different organs in young plants of citrus grown during one month in controlled conditions (growth chamber) using ^{44}Ca as a tracer. Increasing concentrations of Mg significantly reduced ^{44}Ca content in harvested plants, and hence the Ca-uptake efficiency.

S10P17

Study on the relationship between fruit quality, soil nutrient availability and tree nutritional status

Lu X.P., Huang C.N., Xiao Y.M., Nie Q., Zhao X.L., and Xie S.X.

College of Horticulture and Landscape (CHL), Hunan Agricultural University, China. puninglu@126.com

Ponkan is one of the two important mandarin varieties in China because of superior fruit yield and quality, and fresh market value. Aiming to guide precise fertilization and increasing grower income, the relationship between fruit quality, and soil and tree nutrient status was studied within five of the main Ponkan production areas: Yongshun county (YS), Baojing county (BJ), Jishou city (JS), Luxi county (LX) and Xinhua county (XH). The results showed that soil pH value of five areas ranged from 4.4 to 5.4. Water coefficient was significantly higher in XH than that in the other areas. Soil organic matter in JS and LX was lowest and also lower than the

optimum value. Available nitrogen content was highest in XH and the lowest in JS, but all of the areas were lower than the optimum. There was similar trend for leaf nitrogen content. Phosphorus contents in soil and leaves varied in different areas; tree leaf phosphorus from XH and BJ reached the optimum value, while others were lower than that. Soil potassium was the lowest in JS and LX orchards, while leaf potassium content in all area orchards was lower than the optimum value. Magnesium and calcium availability in soil were high in all areas, but very low in leaves. Zinc in all areas reached the optimum value in both soil and leaves. Data of fruit quality analysis indicated that Ponkan fruits from five areas had similar fruit weight, TSS and titratable acid, while fruits from XH presented the highest sugar and lowest acid contents.

S10P18

Boron supply affects growth of citrus and correlates with hydraulic conductivity and gas exchange of rootstock varieties

Mesquita G.L., Mattos Jr. D., Ribeiro R.V., Boaretto R.M., and Quaggio J.A.

Instituto Agronômico (IAC), Tecnologia de Produção, Brasil. gelm_1@hotmail.com

Boron (B) nutrient disorders cause malfunction of the vascular cambium and growth inhibition. The nutrient distribution and its concentration in the leaves of citrus vary among rootstocks, mainly due to the characteristics of roots and water transport in the plant. The objective of this study was to evaluate the effect of B availability on tree growth, and correlate it to hydraulic conductivity and gas exchange of two citrus rootstock varieties. The experiment was conducted in nutrient solution in a factorial design with 1-yr-old Valencia orange trees on two rootstocks: Swingle citrumelo (SW) and Sunki mandarin (SK) supplied with three B levels: poor, adequate and excessive (0, 0.5 e 2.5 mg L^{-1} of B). The hydraulic conductivity of the plant (*KL*) and gas exchange were measured 130 days after beginning the experiment, when plants were collected and separated into roots, stems and leaves for dry mass estimations. The dry mass production, *KL* and CO_2 assimilation (*A*) were differentially affected by the levels of B. Growth and *KL* increased linearly with B rates in the nutrient solution for trees on SW [81.3 g per plant and 3.16 mmol(H2O) $m^{-2}s^{-1}MPa^{-1}$, respectively], whereas *A* was maximum with the adequate nutrient level (3.49 µmol m^{-2} s^{-1}). Excess B caused significant injuries on trees on SK, which showed optimum plant growth, *KL* and *A* with the adequate B level [53.3 g per plant; 6.02 mmol(H_2O) m^{-2} s^{-1} MPa^{-1}, and 6.05 µmol.m^{-2}.s^{-1}, respectively].

S10P19

Silicon uptake in citrus and the validation of an analytical method

Vahrmeijer J.T.[1], Asanzi N.M.[2], and Taylor N.J.[2]

[1]Citrus Research Inernational (CRI), Fruit Production and Quality, South Africa; and [2]University of Pretoria (UP), Plant Production and Soil Science, South Africa. jtv@up.ac.za

The importance of silicon as a beneficial element for improving yield and quality of many crops, including citrus, is well-known. However, whilst analytical methods to determine Si in plant material have been developed and the uptake of Si in other plants is well documented, there is limited information on the uptake and analysis of Si in citrus. Therefore, experiments were conducted to validate an analytical method for Si and to assess the uptake of Si in citrus. Microwave-assisted digestion was used for Si extraction and the Si concentration was determined with an induced couple plasma optical emission spectrum (ICP-OES), which was compared to the standard colorimetric method. Silicon uptake experiments in citrus varieties (Valencia and Clementine) were conducted in winter and summer with two Si formulations, potassium silicate and silicic acid and was applied to the roots (0, 75 and 150 mg kg^{-1}) and as a foliar spray (0, 100 and 500 mg L^{-1}). A correlation of 98% was found between the ICP-OES and the standard colorimetric method.- The uptake study results showed no significant differences (P≤0.05) in Si content for different Si formulations and citrus varieties, but Si content in citrus leaves increased significantly (P≤0.01) with maturity. Silicon absorption increased significantly (P≤0.05) with application rate and Si content in the leaves was significantly higher (P≤0.05) when applied to the roots, than when applied to the leaves. Si uptake was also significantly higher in summer than in winter.

S10P20

Phosphorus deficiency decreases nutrient use efficiency of citrus

Zambrosi F.C.B., Mattos Jr. D., Quaggio J.A., and Boaretto R.M.
Instituto Agronômico (IAC), Solos, Brasil. zambrosi@iac.sp.gov.br

The evaluation of the effects of P availability on the efficiency of nutrient absorption and utilization by citrus rootstocks is required to extend the knowledge on plant responses to P deficiency. Thus, seedlings of citrus rootstocks 'Rangpur' lime, 'Swingle' citrumelo, 'Cleopatra' and 'Sunki' mandarins were grown on nutrient solution with 0.0125; 0.05; 0.2 and 0.8 mmol L-1 of P. After 100 days of P treatments, the rootstocks were harvested and then separated into leaves, stem and roots to estimate plant dry weights, and N, K, Ca, Mg, S and B concentrations. The nutrient accumulation by the citrus rootstocks was proportional to P supply in the nutrient solution. Regardless of treatment, the following pattern of nutrient accumulation by the rootstocks was observed: 'Rangpur' lime > 'Swingle' citrumelo > 'Sunki' mandarin > 'Cleopatra' mandarin. The lowest P concentration in the nutrient solution significantly decreased the efficiency of nutrient absorption and utilization compared to 0.2 and 0.8 mmol L-1 of P. The 'Rangpur' lime was the most efficient rootstock in nutrient utilization for growth, while 'Swingle' citrumelo was the least efficient in nutrient absorption. The results demonstrate that low-P availability decreased the accumulation, efficiency of absorption and utilization for growth of N, K, Ca, Mg S and B by the plants, suggesting that the adequate management of P fertilization is critical to improve nutrient use efficiency in citrus orchards.

S10P21

Enhancement yield, fruit quality and nutritional status of Washington Navel orange trees by application of some biostimulants

El-Shazly S.M.[1], and Mustafa N.S.[2]
[1]Faculty of Agriculture, Alexandria University, Alexandria, Egypt. [2]Agricultural and Biological Division, National Research Center, Cairo, Egypt. samyshazly55eg@yahoo.com

The effect of biostimulants [potassium humate, yeast extract and amino green II (amino acids mixture)] on yield, fruit quality and nutritional status of Washington Navel orange trees were evaluated under field conditions. Potassium humate was applied at 10 and 20 g/tree as soil application. In addition, yeast extract at 0.2 and 0.4% and amino green II at 0.25 and 0.5% were applied as foliar application. The obtained data revealed that all biostimulants increased total yield compared to control. The highest yield was obtained with trees received potassium humate at 20 g/tree. Fruit weight, fruit juice %, total soluble solids (TSS), total sugars and vitamin C contents were markedly increased with biostimulants treatments compared to control. Moreover, high values of such characters were obtained with trees treated with potassium humate at 20g/tree and active dry yeast at 0.4%. Biostimulants treatments enhanced leaf chlorophyll content and leaf mineral contents of N, P, K, Ca and Mg. It was concluded that biostimulants treatments had a promotional effect on yield, fruit quality and nutritional status of Washington Navel orange trees. In this respect, potassium humate at 20 g/tree and active dry yeast at 0.4% were considered promising treatments.

S10P22

Calcium, magnesium and potassium levels in the soil and in the leaf of orange trees on different rootstocks and soil conservation systems in the northwest of Paraná State, Brazil

Gil L. G.[1], Auler P. A. M.[2], and Pavan M.A.[1]
[1]Instituto Agronônico do Paraná (IAPAR), Área de Solos, Brasil; and [2]Instituto Agronônico do Paraná (IAPAR), Área de Fitotecnia, Brasil. lggil@iapar.br

This study aimed to determine the effects of soil preparation, *Brachiaria brizantha* mowing, and rootstocks on soil nutrient availability and leaf Ca, Mg and K contents of orange trees, on a Typic Haplorthox originated from the Caiua Sandstone. The experimental plots were defined by soil preparation: no-tillage (NT), strip-tillage (ST) and conventional tillage (CT); the subplots by mowing management between tree rows: conventional (CM) and mulching (MM), and the subsubplots by rootstocks: Rangpur lime and Cleopatra mandarin. Leaf

and soil samples (0-10,10-20, 20-30 and 30-40 cm depth layers, along tree rows and between rows) were collected from 2005 to 2009 for chemical analyses. Leaf Ca content was higher for the CM and for the Cleopatra mandarin trees. Leaf K was higher for the MM and for the Rangpur ones. Ca and Mg in the 0-10 cm soil layer between rows were higher for NT, and in the lower layers the contents were higher for CT. The MM increased exchangeable K availability in all soil depths, and this management reduced the Ca content down to the 30 cm depth layer. The establishment of orange orchards under the NT system did not cause detrimental effects on soil fertility. On the contrary, it favored the increase of Ca and Mg in the top soil layer. The MM was important not only for the maintenance of adequate K levels in the soil but also for the improvement of the K recycling in the soil system.

S10P23

Response of clementine citrus to foliar potassium fertilization: Effects on fruit production and quality

Hamza A[1], El Guilli M[2], Bamouh A[3], Zouahri A[4], Bouabid R[5], and Lfadili R[6].

[1]Institut National de la Recherche Agronomique (INRA), Agronomy, Morocco; [2]Institut National de la Recherche Agronomique (INRA), Phytopthology, Morocco; [3]Institut Agronomique et Vétérinaire Hassan II (IAV Hassan II), Plant Production, Protection and Biotechnology, Morocco; [4]Institut National de la Recherche Agronomique (INRA), Soil Science, Morocco; [5]Ecole Nationale d'Agriculture de Meknès (ENAM), Soil Science, Morocco; and [6]Institut Agronomique et Vétérinaire Hassan II (IAV Hassan II), Agronomy, Morocco. hamza.abdelhak@gmail.com

Clementine "Cadoux" (*Citrus reticulata Blanco*) is a citrus fruit that is well appreciated by consumers. However, this cultivar tends to produce fruits of small to medium size that are less accepted commercially. In this study, we evaluated the effects of various rates and frequencies of foliar potassium fertilization, as either potassium nitrate (KNO_3) or potassium sulfate (K_2SO_4), on fruit production (fruit size, weight and yield) and quality parameters (skin thickness, firmness, color index, maturity index, juice content, acidity and total soluble sugars). Application rates of tested foliar fertilizers were 5% and 8% KNO_3, 2.5% and 4% for K_2SO_4, applied either two or three times during fruit growth on orchards of three planting densities (D1: 6 x 6 m, D2: 5 x 6 m and D3:- 6 x 3.5 m). The levels of potassium in leaves of clementine var. Cadoux increased by 40% two weeks after the last foliar potassium application. Fruit weight increased with potassium application rate and frequency. Potassium fertilization treatments in three foliar applications showed the best percentages of fruits of extra size class, whatever the source of potassium (KNO_3 or K_2SO_4) or plant density. Foliar applications of potassium increased fruit color, firmness and rind thickness. Fruit juice content, acidity and total soluble sugars were slightly increased by foliar potassium application. Raising potassium concentration and the number foliar applications increased tree fruit production. In terms of efficiency of foliar potassium fertilizers, 4% K_2SO_4 in three applications resulted in a maximum gain of fruits per kg of foliar fertilizer.

S10P24

Impact of biostimulant substances on growth of Washington Navel orange trees

Mustafa N.S.[1], and El-Shazly S. M.[2]

[1]National Research Center, Cairo (Citrus nutrition), Pomology, Egypt; and [2]Pomology Department, Faculty of Agriculture, Alexandria University, Alexandria, Egypt. nabilhotline@yahoo.com

In a field experiment, Washington navel orange trees were treated with three biostimulants [potassium humate, active dry yeast and amino green (amino acids mixture)] to study the effect of these substances on vegetative growth and leaf contents of Na and Cl. Potassium humate was applied at 10 or 20 g/tree to the soil. In addition, yeast extract at 0.2 or 0.4%, and amino green II at 0.25 or 0.5% were applied to the plant leaves. The results showed that tree height, leaf area, leaf fresh and dry weights and volume index were positively affected by the biostimulants treatments. Greater vegetative growth of trees was achieved with potassium humate > active dry yeast > amino green. Data also demonstrated that biostimulant treatments significantly decreased leaf Na and Cl contents compared to untreated control trees. Therefore, such substances (especially potassium humate) can be used to reduce negative impacts of salinity in soil or irrigation water. In general, biostimulants substances had a beneficial effect on growth performance and leaf contents of Na and Cl of Washington navel orange trees.

S10P25

Foliar nutrition with macronutrients for Valencia orange and Murcott tangor trees

Rodríguez V.A.[1], Alayón Luaces P.[1], Píccoli A.B.[2], Chabbal M.D.[3], Giménez L.I.[4], and Martínez G.C.[2]

[1]Facultad de Ciencias Agrarias - UNNE (FCA - UNNE), Producción Vegetal, Argentina; [2]Facultad de Ciencias Agrarias - UNNE (FCA - UNNE), Física y Química, Argentina; [3]Facultad de Ciencias Agrarias - UNNE (FCA - UNNE), Producción Vegetal, Argentina; and [4]Facultad de Ciencias Agrarias - UNNE (FCA - UNNE), Cálculo Estadístico y Biometría, Argentina. cocorodriguez@live.com.ar

In order to achieve quick tree responses, opportune foliar fertilization is used complementary to soil nutrition. The objective of this study was to analyze the effects of different doses of foliar fertilizers on 'Valencia late' sweet orange and 'Murcott' tangor. Field experiments were done during three seasons in commercial orchards in Corrientes, Argentina, on complete block designs with four replications and experimental plots of four trees. Tested treatments were: T1 control; T2 N (12%) 2 L ha^{-1}; T3 N (12%) 4 L.ha^{-1}; T4 N (9%) and P (2.6%) 2 L.ha^{-1}; T5 N (9%) and P (2.6%) 4 L ha^{-1}; T6 N (9.3%), P (2.6%) and K (2.1%) 2 L ha^{-1}; T7 N (9.3%), P (2.6%) and K (2.1%) 4 L ha^{-1}; sprayed three times per season, at pre-flowering, full flowering and last summer sprouting. Leaf concentrations of N, P and K were determined in leaves of fruiting branches obtained in autumn. At harvest, total fruit production was measured and diameter, percentage of juice, soluble solids content, acidity and ratio were determined on a sample of 40 fruits per plot. Variance analysis and Duncan test were performed. In 'Valencia late', compared to the control, all sprayed treatments increased P foliar concentration. Treatment T7 increased yield but fruits had smaller diameter. In 'Murcott', fertilizer treatments increased fruit yield compared to the control, with higher production in T7 and T6 plots; T7 increased P foliar and decreased total soluble solids in juice compared with control. Foliar fertilization with macronutrients allows increased fruit production of 'Valencia late' sweet orange and 'Murcott' tangor.

S10P26

Effects of nutritional trunk injections on sweet orange production

Martínez G.C.[1], Alayón Luaces P.[2], Yfran Elvira M.M.[1], Chabbal M.D.[2], Mazza S.M.[3], and Rodríguez V.A.[2]

[1]Facultad de Ciencias Agrarias - UNNE (FCA - UNNE), Física y Química, Argentina; [2]Facultad de Ciencias Agrarias - UNNE (FCA - UNNE), Producción Vegetal, Argentina; and [3]Facultad de Ciencias Agrarias - UNNE (FCA - UNNE), Cálculo Estadístico y Biometría, Argentina. cocorodriguez@live.com.ar

The objective of this work was to determine the effect of nutritional trunk injections (NI) of N (0.80%), P_2O_5 (0.80%), K_2O (0.70%), CaO (0.07%) and MgO (0.25%) on fruit yield and quality of "Valencia late" orange trees in Corrientes, Argentina. Field experiments were conducted during three years (from 2008/09 to 2010/11 harvest seasons) with NI applications to each side of tree´s trunk, at 20 cm above soil surface. The treatments consisted of soil fertilization (50% spring and 50% autumn) with: (1) 1.5 kg $plant^{-1}$ of a 15-6-15-6 NPKMg formula utilized in the region (half dose, HF); (2) HF plus NI in spring; (3) HF plus NI in spring and summer; (4) HF plus NI in spring, summer and autumn, and (5) 3.0 kg $plant^{-1}$ of 15-6-15-6 (full dose). Statistical design was a randomized complete block with four replications, with two plants per plot used for evaluation of treatment effects. Concentrations of N, P, K, Ca and Mg were determined in leaves of fruiting branches obtained in autumn. Total fruit production was measured, and diameter, percentage of juice, soluble solids content, acidity, ratio and juice color were determined on a sample of 40 fruits per plot at harvest. Variance analysis, Duncan test and principal component analysis were conducted for quantitative variables, whereas Kruskal Wallis test was performed for color. The treatments did not affected fruit quality. However, treatment 4 increased 26.5% fruit yield compared with same dose of soil application (treatment 1).

S10P27

Influence of reduced rate of application of phosphorus and potassium fertilizers on soil and fruit quality in a Satsuma mandarin orchard

Yoshikawa K.Y.[1], Nakamura A.N.[1], Baba A.B.[2], and Kusaba S.K.[3]

[1]Shizuoka Prefectural Research Institute of Agriculture and Forestry (SPRIAF), Fruit Research Center, Japan; [2]Shizuoka Prefecture (SP), Community and Environmental Affairs Department, Japan; and [3]National Agriculture and Food Research Organization (NARO), Institute of Fruit Tree Science, Japan. kiminori1_yoshikawa@pref.shizuoka.lg.jp

The influence of reduced rates of application of phosphorus and potassium fertilizers on fruit quality and soil in a Satsuma mandarin (*Citrus unshiu* Marc.) orchard was investigated. Thirteen-year-old trees grown

on gray upland soil were used. Phosphate and potassium fertilizers were applied at rates of 0% (PK0), 50% (PK1), and 100% (PK2), with respect to the conventional amount of fertilizers (P_2O_5: 120 kg ha^{-1}, K_2O: 240 kg ha^{-1}). Reduction in the rate of application of phosphate and potassium fertilizers had no influence on the sugar and acid contents of the fruits. In addition, reduction in the rate of phosphate application had no influence on the available phosphoric acid content in the soil. Phosphorus concentration in the leaves of PK0 was lower than that of PK1 and PK2; however, the concentration was within the standard range for nutritional diagnosis- The exchangeable potassium content in the soil of PK0 was lower than that of PK2. Potassium concentration in the leaves showed no change in all the treatments. These results show that reduction in the rate of phosphorus fertilizer application is possible in Satsuma mandarin orchards to prevent accumulation of available phosphoric acid in the soil. Since the level of exchangeable potassium in the soil decreased, it is necessary to judge the reduction in the rate of potassium fertilizer application by performing soil diagnosis.

S10P28

Potassium leaching from young lysimeter-grown orange trees

Boman B. J.[1], and Battikhi A.M.[2]

[1]University of Florida (UFL), Indian River Research and Education Center, USA; and [2]University of Jordan (UJ), Soil Science, Jordan. bjbo@ufl.edu

A study was conducted at University of Florida Indian River Research and Education Center to determine the effects of different fertilizer forms on the leaching of potassium to groundwater in the sandy soils common to the area. Lysimeters constructed from polyethylene tanks were filled with Oldsmar fine sand soil. A single 'Midsweet' orange on Carrizo citrange rootstock tree was planted in each lysimeter. Fertilization treatments included conventional applications of dry-soluble fertilizer at 6-week intervals, weekly fertigation applications, and single annual applications of two different controlled release materials. Irrigation (by microsprinklers) and drainage volumes measured with totalizing flow meters were collected weekly for two and a half years. Trees fertilized with controlled release products achieved equivalent growth to trees fertilized by the dry soluble materials and to those fertigated, even though the controlled-release treatment trees received only 44% of N and K as the other treatments. The amount of K leached averaged 39% (fertigated), 45% (controlled release), and 56% (dry soluble) of that applied. The total K leached with the controlled release fertilizer treatments was approximately one-half that of the fertigated trees and one-third that of the dry broadcast treatments. Leaching of K was found to be directly related to the weekly rainfall rate.

S10P29

Effect of organic fertilization on soil organic matter and on root apparatus of citrus trees

Trinchera A.[1], Torrisi B.[2], Allegra M.[2], Rinaldi S.[1], Rea E.[1], Intrigliolo F.[2], and Roccuzzo G.[1]

[1]Centro di ricerca per lo studio delle Relazioni tra Pianta e Suolo (CRA-RPS), ITALY; and [2]Centro di ricerca per l'Agrumicoltura e le Colture Mediterranee (CRA-ACM), ITALY. francesco.intrigliolo@fastwebnet.it

The aim of this work was to verify the effect of organic fertilization on soil organic matter and on root apparatus of mature orange trees [*Citrus sinensi*k] ValenciaLate, grafted on *C. aurantiu*., in citrus orchard organically managed for 15 years. In a randomized block experimental design, compost from "pastazzo" (mixture of citrus pulp and skin), poultry manure, bovine manure, compared to a mineral fertilizer, were applied yearly to the soil (the same N/plant/year). For each plant, an equal volume of soil was sampled at the same distance from the trunk determining, in the sampling areas, the root density (roots weight/soil volume). The root biomass was then separated into primary and secondary roots, calculating the related fresh weight and dry matter. Representative young roots were selected and analyzed by electron scanning microscopy (SEM). As far as soil characterization concerns, the TOC%, TEC%, HA% and FA% were determined and then the related HR% and DH% were calculated. Results showed that organic fertilization, when compared to the mineral one, determines not only an improvement of soil organic matter content and humification parameters, but also an increase of root biomass. The SEM analysis evidenced that organic fertilizers induced the appearance of adventitious buds on the terminal portions of the roots, an improved regularity of the elongating meristematic cells an, an increase of the production of root mucigel.

S10P30

Use of plant-soil-atmosphere sensors on a young Clementine mandarin orchard under regulated deficit irrigation to monitor changes in water relations for better irrigation scheduling

El-Otmani M., El-Fadl A., and Benismail M.C.

Institut Agronomique et Vétérinaire Hassan II, Complexe Horticole d'Agadir, BP: 728 (IAV-CHA), Horticulture, Morocco.
elotmani.mohamed@gmail.com

Water availability for agriculture irrigation is becoming scarce in the Mediterranean Basin. Citrus orchards use as much as 1 000 m^3 ha^{-1}, if not more in situations where flood irrigation is still a common practice. However, producers are looking for ways and strategies to save as much of the resource as possible. For this reason, young Clementine orchard, planted in 2007 in the area of Agadir, Morocco receiving about 200 mm of rain per year, was used to test a regulated deficit irrigation strategy (RDI) supplying a combination of 100%, 75% or 50% of crop evapotranspiration ((ETc) at different stages of fruit development on tree vegetative and reproductive development. Reference ETo was calculated using parameters obtained from a compact weather station installed near the orchard. To monitor soil water statul, Watermark and FDR probes were used. Tree Water relations were evaluated using 1) LVDT sensors for measuring tree diameter microvariations, 2) leaf water potential, 3) trunk sap flow and 4) stomatal conductance. Trunk diameter, sap flow and stomatal conductance were significantly affected by climatic conditions and soil humidity. RDI had an effect on root and canopy development but effect on fruiting varied among years with the well irrigated trees tending to have more fruits at harvest. The results are discussed in relation to water economy and water use efficiency as well as in relation with the accuracy of the sensors and the possibility of their use in irrigation scheduling.

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Session 11

CULTURAL PRACTICES AND MECHANIZATION

S11O01

New method of citrus grafting by the use of laboratory tape (top budding)

Kitamura M.[1], Caisley J.[2], Sakaki H.[1], Fujita K.[1], and Takahara T.[1]

[1]Kumamoto Prefectural Agricultural Research Centre, Institute of Fruit Tree Science, Japan; and [2]Japan Concept Ltd., UK.
jonathan.caisley@japan-concept.co.uk

For propagation of citrus trees in Japan, Bench Grafting is used in spring, and Budding in autumn. Both methods have drawbacks, with high worker skill required for Bench Grafting, and a long process needed for Budding, due to the need to return later in the season to remove foliage left above the budded area. Therefore, we examined Top Budding (TB), a simple new method not requiring expert grafting skills and suitable for mass tree cultivation. TB is used from spring to early summer when the sap of the rootstock is flowing and bark can easily be removed for budding. Buds taken in Feb / March were used and inserted by standard T-budding. The rootstock was cut completely 5 cm above ground, removing all upper foliage (unlike the existing method where the foliage is kept). After grafting, the bud union area was totally sealed with water tight, air porous laboratory film (brand name: Buddy Tape) to prevent dehydration. TB and Bench Grafting showed similar survival rates, but bud break of the scion with TB was slightly later than for Bench Grafting. Growth for Bench Grafting was quicker than for TB in early stages, but by the end of the growing period, both grafts had equal growth. Operation time for TB was clearly shorter than that for the conventional Bench Grafting method, reducing labour time during the grafting process by 40%. During the trial by 5 inexperienced workers, the TB method achieved 100% survival rate and trees showed good growth. It was concluded that TB was a simple and easy citrus tree preparation technique that can be mastered even by non experienced operators with success rates similar to those achieved by skillful Bench Grafting operators.

S11O02

Performance of citrus nursery trees and two-year older trees after transplantation in the field

Zekri M.

University of Florida, USA. maz@ufl.edu

Because citrus production must use more efficient techniques and practices for rapid recovery of capital investment and maximum net returns, a long-term field study was conducted to evaluate the performance of four groups of 'Valencia' orange trees on 'Volkamer' lemon rootstock. Before being planted in the grove, three groups of trees came from a container nursery, transplanted in three different size bags (30-, 45-, or 60-cm-long and diameter) and allowed to grow for two years. The trees of the fourth group were two-year young standard container nursery trees grown in 10 x 10 x 35-cm plastic citripots. For each group, growth, leaf mineral concentration, fruit production and quality were determined and net income per acre was computed. The young nursery trees accumulated more potassium and less calcium in their leaves and had less soluble solids, acid, and juice in their fruit. During the first four years after planting, tree size and fruit production were the highest for the trees coming from the large size bags. It took six years for the young trees coming directly from the nursery to catch up the trees previously grown for two extra years in 60-cm bags.

S11O03

Effect of inarched, two-rootstock trees, on development and yield of 'Valencia' orange orchards

Lima J.E.O.[1], and Lima J.E.P.[2]

[1]Mudas Cítricas Citrolima (CITROLIMA), Director, Brazil; and [2]Rucks Citrus Nursery (RCN), General Manager, USA.
lima@citrolima.com.br

Susceptibility to Sudden Death, Blight and Foot Rot diseases and to nematodes has not prevented 'Rangpur' lime from still being the most used citrus rootstock in Brazil due to its adaptability to poor soils and its drought resistance. Historically, inarching trees on 'Rangpur' lime with rootstocks that are resistant to diseases induced resistance to root and trunk diseases. Experiments were established in 2005 and 2007 to

compare 'Valencia' trees on (1) 'Rangpur' lime, on (2) 'Swingle' citrumelo, or (3) as inarched using these two rootstocks. Inarched trees had the same size as those on 'Rangpur' alone and both types were larger than trees on 'Swingle' alone in the first 5-7 years of the orchards. Inarched trees had similar yields as those on 'Rangpur' lime alone and both types yielded more than trees on 'Swingle' alone. In addition, inarched trees had fruit quality intermediary between that of fruit from trees on 'Rangpur' and that of fruit from trees on 'Swingle' alone.

S11O04

Morphological and yield characteristics of 'Washington' navel orange and 'Tahiti' lime trees produced with buds from floral *versus* vegetative mother shoots

Lovatt C.J.[1], and Krueger R.R.[2]

[1]University of California-Riverside (UCR), Department of Botany and Plant Sciences, USA; and [2]USDA-ARS National Clonal Germplasm Repository (USDA-ARS), USA. carol.lovatt@ucr.edu

Citrus cultivars bear "floral" shoots that produce more inflorescences than vegetative shoots (411 inflorescences, 11 vegetative shoots). Each cultivar also bears "vegetative" shoots that produce fewer inflorescences (208) and more vegetative shoots (202) than floral shoots on a similar number of nodes. Canopy sections in which floral shoots develop contribute a greater percent of tree total yield than those dominated by vegetative shoots. We hypothesized that trees propagated from buds taken from floral shoots would retain the internal physiological status of a floral mother shoot to produce more floral daughter shoots than trees propagated from buds taken from vegetative mother shoots and thus be more productive. Buds from the two types of mother shoots collected from 'Tahiti' lime and 'Washington' navel orange were budded on 'Carrizo' citrange rootstocks. Forty trees were established in a field at UC-Riverside. At age 3 years, 'Tahiti' lime trees propagated from buds from floral mother shoots produced 28% more fruit than trees propagated from vegetative mother shoot buds. Similarly, 3-yr-old 'Washington' navel orange trees produced with buds from floral mother shoot yielded 26% more fruit than trees produced with buds from vegetative mother shoots. At the end of June drop, 4-yr-old 'Washington' navel orange trees propagated from floral mother shoot buds retained 35% more fruit than trees propagated with vegetative shoot buds, but the reverse was true for 'Tahiti' lime.

S11O05

Effect of different pruning severities on incidence of creasing and fruit quality in sweet orange

Hussain Z., Singh Z., and Mandel R.

Horticulture Research Laboratory, (HRL), Department of Environment and Agriculture, School of Science, Faculty of Science and Engineering, GPO Box U1987, Perth, 6845, WA, Australia. zachoundi@yahoo.com

Creasing (albedo breakdown) is a physiological disorder with cracks in the internal white tissue (albedo) causing puffiness of orange peel. It causes considerable economic losses to citrus growers in Australia and other parts of the world. We investigated the effects of pruning severities on incidence of creasing and fruit quality in 'Washington' navel, 'Lane Late' and 'Navelina' sweet oranges. Pruning treatments including control (no pruning), light pruning (25% branches) and severe pruning (50% branches) were applied after fruit harvest on 20th September, 2010 at Gingin, Western Australia. The experiment was laid down following a split plot design, with four replications and single tree as an experimental unit. Thirty five ripe fruits per tree were harvested randomly around the tree canopy to determine the incidence of creasing and fruit quality. Results showed that both pruning treatments have reduced percentage of creased fruit and creasing index in all three cultivars as compared to control. Severe pruning was more effective in reducing creasing in all cultivars than light pruning. The effects of pruning on soluble solids and acid ratio, ascorbic acid, total antioxidants, individual sugars and organic acids in the juice will also be presented and discussed. In conclusion, severe pruning was more effective in reducing the incidence of creasing and enhancing the fruit quality in sweet oranges.

S11O06

Reduction of fruit splitting and the incidence of creasing of 'Nova' mandarin and 'Washington' navel orange by NAA sprays

Greenberg J.[1], Kaplan I.[1], Tagari E.[1], Fainzack M.[2], Egozi Y.[2], and Giladi B.[2]

[1]Ministry of Agriculture, Extension Service, Israel; and [2]Mehadrin-Prior, Israel. yogreen@shaham.moag.gov.il

'Nova' mandarin and 'Washington' navel orange in Israel suffer from peal splitting, causing fruit loss of up to 50,% and from creasing, disqualifying the fruit for fresh marketing. The effects of naphthaleneacetic acid (NAA 300mg/L), tank-mixed with 'Bonus-NPK' fertilizer (5%), on the yield, fruit number, fruit size, fruit quality, fruit splitting and creasing were studied. In 'Nova' mandarin, different application times were examined, on small fruitlets (15-20 mm in diameter)-when NAA causes fruit thinning, and on larger fruitlets (26-32 mm in diameter) when NAA does not affect fruit abscission. NAA spray on large fruitlets reduced creasing from 37% in control to 10% in sprayed trees. Moreover, fruit drop due to splitting was also reduced by 14%. In 'Washington' navel orange, NAA spray on large fruitlets (30 mm) reduced creasing by 30%, while the common spray with giberellic acid (20 mg/L) in August reduced creasing by 20%. In both varieties, NAA sprays on small fruitlets reduced fruit number and yield, while spraying larger fruitlets had no effect on fruit number or yield. The NAA sprays had no significant effects on the percent of juice, total soluble solids and the acid content of the fruit. The data suggest that NAA treatment is effective in reducing creasing and splitting when sprayed on large fruitlets, at the stage that it does not affect fruit size and fruitlet thinning anymore. Results and their implication regarding cultural practices are discussed.

S11O07

On-farm citrus trials in Australia: Effective extension for commercial assessment

Falivene S.[1], Connolly K.[1], and Cannard M.[2]

[1]NSW Department of Primary Industries (NSW DPI), Intensive Industries, Australia; and [2]Murray Valley Citrus Board (MVCB), Australia. steven.falivene@dpi.nsw.gov.au

Many products and practices have been promoted to the citrus industry and little is known about their performance under Australian conditions. Some of these products have been trialed within Australia. Performance of some of these products on farm has been highly variable causing confusion in the industry. The variability is probably due to differences in site (e.g. soil, climate, variety) and management (e.g. irrigation, nutrition). This project assesses the cost benefit of a number of citrus practices promoted to industry on grower's properties. The project utilizes active learning principles by facilitating grower participation with trials (planning, maintenance & reporting). Over 20 trials have been implemented in the past two seasons. Trials included ground and foliar potassium applications, pruning, foliar fruit size sprays, kaolin clay foliar sprays and crop regulation. Trials have resulted in cost saving with reduced potassium application. The project developed tools and methods to enable efficient and effective in-field collection and analysis of data. This enabled the project to implement more trials. Single tree replicated trials (RCBD) are the favored method to conduct trials. Grower feedback has been very positive indicating that it has helped them make improved and cost effective management decisions. On-farm trials are a very effective extension method to obtain fast assessment and adoption of best management practices suited to site conditions.

S11O08

Evolution of citrus mechanical harvesting in Florida – Lessons for the future

Roka F.M.

University of Florida (UF), Southwest Research and Education Center, USA. fmroka@ufl.edu

Florida citrus growers have invested more than $40 million since 1995 to develop mechanical harvesting systems. Technological improvements allowed mechanically harvested acreage to increase steadily between 1999 and 2009 and reach more than 14,000 hectares. Since 2009, however, the trend for mechanical harvesting has been downward and by the end of the 2010-11 season, the number of hectares mechanically harvested had fallen by two-thirds. Reasons behind the movement away from mechanical equipment include

horticultural, engineering, and economic. The spread of Huanglongbing (HLB, *ex* Greening) has reduced the overall tree health in Florida and has heightened growers' long-standing concern over the added physiological stress that comes with mechanical harvesting equipment. Equipment performance, as measured by fruit recovery percentage, has declined as tree canopy uniformity has deteriorated from effects of various tree diseases, particularly from HLB. As the performance efficiencies of harvesting equipment eroded, the unit costs of mechanical harvesting increase. The purpose of this paper is to further explore these factors and discuss how the objectives of growers and juice processors need to be integrated into future designs of mechanical harvesting systems. Data on the ownership and operational costs of existing equipment strongly suggests that the potential for harvest cost savings through mechanical harvesting systems remains significantly high.

S11P01

Study on mushroom dregs application in citrus nursery

Guan B., Chen K.L., He J., and Liu J.J.

Horticulture Research Institute, Sichuan Academy of Agricultural Sciences (HRI, SAAS), Centre of Fruit Research, China. chen-kl@163.com

Peat is an important substrate widely used in citrus nurseries in China. The commonly used formula is peat:sand/soil:husk of rice = 4:4:2 or peat:soil = 6:4. Use of peat was restricted during recent years. Furthermore, mushroom producing houses produced great quantities of mushroom dregs at harvest which were often disposed of as garbage. The aim of this study is to investigate the feasibility of using mushroom dregs instead of peat in citrus nurseries with nutritional bags. 'Ziyang-xiangcheng' (*Citrus junos*), an important resistant rootstock to alkaline soil, and five different mushroom dregs were used in this experiment. The trial proportion of the seedling substrate was mushroom dregs:soil:peat = 1:2:2, 1:1:1, and 1:1:0. There were 2 controls, control 1 had mushroom dregs:soil:peat = 0:1:1, and control 2 was only soil. Each treatment had three replicates with 100 seedlings per replicate. The effects of mushroom dregs on the growth of seedlings in the nutritional bags were evaluated. The results showed that 'Ziyang-xiangcheng' seedlings can survive and grow well in most media. The seedlings height of 11 mushroom dreg treatments and seedlings diameter of 9 treatments were superior to control 1, and all mushroom dregs treatments were superior to control 2. Therefore, it is feasible and economical to apply certain mushroom dregs instead of peat in the substrate of citrus container seedlings.

S11P02

Effect of soil preparation systems, mulching and rootstocks on 'Pera' orange (*Citrus sinensis*) performance in the northwest of Parana State, Brazil

Auler P.A.M.[1], Fidalski J.[2], Gil L.G.[2], and Pavan M.A.[2]

[1]Instituto Agronômico do Paraná (IAPAR), Área de Fitotecnia, Brazil; and [2]Instituto Agronômico do Paraná (IAPAR), Área de Solos, Brazil. aulerpe@iapar.br

The objective of this work was to evaluate the effect of minimum soil preparations and mulch management on the performance of orange trees in a Typic Haplorthox from Caiua Sandstone soil type with lower chemical fertility and water storage capacity, and higher susceptibility to erosion. The experiment was established in an area located in the Ipiranga farm (Cocamar), at Paranavaí municipality, Parana State, Brazil. The experiment was laid out as a split-split plot design, with 12 treatments and 4 replications. The main plots were used to study the soil preparations: conventional with tillage in total area, and minimum preparation in strip and direct planting with partial tillage. In the subplots, the management of the tree rows, with and without mulch was adopted. In the split-split plots the rootstocks 'Rangpur' lime (*Citrus limonia*) and 'Cleopatra' mandarin (*C. reshni*) were used. The 'Pera' orange trees were planted in 2003 with a spacing of 6.8 m x 3.5 m. The mulch was established in the rows from mechanically mowing the *Brachiaria brizantha* weeds that grew between rows. There was no- effect of soil preparations on fruit production and average fruit weight of the first six year crops. Mulching increased soil K levels and water holding capacity and reduced the need for weed control. In the trees, mulching kept the fruit production under 'Rangpur' and reduced it under 'Cleopatra' rootstocks. The minimum soil preparations evaluated showed an important management for establishing orange orchards in this region. The 'Rangpur' rootstock was more adapted to the mulch management treatment used in this study than was 'Cleopatra' rootstock.

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S11P03

Evaluation of rootstocks for the 'Tahiti' lime in the municipality of Jaíba, Brazil

Alves R.R., Machado D.L.M., Salomão L.C.C., Siqueira D.L., and Silva S.D.R.
Universidade Federal de Viçosa (UFV), Departamento de Fitotecnia, Brazil. lsalomao@ufv.br

The 'Tahiti' lime (*Citrus latifolia*) is one of the most produced fruits in the irrigated region of Jaíba perimeter, Minas Gerais State. The fruit is for the domestic market and export. However, 'Rangpur' lime (*C. limonia*) is the only rootstock used in the region. Rootstock diversification is important. The aim of this study was to evaluate the influence of twelve cultivars of rootstocks on the development and fruit quality of 'Tahiti' lime grown in the municipality of Jaíba (latitude: -20° 15' 18'' and longitude: -43° 40' 28''). The experimental design consisted of randomized blocks, with twelve treatments (rootstocks) and five replications. The trees were evaluated for annual productivity, diameter of rootstock, diameter of the scion, and height and diameter of the canopy. The fruits were evaluated for color, soluble solids content in the juice, juice titratable acidity, percentage of juice and levels of vitamin C. The largest productivity was induced by rootstocks 'Volkamer' lemon (*C. volkameriana*), 'Cleopatra' mandarin (*C. reshni*) and 'Citrandarin 1710' (*C. reticulata* x *Poncirus trifoliata*). The greatest tree height and best fruit quality were observed in trees grafted on 'Limeira' trifoliate (*P. trifoliata*), 'Citradia 1708' (*C. aurantium* x *P. trifoliata*), 'Cleopatra' mandarin and 'Citrandarin 1710'. The latter two had better potential as alternative rootstocks to 'Rangpur' lime.

S11P04

Development and production of 'Tahiti' lime IAC-5 grafted on 'Flying Dragon' (*Poncirus trifoliata* var. *monstrosa*), grown in high planting densities

Machado D.L.M., Alves R.R., Siqueira D.L., Salomão L.C.C., and Silva S.D.R.
Universidade Federal de Viçosa (UFV), Fitotecnia, Brazil. siqueira@ufv.br

The high citrus density planting using the rootstock 'Flying Dragon' (*Poncirus trifoliata* var. *monstrosa*) is possible because it is a rootstock that has the ability to induce dwarfing and produce small size trees, resulting in greater ease of orchard management and increased productivity of the plantings. Thus, the objective of this work was to evaluate tree growth and fruit quality of 'Tahiti' lime (*Citrus latifolia*), cultivar IAC-5, grafted on 'Flying Dragon' using spacings of 1 m, 1.5 m, 2 m, 2.5 m or 3 m between trees in the row by 6 m between tree rows. The experiment was conducted in the city of Jaíba (latitude 20° 15' 18'', longitude 43° 40' 28'') in a randomized block design with six repetitions. The trees were evaluated for annual productivity, diameter of the rootstock and of the scion, and height and diameter of the canopy. The fruits were evaluated for juice content, total soluble solids, titratable acidity and vitamin C. In the period 2010-2012, productivity per hectare in 6 m x 1.5 m spacing was higher than that of the other spacings. Effect of spacing was minor on fruit quality, being that the spacing 6 m x 3 m provided greater contents in fruit juice, vitamin C and total soluble solids. No significant differences were found regarding tree growth characteristics between treatments. The results suggest the possibility that increased planting density will be used in future citrus plantings using 'Flying Dragon' rootstock.

S11P05

Effect of shade screen on production, fruit quality and growth parameters in 'Fino 49' lemon trees grafted on *Citrus macrophylla* and sour orange

Gimeno V.[1], Simón I.[2], Martínez V.[1], Lidón V.[2], Shahid M.A.[3], and García-Sánchez F.[1]
[1]Centro de Edafología y Biología Aplicada del Segura (CEBAS), Plant nutrition, Spain; [2]Escuela Politécnica Superior de Orihuela (EPSO), Spain; and [3]University of Sargodha, Pakistan. vicente@cebas.csic.es

Spain ranks as the second-largest lemon fruit producing country in the world and it is one of the largest exporters. About 80% of the production is located in the arid southeast (Murcia and Alicante). In citrus, it is known that 50% shade screens reduce leaf temperature and light intensity at midday in summer and spring periods in these areas, resulting in an increase in the photosynthetic leaf water use efficiency (WUEphy = ACO2/Eleaf). In this experiment the effect of shading with Aluminet-50 screen (which reduces about 50% of incident photosynthetically active radiation) on the production, fruit quality, leaf water relations, leaf mineral

composition, and growth parameters of 'Fino 49' lemon trees grafted on two rootstocks, *Citrus macrophylla* and sour orange, were studied. This study was carried out in Santomera (Murcia) over a four-year period (2008-2011) using seven-year old trees at the beginning of the experiment. Shade screen had similar effect in trees grafted on both rootstocks. Shading increased the height and canopy perimeter producing trees with a greater canopy volume than that under full sunlight (increase of about 60% compared with full sunlight trees). Despite that, shading reduced fruit production. Trees under full sunlight produced in total 81 and 45 kg/tree, while trees under shade produced 70 and 33 kg/tree, respectively for *C. macrophylla* and sour orange. Leaf water status according to leaf water potential (Ψw) and relative water content (RWC) was greater in trees under shade screen. Shading affected the physical characteristics of the fruit from trees on *C. macrophylla* as fruits under shade had less fresh weight and smaller width than fruits from trees under full sunlight. Leaf mineral concentration was not affected by shading, except for B and Mn where the concentration increased by shading.

S11P06

Effect of liquid fertilizer applied by injection into the soil on quality of 'Tarocco' blood orange

He J., Chen K.L., Guan B., and Liu J.J.

Horticulture Research Institute, Sichuan Academy of Agricultural Sciences (HRI, SAAS), Centre of Fruit Research, China.
chen-kl@163.com

Experiments using four different treatments of liquid fertilizer (No. 1 to No. 4) and one treatment of compound fertilizer ($N:P_2O_5:K^2O$ = 15:15:15) (No. 5) were conducted continuously for 3 years to compare the fertilization effect on fruit quality of 'Tarocco' orange. The liquid fertilizer (containing humic acid and dissolved N, P, K) was injected into the soil by pressure increasing device to a depth of 20 cm-30 cm. The results indicated that the fruit of all liquid fertilizer treatments had higher content of total soluble solids (TSS) and total sugars than those of compound fertilizer. The content of TSS increased by 13.5%, 14.4%, 7.2% and 2.7% and total sugars increased by 25.9%, 24.7%, 11.5% and 6.7%, respectively for treatments No. 1, 2, 3 and 4, compared to those of fruit from treatment No. 5. The content of total acid for liquid fertilizer was different. Treatment No. 1 and No. 2 produced lower total acid content in fruit than did treatment No. 5 while treatments No. 3 and No. 4 produced higher total acid content. The fruits of treatments No. 1, 2, 3 and 4 also had higher vitamin C content than fruit from No. 5 treatment. In short, the fruit quality of 'Tarocco' blood orange could be improved by liquid fertilizer injection into soil.

S11P07

Effect of branch girdling and ringing on carbohydrates, chlorophyll fluorescence and SPAD index in leaves of 'Tahiti' acid lime

Santos D.[1], Siqueira D.L.[1], Salomão L.C.C.[1], and Cecon P.R.[2]

[1]Universidade Federal de Viçosa (UFV), Fitotecnia, Brazil; and [2]Universidade Federal de Viçosa (UFV), Estatística, Brazil.
lsalomao@ufv.br

This work evaluated the effects of girdling and ringing on the dynamics of carbohydrates, chlorophyll fluorescence and SPAD index in leaves of 'Tahiti' acid lime. Three year-old trees cultivated in the subtropical climate of Minas Gerais, Brazil, were used in the experiments. These practices were applied at 8 dates: June 30th 2009, July 15th 2009, July 30th 2009 and August 14th 2009 and June 15th 2010, July 5th 2010, July 25th 2010 and August 14th 2010 plus a control (not girdled nor ringed). Girdling and ringing were both performed on the main branches of the trees, 10 cm above their insertion on the trunk. Greater contents of starch were observed until 50 to 60 days after girdling on both 2009 and 2010 crops, returning to normality around day 120 due to the reestablishment of phloem transport. Unlike girdling, the responses obtained with ringing were mostly similar to those observed in control trees. Girdling reduced the ratio of variable to maximal chlorophyll fluorescence (Fv/Fm relationship) in the 2009 and 2010 years. In 2009, trees subjected to ringing presented maximum values of Fv/Fm similar to the ones of control trees on day 90 after incision. In 2010, plants subjected to ringing did not present variation in Fv/Fm relationship over time, similar to the control plants. Regarding SPAD index, results presented similar trends in both years. In the 2009 treatments, all girdled trees presented a reduction in the index, reaching minimal values around days 80 to 90 after incision. In 2010 treatments, those values were reached around days 100 to 110.

S11P08

Production of 'Tahiti' acid lime after branch girdling or ringing

Santos D., Siqueira D.L., Salomao L.C.C., and Cecon P.R.

Universidade Federal de Viçosa (UFV), Fitotecnia, Brazil. siqueira@ufv.br

This work evaluated the effects of girdling and ringing on 'Tahiti' acid lime flowering, rate of flower drop and fruit set. Trees of three years of age were used. They were grown in the subtropical region of the state of Minas Gerais, Brazil. These cultural practices were applied at 8 dates: June 30th 2009, July 15th 2009, July 30th 2009 and August 14th 2009 and June 15th 2010, July 5th 2010, and August 14th 2010 plus a control (not girdled nor ringed). Girdling and ringing were both performed on the main branches of the trees, 10 cm above their insertion on the trunk. Regardless of the year and time of application, girdling and ringing did not alter rate of flower drop and time of flowering and fruit harvest. Fruit set (PFS), number of fruit produced (NFP) and fruit production (PPP) of girdled trees were greater than those for control trees, whereas average mass (AMF) and average diameter of fruits (ADF) were smaller. Ringed plants did not present differences in relation to the control trees. Regardless of the time when the treatments were applied, in 2009 PFS was 3.19 times greater in girdled plants in comparison to the other treatments. Girdling also caused a reduction of 43% in flower abscission. This reduction was responsible for an increase of 117% in PFS, 117% in NFP and 84% in PPP in relation to ringed trees. However, as fruit set increased in girdled trees, AMF was reduced by 12% compared to fruit from ringed or control trees.

S11P09

Horticultural package for high quality 'Tahiti' lime production

Mourão Filho F.A.A.[1], Stuchi E.S.[2], Cantuarias-Avilés T.[1], Espinoza-Núñez E.[1], Bremer Neto H.[1], Bassan M.M.[1], and Silva S.R.[1]

[1]Universidade de São Paulo (USP), Departamento de Produção Vegetal, Brazil; and [2]Embrapa Mandioca e Fruticultura (EMBRAPA), Diretoria Científica, Brazil. francisco.mourao@usp.br

The production and international marketing of the 'Tahiti' lime (*Citrus latifolia*) have significantly expanded in recent years. On the other hand, increasing production costs and the occurrence of novel destructive diseases have motivated the development of more competitive and efficient systems for high quality fruit production. During the past years, four experiments were conducted in Southern Brazil to optimize fruit yield and quality of 'Tahiti' lime. This work summarizes the most important achievements from these experiments in order to propose a horticultural package for high quality 'Tahiti' lime production. The main results from these experiments indicate that dwarfing rootstocks may lead to early-bearing, higher yield efficiency and higher fruit quality of 'Tahiti' lime. Although some other rootstocks may induce high drought tolerance and higher cumulative yield, in this horticultural package the use of irrigation was essential for even higher efficiency. On the other hand, plant size, fruit yield and quality were directly and significantly affected by the 'Tahiti' lime scion selection. Fruit quality and conservation were also significantly influenced by the harvest method. The combination of these practices, including the correct choice of the scion selection/rootstock combination, the use of irrigation and the adequate harvest methods may lead to increased production of high quality 'Tahiti' limes.

S11P10

Field evaluation of two canopy shake systems, OXBO 3210 and OXBO 3220, on citrus orchards in Andalusia (Spain)

Arenas Arenas F.J., Salguero A., and Hervalejo A.

Instituto de Investigación y Formación Agraria y Pesquera (IFAPA). Centro Las Torres-Tomejil, Consejería de Agricultura, Pesca y Medio Ambiente. Junta de Andalucía, Spain. fjose.arenas@juntadeandalucia.es

Spanish citrus orchards are threatened by the globalization of the market and the existence of countries with cheaper labor, especially those orchards destined to juice industry in which manual harvest represents about 50% of the final production costs. In this situation mechanical harvesting is a promising way to decrease harvest

cost and thereby increase the profit margin in production of citrus fruit for processing. However, adoption of mechanical harvesting requires a major effort in the selection and adaptation of existing equipment, as well as in the design of the plantations. In 2009, several field experiments using two continuous canopy shake systems, OXBO 3210 and OXBO 3220, were conducted in unadapted citrus orchards to evaluate the effect of harvester amplitude (CPMs) and harvester speed on fruit removal efficiency, tree damage and fruit quality. The results suggest that a suitable adaptation of citrus plantations (continuous hedgerows with high canopy and bottom canopy pruned to a height of 1 m above the ground) together with an appropriate calibration of the equipment, would allow to obtain high fruit removal efficiency without causing considerable damage to the tree or affect the physical integrity of the fruits for processing. Mechanical harvesting had no effect on internal fruit quality.

S11P11

Production seasonality of 'Tahiti' lime in three consecutive harvests under semiarid climatic conditions

Maia V.M., Álvarez J.M., Oliveira F.S., Gomes F.H.C., Azpiazú I., and Salles B.P.A.

Universidade Estadual de Montes Claros (UNIMONTES), Ciências Agrárias, Brasil. victor.maia@unimontes.br

Cultivation of 'Tahiti' lime is increasing significantly in the Minas Gerais State, due to its good adaptation to the semiarid climate of the region. This work aimed to evaluate the seasonality of irrigated 'Tahiti' lime production using three consecutive harvests under semiarid conditions. The experiment was conducted in a commercial orchard, located in Northern Minas Gerais (altitude of 449 m, latitude 15°05'34"S, and longitude 43°58'44"W). The orchard was planted in January 2007 and the trees started producing in December 2008. The evaluations were made when fruits were harvested, starting the beginning of December 2008 until December 2011. All the fruits from all the trees were collected weekly, obtaining the total yield for each month throughout the year. These fruits were harvested when they reached a diameter of 54 mm. Determinations were made of total production, yield, and fruit was sorted for export, domestic market and discards. Total yield was calculated by adding the weight of the fruits intended for the export, that for the domestic markets and that for the discards for each month. Production of 'Tahiti' lime occurs in all months of the year under semiarid climatic conditions. The yield obtained in the first, second and third year of production was 11,611, 8,809 and 12,102 $Kg \cdot ha^{-1}$, respectively. There are peaks of production in May, June and November.

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S11P12

Enhancement of citrus value chain production in Pakistan and Australia under the AusAid Program

Khurshid T.[1], Ahmad I.[2], Jaskani M.J.[3], Nabi G.[4], Tahir T.[5], Ali W.[5], Rahman A.[6], Khan M.A.[7], and Rahman H.[5]

[1]Dareton Primary Industries Institute (DPII), New South Wales. Department of Primary Industries, Australia; [2]FAO Representation NARC Premises Park Road Islamabad (FAO), Program Development, Islamabad; [3]University of Agriculture Faisalabad (UAF), Horticulture, Pakistan; [4]Agriculture Research Institute, Tarnab (ARI), Horticulture, Pakistan; [5]National Agricultural Research Centre (NARC), Horticulture, Pakistan; [6]Citrus Research Institute, Sargodha (CRI), Citrus Physiology, Pakistan; [7]Fruit and Vegetable Development Project (FVDP), Horticulture, Pakistan. tahir.khurshid@dpi.nsw.gov.au

Citrus is a major fruit crop in Pakistan, and 'Kinnow' mandarin is the dominant cultivar. Currently 53% of citrus fruit produced in Pakistan is consumed domestically, and *ca*. 10% is exported. A small proportion is processed, and postharvest losses are high. Export of 'Kinnow' mandarin from Pakistan is trending upwards, but the industry's capacity to supply export markets is limited. A constraints analysis conducted under the auspices of the Agricultural Sector Linkages Program during 2006 suggested that the development of the Pakistan citrus industry was constrained by poor nursery practices, dependence on a limited range of varieties, poor irrigation and crop management practices. A project led by NSW DPI and collaborative organizations in Pakistan including, the Pakistan Agriculture Research Council, Islamabad, the Citrus Research Institute, Sargodha, the University of Agriculture, Faisalabad, the Agriculture Research Institute, Tarnab, Peshawar and Fruit and Vegetable Development Project, Lahore has been funded by AusAid to increase citrus production in Pakistan through transfer of improved orchard management techniques. The overall aim of this project is to improve mandarin and orange productivity in Pakistan through germplasm transfer to extend the growing

season and test a range of varieties in the hotter climate of Pakistan, and provide training in the areas of nursery production, irrigation and orchard management. Technology transfer is taking place *via* small to medium scale demonstration sites in citrus producing regions in Pakistan, and *via* more formal training of citrus research and extension personnel in Pakistan and Australia.

S11P13

Technology and production costs for organic *versus* conventional 'Navelina' citrus orchards

Reis D.[1], Marreiros A.[2], Fernandes M.M.[2], Mourão I.[3], and Duarte A.[4]

[1]Instituto Politécnico de Viana do Castelo (IPVC), Escola Superior Agrária de Ponte de Lima, Portugal; [2]Direcção Regional de Agricultura e Pescas do Algarve (DRAPALG), Portugal; [3]Centro de Investigação de Montanha/Instituto Politécnico de Viana do Castelo (CIMO/IPVC), Escola Superior Agrária de Ponte de Lima, Portugal; and [4]Institute of Mediterranean Agricultural and Environmental Sciences - University of Algarve (ICAAM/UAlg), Faculty of Sciences and Technology, Portugal. dhcreis@gmail.com

The organic production is often associated with higher production costs when compared to conventional farming but other authors have reported a decrease in production costs for organic farming. In this study we describe the operations carried out over a year in an organic orchard of 'Navelina' orange located in the southern Portuguese region of Algarve. In addition, we describe the operations performed in two conventional orchards, located near the organic orchard. The corresponding production costs were determined. The organic orchard was managed quite differently from conventional orchards. The differences were significant in relation to weed control, fertilization and pest and disease control. Although some biological orchard operations are very expensive, the total production costs are similar in organic and conventional orchards. Yield was also similar in both cultivation systems. The data obtained allowed for an evaluation of the economic benefits of organic farming in this cultivar.

S11P14

Prediction of moving northward of favorable regions to cultivate subtropical citrus by estimation of changes in annual mean and minimum air temperature in Japan

Sugiura T., Sakamoto D., Sugiura H., Koshita Y., and Asakura T.

National Agriculture and Food Research Organization (NARO), Institute of Fruit Tree Science, Japan. sugi@affrc.go.jp

Satsuma mandarin (*Citrus unshiu*) is the leading citrus variety in Japan. It often suffers high-temperature damage such as sunscald, coloring disorder and puffy fruit. It has been predicted that satsuma mandarin production in Japan will become difficult under increasing temperatures resulting from global warming. Because fruit trees need several years to grow until they reach the productive age and once they start producing, they are kept for many years, it is important to make planting plans for the long term and incorporating climate change in the decision making. This study is an examination of whether 'Tankan' (*Citrus tankan*), which in the past was the major subtropical citrus in Japan, can be grown in the areas where satsuma mandarin is grown. To develop maps showing the most favorable regions to cultivate 'Tankan' in the future, the annual mean air temperature and the lowest air temperature in one year were estimated by using mesh temperature data (approximately 1 by 1 km resolution) that was derived from SRES-A1B emission scenario and a general circulation model, MIROC3-HiRes. Results indicate that 'Tankan' can be basically cultivated in the region where the satsuma mandarin cultivation became difficult. Subtropical citrus production may be selected as one of the adaptation plans after 40 years in many of the present citrus zones with the exception of inland area where cold damage will often occur.

S11P15

INTA´s Citrus Improvement Program

Costa N., Plata M.I., and Anderson C.

Estación Experimental Agropecuaria Concordia (INTA), Argentina. anderson.citrus@gmail.com

INTA´s Citrus Improvement Program was established 28 years ago as an industry insurance policy. Since 1984, the Concordia Experiment Station maintains the citrus mother trees genetically and sanitarily

checked. The objective is to secure propagating material of commercial varieties for industry needs. Varieties are selected from research projects and/or introductions and are subject to quarantine controls and a sanitation process. The selected trees are shoot-tip grafted and indexed for Tristeza, Psorosis, Exocortis, Cachexia, Citrus Variegated Chlorosis, Canker and Huanglongbing. Two hundred and thirteen scion and rootstock varieties are maintained and evaluated annually for their agronomic characteristics and sanitary status by visual observation and biological, immunochemistry and/or molecular diagnostics. Data obtained on origin, botanical characteristics, agronomic performance, sanitary status and availability of propagation material is recorded. The information is available at INTA's website (www.inta.gov.ar/concordia). Basic material is exported to citrus producing countries and sent to germplasm banks on request. The isolation, annual evaluations, pest monitoring and disease diagnosis guarantee the quality of the material offered by the Program to Argentine citrus industry.

S11P16

Labor-saving practices for 'Shatangju' mandarins in China

Ma P.Q., and Wu W.

Key Laboratory of South Subtropical Fruit Biology and Genetic Resource Utilization, Ministry of Agriculture, Fruit Research Institute, Guangdong Academy of Agricultural Sciences, China. mapeiqia@163.com

'Shatangju' mandarin (*Citrus reticulata*) is one of most important citrus variety in Guangdong, China. Some current practices, like excessive fine pruning of the tree, and manual summer shoot tip removal to avoid fruit drop, are expensive and inefficient. Experiments have been carried out using simpler branch pruning methods, restraining summer shoot growth using chemicals or avoiding tip removal. The impact of such new practices on production costs and fruit quality has been investigated. Results showed that there were lower costs and better yields with the new method of branch pruning, due to 32% reduction of labor costs (4230 yuan/ha *versus* 6225 yuan/ha with traditional pruning) and 17% increase of fruit set. Moreover fruit appearance and quality were improved. Furthermore, chemical summer shoot restriction significantly increased fruit retention (from 61.2% to 84.6%). Fruit set was also increased. However, the chemical induced blemishes on some fruit and apparently produced phytotoxicity. On the other hand, keeping summer shoot growth greatly reduced cultivation costs, but decreased production by 57.1%.

S11P17

FruTIC: An information and communication tool for an integrated health management of citrus groves

Garrán S.M.[1], Stablum A.[2], Franco S.[2], Ibarrola S.[2], Mika R.[1], Milera S.[2], and Marnetto S.[2]

[1]Instituto Nacional de Tecnología Agropecuaria (INTA), Province of Entre Ríos, Argentina; and [2]Asociación Cultural para el Desarrollo Integral (ACDI), Province of Santa Fe, Argentina. smgarran@correo.inta.gov.ar

In order to allow the implementation of integrated management practices (IPM), an information and communication platform has been developed for the citrus growing area of the Uruguay River, in Argentina. With this objective in mind, an environmental and phenological monitoring network has been established throughout the whole region. This system, named FruTIC and based on the new ICTs, is accessible via internet through its website (http://www.frutic.org.ar) as well as through automated alerts and warnings implemented via SMS and e-mails. Information is hourly updated all year around, providing historical and current data and also forecasts and models on the evolution of most of the environmental and phenological variables required for fulfilling IPM practices. The system allows the access to both referential as well as grove personalized information on these variables. It also facilitates the technical administration of citrus groves since it allows that all recommendations and practices performed on every commercial plot be registered on line, as well as providing automated reports of all management and monitoring practices performed. This documentation is also needed for traceability requirements and the certification of the production process. FruTIC is an innovative system for the management of the citrus crop, providing elements for a precision, more sustainable and less contaminated citrus industry. It is also able to be applied on any other citrus growing areas and crops.

S11P18

A phytosanitary evaluation method (MEF) for commercial citrus groves.

Garrán S.M.[1], Mika R.[1], Boca R.T.[2], Beribe M.J.[3], and Pérez A.[4]

[1]Instituto Nacional de Tecnología Agropecuaria (INTA), Entre Ríos, Argentina; [2]Instituto Nacional de Tecnología Agropecuaria (INTA), Instituto de Clima y Agua (CNIA), Argentina; [3]Instituto de Producción Animal (CNIA), Buenos Aires, Argentina; and [4]Facultad de Agronomía de la Universidad Nacional de Buenos Aires (FAUBA), Argentina. smgarran@correo.inta.gov.ar

Precision agriculture means site specific management. In order to fulfill this concept, information is needed on main factors affecting yield and fruit quality within the orchard. A phytosanitary evaluation method (MEF) has been recently developed in order to fulfill the identification of main biotic and abiotic factors affecting yield and fruit quality within a citrus grove and also estimating physical and economic losses attributed to each one of these factors as well as their spatial distributions. An improved version of this method will be presented which is based on field sampling of citrus commercial plots performed close to harvest time. Diagnosis and quantification of yield and fruit quality losses are now obtained simultaneously through the same field inspections performed by trained personnel and based on a systematic georeferenced tree sampling together with a multistage random sampling of their fruits. Fruit number of each sampled tree is estimated as well as main factors causing yield constraints are visually identified and categorized according to their relative magnitude. Fruit quality data are also obtained by categorizing every sampled fruit on trees according to Commercial Grading Standards, identifying and listing main factors affecting fruit grade and measuring fruit equatorial diameter. Data obtained from both yield and fruit quality samplings together with updated market prices for the different Commercial grades are then processed through algorithms and geostatistics. Since the method also provides information about the spatial distribution of different pests and diseases within the plot, it can be considered suitable to be used as a basic tool in precision fruit production.

S11P19

Micro budding of commercial citrus cultivars of India

Vijayakumari N.

National Research Centre for Citrus (NRCC), Tissue culture, India. narukullav@gmail.com

Commercial propagation of citrus in India and in many other countries is based on the traditional shield/T budding technique. The major factors that influence the performance of this technique are related to the use of appropriate rootstocks (1 year old 'Rough' lemon, 1 ½ to 2 year old 'Rangpur' lime) and adequate scion wood (round twigs having white streaks which are season restricted), and the occurrence of favorable climate conditions. The method takes long time (around 22 months) to produce marketable bud grafts. "Micro budding" is a faster propagation technique (approximately one year long) that can reduce nursery phases and reduce grower expenses by saving time and space. It has been developed for the first time in India at NRCC, using *Citrus reticulata* Cv. 'Nagpur' mandarin on 5 month old commercial citrus rootstocks of Central India. The technique can be easily extended to other citrus species (i.e. sweet orange, acid lime, 'Kinnow' mandarin). It reduces enormously labour and maintenance costs during the commercial propagation and can be performed in low cost green houses. It can also be used for fast biological screening of virus, viroids and bacteria, and in any many disease inoculation and expression studies. Thanks to the use of micro budding, NRCC has multiplied and released around 15,000 high quality plants of 'Nagpur' mandarin to citrus growers of Central India, within a time span of 11 - 12 months. Prior to commercial release, these micro budded plants have been evaluated at NRCC in replicated field trials. This paper reports the complete process and how field tests were performed.

S11P20

Evaluation of organic growing media and bio-control agents in the production of certified citrus rootstocks

Yaseen T.[1], Dongiovanni C.[2], Roccuzzo G.[3], Ippolito A.[4], and D´Onghia A.M.[1]

[1]Centre International de Hautes Etudes Agronomiques Méditerranéennes (CIHEAM/IAM.B), Italy; [2]Centro di Ricerca e Sperimentazione in Agricoltura, Basile Caramia, (CRSA), Italy; [3]Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee (CRA-ACM), Catania, Italy; and [4]Dipartimento di Biologia e Chimica Agro-forestale ed Ambientale, Università degli Studi di Bari Aldo Moro, Italy. donghia@iamb.it

Citrus groves for organic production shall fulfill the European Regulation on organic production and labeling of organic products (EC No 889/2008), which requires the use of citrus seedlings grown under organic

management. Due to the lack of nurseries able to comply with these requirements, a study was financed by the Italian Ministry of Agriculture (MiPAAF) regarding the preparation of suitable organic media for growing citrus rootstocks and for the evaluation of the commercial bio-product Clonotri (*Trichoderma harzianum* and *Clonostachys rosea*), and this to enhance plant vigour and protect the trees against soil-borne pathogens. Substrates were prepared and evaluated using the growing medium of IAM-Bari (IAMB mix) as control, since this substrate has been used with success for the production of citrus plants. Considering that peat moss is a non-renewable natural resource, commercial and experimental composts for organic production (ECOS and Compas, respectively) and coconut fiber were individually evaluated for reducing the peat moss quantity (35%) in IAMB mix. Moreover, a Guano-based fertilizer was added to the 3 growing media and the elemental sulfur used for pH adjustment. Trials were carried out in an insect proof screenhouse for the premultiplication of the trees in the Apulian certification programme. About 3-4 month-old 'Volkameriana' lemon, 'Troyer' citrange and sour orange seedlings were transplanted to the 3 growing media and the IAMB mix. All growing media were evaluated individually and in combination with Clonotri, using 20 replicates per treatment per rootstock. Plant growth, trunk diameter and dry weight were evaluated at the end of the experiment. Significant increase in vegetative parameters was obtained in all citrus rootstocks grown in the Compas-based growing medium. However, no significant results in enhancing plant growth were achieved by the combination of Clonotri.

S11P21

The diversification of agriculture in the new Cuban Citriculture

Cueto J.C.[1], Piñero J.P.[2], Ortega I.O.[3], and Torres L.T.[1]

[1]Grupo EmpresTimes Fruticola (GEF), Direccion Técnica y de Desarrollo, Cuba; [2]Empresa Cítricos Ceiba, Dirección, Cuba; and [3]UBPC 24 de Febrero, Cooperativa de Producción, Cuba. despacho@iift.cu

The Cuban citrus production is developed by the specialized enterprises of Fruit Culture Enterprise Group (GEF) mainly; those enterprises are located all over the country under a great diversity of climatic conditions. The existing climatic and soil diversity also permitted the successful development of many other horticultural crops, like other fruits and crops with short production cycle. After the invasion of HLB disease to Cuba, the yields of the old and affected citrus groves have been decreasing quickly and the financial capabilities of the producers went down. The productive infrastructure and the industrial capabilities for the citrus fruit processing are not exploited efficiently during the whole year. Cuba has the goal to recover its citriculture through the diversification of the plantations, with plantings mainly in new areas dedicated to citrus production. However, in the Cuban conditions, these decisions and practices have very little background information available. In this paper we summarize the principal experiences obtained in some Cuban enterprises in the last three years (2009-11) about use of intercropping with minor crops in citrus plantations. This approach is analyzed in details using the results obtained by one citrus production cooperative at "Ceiba Enterprise".

S11P22

Presence of weed biotypes with suspected resistance to glyphosate in agroecosystem citrus orchards in Cuba: a latent threat

Otero L.[1], and de Pardo R.[2]

[1]Institute of Research in Tropical Fruitculture, Cuba; and [2]University of Córdoba, Department of Agricultural Chemistry, Spain. lilianotero@iift.cu

In all the citrus orchards in Cuba, in the last few years, there has been evidence of weeds escaping from glyphosate control at doses which normally controlled them (1440 $g \cdot ha^{-1}$) in the four annual frequencies of application dictated by the technologies set up for this crop. Species like *Melochia pyramidata*, *Dichanthium annulatum*, *Momordicha charantia*, and *Chamaesyce prostrata* show an evasive behavior towards this herbicide, which has had an exponential trend in its preference and use by agricultural enterprises for over 10 years. Some weeds like *Bidens pilosa* and *Lepidium virginicum* have been problematic in enterprises in various areas of the country (Ceiba in La Habana and Arimao in Cienfuegos) where they occur with an occupation of

over 50% of the infested fields. The frequent presence of *Echinochloa colona* and *Eleusine indica* is of interest if we take into account that they have been reported as being resistant to glyphosate in several countries like Argentina, Australia and the USA. This work describes the most suspicious species in the different regions of the country with the aim of warning about a situation, which in the immediate future could complicate the management of citrus orchards.

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CITRUS HLB AND OTHER BACTERIAL DISEASES

S12O01

New insights into the citrus Huanglongbing complex and potential solutions to this devastating disease

Duan Y.P., Zhou L.J., Zhang M.Q., Vahling-Armstrong C., Hao G.X., Zou H.S., and Hoffman M.
USDA-ARS-USHRL, USA. Yongping.Duan @ ars.usda.gov

Citrus Huanglongbing (HLB) is a century-old and emerging disease that impedes citrus production worldwide. *Candidatus* Liberibacter asiaticus (Las) is the globally prevalent species of HLB bacteria. Here we describe molecular characterizations of Las, and our newly-developed control methods. From a genomic standpoint, we revealed that Las has a significantly reduced genome and unique features adapted to its intracellular lifestyle. For such a small genome, a large fraction, about 1/15, originates from prophages/phages. There are at least two prophages in the Florida isolates. Frequent recombination and reassortment of these prophages/phages may contribute to the evolving diversity and plasticity of the bacteria. There are at least 8 different types of Las populations that exist in different hosts, different geographical origins, and in a single infection. Furthermore, the different Las populations may be responsible for the titer variations, such as the extremely low-titer seen with seed-transmitted Las bacteria from citrus and of infected *Murraya paniculata*. Using functional genomics, we have revealed several aspects of Las biology that may contribute to its pathogenicity including a high affinity zinc uptake system (ZnuABC), which allows Las to compete with its host for the limited zinc supply and an ATP translocase that facilitates parasitization of the host's energy supply. In addition, Las encodes two novel autotransporter proteins that target mitochondria and may modulate host energy biosyntheses. Although no flagellum has yet been observed, Las encodes a functional flagellin that triggers the citrus basal defense response. In spite of the difficulty with HLB control, our newly-developed thermotherapy and chemotherapy methods eliminated or suppressed Las bacteria in the- infected plants, providing potential solutions for this devastating disease.

S12O02

Tissue-print and squash real-time PCR for direct detection of *Candidatus* Liberibacter spp. in citrus plants and insect vectors

Bertolini E.[1], Lopes S.[2], Felipe R.T.A.[3], Teresani G.[1], Mourão F.A.A.[3], Colomer M.[4], Bové J.M.[5], López M.M.[1], and Cambra M.[1]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Protección Vegetal y Biotecnología, Moncada, Valencia, Spain; [2]Fundo de Defesa da Citricultura (FUNDECITRUS), Plant Pathology, Araraquara, São Paulo, Brazil; [3]Universidade de São Paulo (USP), Produção Vegetal, Piracicaba, São Paulo, Brazil; [4]Plant Print Diagnostics S.L. (PPD), Technical Department, Guadassuar, Valencia, Spain; and [5]Institut Nationale de la Recherche Agronomique (INRA) and Université Victor Ségalen, Bordeaux, France. mcambra@ivia.es

Huanglongbing (HLB), the most severe disease of citrus, is seriously threatening the industry worldwide. The accurate detection of the *Candidatus* Liberibacter species associated with HLB, is essential for control and management of the disease. Real-time PCR is a very appropriate technique for sensitive and specific detection, but the need for nucleic acids purification, greatly limits the number of samples that can be processed and highly increases the time as well as the cost of the analyses. Direct real-time PCR detection of *Ca*. Liberibacter, overcoming nucleic acids purification, is successfully achieved by systems of sample preparation such as tissue-print, spot or squash methods on membranes. New, universal *Ca*. Liberibacter primers were designed, patented and used with TaqMan probe for accurate detection of plant and psyllid-vector targets immobilized on nylon or paper membranes. A complete kit based on this technology was successfully validated in an international ring-test and diagnostic parameters were estimated. HLB targets were amplified from 35% of 1,359 squashed *Diaphorina citri* individuals having fed on HLB infected plants in Brazil. All samples (1,013) collected from citrus trees during extensive surveys in Canary Islands, Spain, where *Trioza erytreae* is widely present, tested negative; the bacterium was not detected in citrus or in psyllid species. The developed method demonstrated high efficiency in detecting the HLB bacterial agents in routine analyses of the many samples required for prevention and efficient management of the disease.

S12O03

A new insect vector of *Candidatus* Liberibacter asiaticus, Cacopsylla (Psylla) citrisuga (Hemiptera: Psyllidae)

Cen Y.[1], Gao J.[2], Deng X.[3], Xia Y.[4], Chen J.[5], Zhang L.[1], Guo J.[2], Gao W.[1], Zhou W.[3], and Wang Z.[2]

[1]Lab. of Insect Ecology, South China Agricultural University, China; [2]Institute of Tropical and Subtropical Cash Crops, Yunnan Academy of Agricultural Science, China; [3]Citrus Huanglongbing Research Laboratory, South China Agricultural University, China; [4]Center for Integrated Pest Management, North Carolina State University, USA; and [5]Crop Diseases, Pests and Genetics Research, San Joaquin Valley Agricultural Sciences Center, United States Department of Agriculture, USA. cenyj@scau.edu.cn

Nymphs and adults of the pomelo psyllid (Cacopsylla (Psylla) citrisuga Yang & Li) were collected from Huanglongbing (HLB) symptomatic and Candidatus Liberibacter asiaticus (Las)-positive lemon trees (Citrus limon (L.) Burm.F.) in Yunnan Province, China. DNA was extracted from groups of psyllid nymphs, individual late-stage nymphs and groups of adults, and the samples were analyzed with nested-PCR (primers:1500R/27F, OI1/OI2c). The results showed that 10 out of 24 group-nymphs samples, 12 out of 29 individual-late-stage nymph samples, 2 out of 3 group-adult samples were Las-positive. DNA was also extracted from group-nymph of the 1st, 2nd, 3rd, 4th and 5th instar, then subjected to nested-PCR detections. One out of 4 1st instar, 2 out of 4 2nd instar, 1 out of 3 3rd instar, 1 out of 2 4th instar, and all 3 5th instar nymph samples were Las-positive. The amplified DNA fragment was sequenced and was 99% similar to the 16S rRNA gene of Las strain psy62 in GenBank. Transmission study showed that 12 out of 60 healthy lemon young plants were Las-positive after being fed by psyllid nymphs and adults collected from infected trees for about 1 month. These results demonstrate that in addition to Asian citrus psyllid (Diaphorina citri Kuwayama), C. (P.) citrisuga is another insect vector of Las.

S12O04

On the identity of orange jasmine and its relevance to Huanglongbing and *Diaphorina citri.*

Holford P.[1], Nguyen C.H.[1], Beattie G.A.C.[1], Haigh A.M.[1], Hasick N.J.[1], Mabberley D.J.[2], and Weston P.H.[2]

[1]Univeristy of Western Sydney, School of Science and Health, Australia; and [2]Royal Botanical Gardens, Sydney, Australia. p.holford @ uws.edu.au

Orange jasmine, a common ornamental, is a favoured host of *Diaphorina citri* Kuwayama, the principal vector of *Candidatus* Liberibacter asiaticus, the most widespread of three liberibacters that cause Huanglongbing. Orange jasmine is also a transient host of the disease, but its identity has been subject to conjecture since 1747. It is widely regarded as *Murraya paniculata* (L.) Jack, but also as *Murraya exotica* L. Our morphological (mostly leaves and leaflets) and molecular studies (6 chloroplast and the ITS rRNA operon regions) of the genus indicated that it is *Murraya exotica*, probably from Southern China/Northern Vietnam, and that *Murraya paniculata* represents a species that occurs in the Indonesian archipelago. The study also determined that a species, *Murraya asiatica ineditus*, previously regarded as *Murraya paniculata*, occurs in mainland Asia and that a fourth species, *Murraya ovatifoliolata* (Engl.) Domin., includes taxa formerly regarded as varieties of *Murraya paniculata*: *Murraya ovatifoliolata* var. *ovatifoliolata ineditus* (small and large leaflet forms in Australia) and *Murraya ovatifoliolata* var. *zollingeri ineditus* (from the eastern Indonesian archipelago). Two possible hybrids, *Murraya* × *omphalocarpa ineditus* from Orchid Island, Taiwan and *Murraya* × *cycloopensis ineditus* from Papua were identified. *Murraya exotica* is naturally associated with low altitude maritime and riparian acid red soils, and *Murraya asiatica* with limestone hills. Of the four identified species, *Murraya exotica* is the only one known to have been introduced to the Americas. There are no records of *Murraya paniculata* or *Murraya asiatica* as hosts of liberibacters, nor of *Murraya asiatica* as a host of *Diaphorina citri.*

S12O05

Effect of HLB on the expression of calcium signals related genes

Parra C.C.[1], Kunta M.[2], and Louzada E.S.[1]

[1]Texas A&M University-Kingsville (TAMUK), Citrus Center, USA; and [2]Texas A&M University- Kingsville (TAMUK), Citrus Center, USA. eliezer.louzada @ tamuk.edu

Citrus has been challenged for a long time by innumerable biotic and abiotic threats, and it has so far survived. However, the introduction of the bacterium *Ca*. Liberibacter *asiaticus* (CLas), causal agent of citrus greening

or Huanglongbing (HLB) and its vector, the Asian Citrus Psyllid (ACP), has completely changed this scenario and the industry now faces one of its worst nightmares. It is well know that Calcium is one of the most important elements in the plant that generate signals to be transduced into response to counteract basically any kind of biotic and abiotic stresses. No information is currently available on the effect that HLB has on genes regulating calcium signals, however, nutrient leaf analysis shown that Calcium is one the elements that is reduced upon CLas infection. The objective of this research is to study the effect of CLas infection on the expression of genes involved in Calcium dynamic in HLB infected Valencia sweet orange young and mature leaves, asymptomatic and symptomatic, compared to non-infected Valencia orange control. Results by real-time PCR obtained to date shows strong reduction in expression of a calcium ion channel in young and mature infected asymptomatic leaves compared to health control. Mature symptomatic leaves also had reduced expression compared to control, but more expression than young and mature asymptomatic leaves. -Results for expression of 15 genes involved in calcium signals will be presented.

S12O06

Transcriptional genomics and proteomics in citrus roots infected by *Candidatus* Liberibacter asiaticus.

Zhong Y.

Institute of Fruit Tree Research, Guangdong Academy of Agricultural Science (GAAS), Citrus Breeding, China. zhongyun99cn@163.com

Jiangxired tangerine root samples at 50 wpi were used in RNA-sequencing. Some DEGs were confirmed by qPCR. Results showed that: 1) 3956 genes were differentially expressed, among which 1840 were up-regulated and 2116 down-regulated. 65 DEGs were identified with more than 10-fold changes. 2) SA signal transduction pathway related genes were up-regulated, the key gene of this pathway, NPR1 was up-regulated 1.6 times, and the downstream PR1 gene was up-regulated 2.4 times. 3) JA signal transduction pathway related gene didn't have notable responses to HLB infection. 4) Sucrose invertase was up-regulated 1.7 times, while the sucrose synthase were down-regulated 1.8 times. This may indicate that the sucrose level in HLB infected roots decreased as result of plugged phloem. Furthermore, ferredoxin expression was down-regulated 2.2 times, which may be related to nutrition deficiency, that is commonly associated with HLB infected trees.

Comparative proteomic approach was also applied to decipher the pathogenic process of HLB in affected red tangerine roots using iTRAQ technique. Results showed that: 1) The 1445 identified proteins mainly ranged from 20 to 70kDa or larger than 100kDa. 2) plugged phloem related protein SEO was up-regulated by 1.67 times, and this protein may function in inhibiting infection of the HLB bacteria and hampering the long distance transport of sucrose. Subtilisin-like protease was up-regulated with a fold change of 282.09 and the gene of this protein was also up-regulated 3.14times, which suggested that the enzyme play important roles in inhibiting the infection of HLB bacteria and protecting the root meristematic zone.

S12O07

Resistance and tolerance to Huanglongbing in *citrus*

Stover E.[1], McCollum G.[1], Driggers R.[1], Duan Y.P.[1], Shatters, Jr. R.[1], and Ritenour M.[2]

[1]USDA/ARS, US Horticultural Research Laboratory (USDA/ARS, USHRL), USA; and [2]University of Florida, Indian River Research and Education Center (U of F, IRREC), USA. ed.stover @ ars.usda.gov

Huanglongbing (HLB) is severely impacting Florida citrus. Productivity declines in many HLB-affected genotypes, often with greatly thinned canopies. Fruit size and quality are often adversely affected as the disease advances. HLB was assessed in diverse cultivars in commercial groves with high HLB-incidence. Temple had the lowest HLB symptoms and Liberibacter (Las) titer, while Murcott and Minneola had the highest. The USDA Ft. Pierce, FL farm is managed to reveal genotype responses to HLB. Some current cultivars and hybrid seedlings demonstrate resistance/tolerance, at least to strain(s) of Las present. C. trifoliata is the best documented citrus resistance source with Las titers suppressed even when C. trifoliata is grafted onto severely-infected rootstocks. Some cultivars and hybrids have abundant foliage symptoms, but full canopies and seemingly normal fruit set and size. In 3-years of data from a replicated trial of Triumph(T), Jackson(J), Flame(F), and Marsh(M), HLB symptoms were severe in all trees and Liberibacter titers were similar. However, F&M were almost completely defoliated in some years while T&J had full canopies. Cumulative fruit/tree was

greater for T&J (255&220) than for F&M (29&66). T&J fruit met commercial standards and had normal size but F&M fruit were unacceptable with many small and misshapen. Evidence mounts that useful resistance/ tolerance to HLB is present in cultivated citrus and this is a focus of the USDA citrus breeding program.

S12O08

A comparison of different methods to evaluate host resistance or tolerance to Huanglongbing, caused by *Candidatus* Liberibacter asiaticus.

Bowman K.D., and Albrecht U.

United States Department of Agriculture, Agricultural Research Service (USDA, ARS), United States Horticultural Research Laboratory, USA. kim.bowman @ ars.usda.gov

One objective of the USDA-ARS citrus breeding program is the development of cultivars with resistance or tolerance to Huanglongbing (HLB) disease, caused by *Candidatus* Liberibacter asiaticus (Las). Both conventional breeding and transformation are being used to generate new clones that are tested for response to the pathogen. We will present the results from 20 separate experiments to test host resistance or tolerance to Las infection. Natural infection in the field occurs through the vector Asian Citrus Psyllid (ACP), but field infection is much slower than desired for efficient studies. Greenhouse inoculation through ACP or graft is much more controlled and rapid, but also presents certain challenges and limitations. In our studies, host trees that became infected with Las in the greenhouse and field developed symptoms that were measured, including effects on leaf morphology, tree growth, and cropping. Host gene expression and metabolic changes were studied, and specific responses documented. Measurements of Las bacteria titer over time were also used to assess the resistance or tolerance of the host. Typical susceptible cultivars became PCR positive for Las as early as 5-8 weeks after graft inoculation, while field infection of trees by even heavy populations of ACP could take 1-2 years or more. Symptom development typically lagged considerably behind PCR detection of the pathogen. Several host clones with resistance or tolerance to Las infection were included in the studies.

S12O09

Evaluation of transgenic citrus for disease resistance to HLB and Canker

Dutt M.[1], Barthe G.A.[1], Orbovic V.[1], Irey M.[2], and Grosser J.W.[1]

[1]University of Florida (UF), Citrus Research and Education Center, USA; and [2]United States Sugar Corporation, USA. manjul@ufl.edu

Transgenic citrus scion and rootstock cultivars were transformed with gene(s) encoding antimicrobial peptides or systemic acquired resistance (SAR) proteins. Each gene was under control of an enhanced CaMV 35S promoter. Several genes were also under control of a phloem specific *Arabidopsis* SUC2 (*At*SUC2) promoter. A number of clones of each transgenic line were evaluated for resistance to Huanglongbing (HLB, caused by Candidatus Liberibacter asiaticus) and Citrus Canker (caused by Xanthomonas citri ssp. citri). 600 trees planted in spring 2009 in a heavily HLB and canker infected Martin County grove were tested using qPCR for infection to HLB after 30 months in field. 396 trees tested negative for the HLB bacterium. Approximately 200 PCR negative trees were observed to be healthy and flushing after 40 months in the field and were again evaluated using qPCR. In addition, we observed several trees containing one of our antimicrobial constructs to be tolerant to citrus canker. In a separate experiment, 345 transgenic trees and controls were placed in a greenhouse containing free flying HLB-infected Asian Citrus Psyllids (ACP) during April 2011. All trees were evaluated for infection after 12 months by qPCR, and 80% of the transgenic trees tested negative for the bacterium. These results suggested that some of the antimicrobial peptides could provide varying levels of resistance against both HLB and canker.

S12O10

California's response to the first detection of HLB.

Luque-Williams M.J.

California Department of Food and Agriculture (CDFA), USA. mlwilliams@cdfa.ca.gov

Since the first detection of the Asian Citrus Psyllid in California in August 2008 (along the border with Mexico) the California Department of Food and Agriculture has implemented, in addition to the long-standing ACP

trapping program, visual surveys, psyllid collections and chemical treatments within a 400m radius of all psyllid finds. Psyllids collected in these surveys were tested for HLB. Despite the surveys and suppression efforts within two years the psyllid had spread throughout five Southern California counties, principally in Los Angeles County, but not to the Central Valley where the majority of commercial citrus is located. In March 2012 a psyllid collected from a grafted pummelo (of unknown origin) during a visual survey in Los Angeles County tested positive for HLB, the first such find in California. Plant material collected from the tree (exhibiting classic HLB leaf symptoms) confirmed that the tree was positive for HLB. A trace forward and trace back of the HLB positive tree was initiated to determine the original source of the budwood of the HLB positive tree (as yet unsuccessfully). Eradication of the tree ensued paralleled by the CDFA developed HLB-positive protocol previously established. All citrus and citrus relatives, ACP nymphs and adults within a 800m radius were collected and tested. With the positive HLB find the Citrus Pest Disease Prevention committee, working with CDFA, has provided the funding for HLB testing of all host plants and ACP (adults and nymphs) in the first 500 m around the find every two months for a year and the testing of ACPs in the next 500m around the find and the host plants that show symptoms. To date no other HLB-positive psyllids or trees have been detected.

S12O11

Comparative study of different host range strains of *Xanthomonas citri* subsp. *citri*: Chemotaxis and biofilm formation.

Sena M.[1], Ferragud E.[1], Redondo C.[1], Johnson E.G.[2], Graham J.H.[2], Girón J.A.[3], and Cubero J.[1]

[1]Instituto Nacional de Investigaciones Agrarias y Agroalimentarias (INIA), Plant Protection, Spain; [2]University of Florida (UF-CREC), Citrus Research and Education Centre, USA; and [3]University of Florida (UF-EPI), Emerging Pathogen Institute, USA. sena.marta @ inia.es

Xanthomonas citri subsp. *citri* strain A (*Xcc* A) is one of the causal agents of Citrus Bacterial Canker (CBC). Bacterial strains within this species are classified according to differences in host range as well as phenotypic and genotypic characteristics. *Xcc* A has a broad host range within the genus *Citrus* and close relatives, whereas *Xcc* A* and *Xcc* A^W strains are limited to Key Lime (*Citrus aurantifolia*). The aim of this study was to compare the chemotactic profile and biofilm formation of *Xcc* A, A* and A^W strain types compared to *Xanthomonas alfalfae* subsp. *citrumelonis,* causal agent of Citrus Bacterial Spot, and *Xanthomonas campestris*, causal agent of Black Rot in crucifers. Our results suggest differences in the chemotactic profile between broad and limited host range *Xcc* strains and xanthomonads from other hosts. Differences in biofilm formation were found among the strains and the inoculated host. At the early stages of biofilm formation, *Xcc* A produced an interwoven fibrillar structure while *Xcc* A^W fibres had a linear structure. TEM revealed that surface motility structures on cells varied; *Xcc* A formed lateral appendages at the edge of the bacterial colony while *Xcc* A^W possessed a single polar flagellum. Purification and sequencing of *Xcc* surface proteins revealed the presence of type IV pilus associated with biofilm and twitching motility in other bacterial species. Current studies are focused on the role of this protein in biofilm formation and the possible differences among the *Xanthomonas* strains.

S12O12

A new minisatellite-based scheme for the global surveillance of *Xanthomonas citri* pv. *citri*, the causal agent of Asiatic Citrus Canker.

Magne M.[1], Leduc C.[1], Vernière C.[1], Vital K.[1], Gordon J.[2], Escalon A.[1], Guérin F.[2], Gagnevin L.[1], and Pruvost O.[1]

[1]CIRAD (CIRAD), UMR PVBMT, Réunion, France; and [2]Université de la Réunion, UMR PVBMT, Réunion, France. olivier.pruvost @ cirad.fr

Xanthomonas citri pv. *citri* (*Xcc*), the causal agent of Asiatic Citrus Canker, is an internationally major bacterial pathogen currently not reported from countries in the Mediterranean basin, including the EU. Some pathological diversity among strains was reported, leading to the description in *Xcc* of pathotypes differing in host range and aggressiveness. *Xcc* is recognized as a monomorphic pathogen and the very low intra-pathovar diversity at housekeeping genes precludes the use of the increasingly popular MLST (MultiLocus Sequence Typing) for the global molecular surveillance of *Xcc*.

We developed a new MLVA (MultiLocus Variable number of tandem repeats Analysis) scheme. It targeted 51 minisatellite loci dispersed over the *Xcc* genome with tandem repeat size ranging from 10 to 217-bp, thus allowing the use of standard agarose gel electrophoresis in laboratories that are not equipped with a genotyper. Among these loci, 34 were found to be polymorphic within a strain collection composed of strains from all continents where *Xcc* has been recorded, including recent outbreaks in Africa where the bacterium is reemerging. This typing scheme displayed a maximal typeability and repeatability. The produced data support the usefulness of this new MLVA scheme for global surveillance of *Xcc*.

S12O13

Analysis of microRNAome of Chinese citron 'C-05' resistant to Citrus Canker disease.

Dai S.M., Chen Y.M., Li D.Z., Li F., Yan J.W., and Deng Z.N.

Hunan agricultural university, China. dsm531@126.com

MicroRNAs (miRNAs) are small RNAs (sRNAs) 20-24 nucleotides in length, and commonly exist in organism. During the last 20 years, research on isolation and function identification of miRNAs made the people understand regulation of gene expression more deeply. Some research showed miRNAs played important role in resistant response of plant to pathogenic bacteria. Citrus Canker is a quarantine bacterial disease, which seriously affect the development of citrus industry. By field trials for many years, we obtained a special material ('Chinese Citron' C-05) resistant to citrus canker which would provide a good base to carry on the research about resistance of citrus to this disease. In this research, Illumina/Solexa deep sequencing technology was applied to analyze microRNAome of Chinese Citron C-05 and susceptible Bingtangcheng (as control). Comparing with Bingtangcheng, miRNAs profile of Chinese Citron C-05 had 69 miRNAs only expressed before inoculation of pathogen bacteria, 64 miRNAs after inoculation and before appearance of hypersensitive necrosis reaction, 74 miRNAs after appearance of reaction. Moreover, through prediction and function analysis of target genes, results showed 69 metabolic pathways were regulated by these differentially expressed miRNAs only in Chinese Citron C-05. Among these pathways, we were interested in 3 pathways related to plant resistance which were helpful to reveal molecular mechanism of 'Chinese Citron' C-05 resistant to citrus canker. Now, we were verifying miRNAs regulated these 3 pathways and expression of target genes.

S12O14

The role of auxin in the citrus defense to early infection by *Xylella fastidiosa*.

Rodrigues CM, Souza AA, Takita MA, and Machado MA.

Centro de Citricultura Sylvio Moreira, IAC, Laboratory of Biotechnology, Brazil. marcos@centrodecitricultura.br

Many studies have shown the involvement of plant hormones in plant responses to biotic and abiotic stresses. Recent studies indicate that auxin has an important role in in stress responses. Previous results from our group showed induction of some genes involved in auxin pathway in Ponkan mandarin (*Citrus reticulata* Blanco), tolerant to citrus variegated chlorosis, one day after challenge with *X. fastidiosa*, the causal agent of this disease. Thus, the study aimed to evaluate the differential expression of some genes involved in this pathway in mandarin and the susceptible sweet orange (*Citrus sinensis* L. Osb) in early stage of infection. The genes evaluated were: *IAA9* (*Aux/IAA*), *ARF6*, *TIR1*, *UBA1*, *UBC2*, *E3*, *SKP*, and *BIG*. The RNAs from mandarin and orange infected or not (control) with the bacteria were extracted. RT-qPCR analyses were carried and as a result, all genes were significantly induced in mandarin. However, in sweet orange it was observed significant induction just for *UBC2* and *UBA1* genes. Because there was also a repression in expression of *E3*, *SKP,* and *ARF6*, ubiquitination may be involved in other pathways. It was evident in this work the importance of differential expression of genes involved in auxin metabolism in resistance and susceptibility to *X. fastidiosa* of mandarin and orange, respectively. In addition, the results indicate that this pathway plays an important role in the defense response of mandarin in the initial stage of infection by *X. fastidiosa*.

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S12P01

Yield loss modeling of "*Candidatus* Liberibacter asiaticus" on Persian lime (*Citrus latifolia*) in southern Mexico

Flores Sanchez J.L.[1], Mora Aguilera G.[1], Loeza Kuk E.[2], Domínguez Monge S.[1], Acevedo Sánchez G.[3], and López Arroyo J.I.[4]

[1]Colegio de Postgraduados (Colpos), Fitopatología, México; [2]INIFAP-Campo Experimental Mocochá, Yucatán (INIFAP), Fitopatología, México; [3]Laboratorio de Análisis Epidemiológico de Plagas Reglamentadas (SINAVEF Lab), Fitopatología, Colpos, México; [4] INIFAP -Campo Experimental General Terán, Nuevo León (INIFAP), Entomología, México. flores.jorge@colpos.mx

In Mexico, Huanglongbing caused by *Candidatus* Liberibacter asiaticus (Las) was detected in Yucatan in 2009 mainly on *Citrus latifolia* and *C. aurantifolia*. An early infected Persian lime orchard was selected to estimate the yield loss caused by Las for purpose of decision making on disease management. A restricted random block design with paired healthy and diseased trees, verified by real time PCR, was established to assess eight morphologic and organoleptic fruit variables in 2011. Weight, size, peel thickness, juice volume and pH were statistically higher on healthy trees (T1) and asymptomatic branches (T3) of positive trees with regard to symptomatic branches (T2) (Tukey, p= 0.05). Differences were not found between T1 and T2 with exception of fruit size. Brix and titratable acidity were not different on any treatment. Las induced higher detrimental effect on weight (17.3%) and juice volume (18.6%) being dependent on disease severity (*x*) (0=healthy, 1=25, 2=50, 3=75, 4=100% canopy symptoms): *Y*weight = 217.2-4.2*x* 0.03x^2, R^2= 0.86; *Yvolume* = 645.4-11.7*x* 0.09x^2, R^2= 0.82. Yield detrimental effect was associated with bacterial concentration base on its relationship with severity: *Y*[bacteria] = 1.174 0.8*x*-0.0067x^2, R^2 = 0.91. HLB-Per 1.0, a MS Excel program, was developed to estimate the impact on production and profit considering different Las infection periods. The model was also validated with *C. aurantifolia.* This is the first yield loss report on Persian lime.

S12P02

Seasonal detection of Huanglongbing symptomatic trees and *Diaphorina citri* in citrus groves in Sao Paulo State, Brazil

Bassanezi R.B.

Fundo de Defesa da Citricultura (Fundecitrus), Departamento Científico, Brazil. rbbassanezi@fundecitrus.com.br

Citrus Huanglongbing (HLB) was first reported in Sao Paulo State (SPS), Brazil, in 2004 and since that the management of this disease has been based on detection and elimination of symptomatic trees and monitoring and control of the insect vector Asian Citrus Psyllid (ACP), *Diaphorina citri*. To maximize the labor and financial resources for best HLB management it is important to determine the periods when HLB-symptomatic trees and ACP are detected by scouting teams in the field. To analyze the seasonality of HLB-symptomatic trees detection, data of monthly scouting of 100% of citrus trees in all citrus blocks of 24 citrus groves in 20 municipalities of Southern, Southwestern, Eastern and Center regions of SPS, from 2005 to 2011, were used. For ACP, data of fortnightly assessments of yellow stick traps placed in four groves without insecticide application in Eastern and Center regions of SPS, from 2005 to 2011, were used. Although HLB-symptomatic trees were detected in all months, 79% of total amount of detected diseased trees was found from February to August and less than 10% from October to December. ACP was also detected in all months, but there were two picks of detection in October and December, corresponding to spring and summer main citrus vegetation. More than 80% of ACP was detected from August to January. Due to the simultaneous presence of considerable amount of ACP and symptomatic trees in August and January, these months could be key periods for HLB management.

S12P03

Identification of "*Candidatus* Liberibacter" and phytoplasma and evaluation of their association with citrus Huanglongbing in China

Su H.N., Wang X.F., Tang K.Z., Li Z.A., and Zhou C.Y.

National Engineering Research Center for Citrus, Citrus Research Institute, Southwest University, College of Plant Protection, China. huanansu211@163.com

Citrus Huanglongbing (HLB) is one of the most devastating citrus diseases worldwide. Prior to 2004, "*Candidatus* Liberibacter" spp. was considered as the putative causal agents of HLB, though Koch's postulates

have not been completely fulfilled. Pigeon pea witches'-broom phytoplasma and Aster yellow phytoplasma were detected from citrus samples with characteristic HLB symptoms from Brazil and China, respectively. These two phytoplasmas might also be associated with HLB. In order to elucidate the "*Ca*. Liberibacter" spp. and the phytoplasma association with HLB in the P. R. China, 472 samples were collected from Guangdong, Guangxi, Yunnan, Guizhou, Sichuan, Chongqing, Jiangxi, Fujian, and Zhejiang Provinces from 2008 to 2011. The symptoms included leaf chlorosis, blotchy mottle, midrib yellowing, zinc nutritional deficiency, iron nutritional deficiency and fruit colour inversion. Using primer set OI1/OI2c, PCR products digested with *Xba* were used for identifying "*Ca.* Liberibacter asiaticus" and *"Ca.* Liberibacter africanus". Primer set GB1/GB3 was used for the detection of "*Ca.* Liberibacter americanus". Nested PCR with phytoplasma-universal primer sets P1/P7 with fU5/rU3 and R16mF2/R16R1 with R16mF2n/R16R2 were used for the detection of phytoplasma. Of the 472 samples, 261 were positive for "Ca. Liberibacter asiaticus". Samples with symptoms of blotchy mottle, zinc nutritional deficiency and fruit colour inversion had higher detection rate than those one with other symptoms. However, all samples were negative for *"Ca.* Liberibacter africanus", "*Ca.* Liberibacter americanus" and phytoplasma. Our results suggested that *"Ca.* Liberibacter asiaticus" was highly associated with HLB in China, and phytoplasmas might not be closely related with the etiology of HLB.

S12P04

Rapid on site detection of the HLB/Citrus Greening causal agent "*Candidatus* Liberibacter asiaticus*"* by AmplifyRP™, a novel rapid isothermal nucleic acid amplification platform.

Russel P.[1], Amato M. A.[2], and Bohannon R.[1]

[1]Agdia Inc., United States; and [2]Agdia Biofords, France. marcos.amato@biofords.com

Huanglongbing (HLB), also called Citrus Greening, has become the most serious threat to the citrus industry worldwide. Although the disease first emerged in the southeastern part of Asia, it is now well established all along the American continent, including Brazil, Florida and California which are the world most important citrus production areas. In the Asian and American continents the disease is associated with the insect-vectored, obligate intracellular bacterium "*Candidatus* Liberibacter asiaticus". The bacterium resides in the host phloem cells and it is transmitted by the Asian Citrus Psyllid, *Diaphorina citri*. Early pathogen detection is the key success factor to protect free areas from pathogen introduction. Unfortunately, the disease has a long incubation period, during which pathogen concentration is very low and non-systemically distributed. This makes diagnostic, disease control and eradication programs extremely difficult. Early detection is not possible by using traditional serological methods. Agdia has recently developed a new rapid molecular detection technique, AmplifyRP, allowing PCR-level sensitivity detection within minutes, in the field. AmplifyRP uses a Recombinase-polymerase methodology for DNA amplification at a single temperature. In contrast to conventional or real-time PCR, AmplifyRP has no DNA purification requirements, requires no thermocycling, and results can be read using small and user-friendly devices. A portable florescence reader or a lateral flow device (similar to Agdia's ImmunoStrip®) can be used to visualize results in as little as 30 minutes, compared to several hours for conventional PCR. AmplifyRP eliminates the need for expensive PCR equipment, a large number of chemicals reagents, and the need for technically trained staff. Given its characteristics, AmplifyRP is the ideal tool to monitor HLB progression by early detection of "*Ca*. Liberibacter asiaticus" in citrus trees or in vector insects.

S12P05

Diversity of "*Candidatus* Liberibacter asiaticus", "*Candidatus* Liberibacter africanus" and "*Candidatus* Liberibacter americanus" based on 23S/5S rDNA sequences

Liao H.[1], Bai X.[2], Li Y.[3], Chen C.[4], Yang L.[5], Xu N.[6], Huang H.[6], and Wang X.[6]

[1]Horticulture Research Institute of Guangxi Academy of Agricultural Sciences (HRIGAAS), Citrus Research Group, China; [2]Department of Agriculture of Guangxi (DAG), Citrus Research, China; [3]Guangxi Crop Genetic Improvement and Biotechnology Lab (GCGIBL), Citrus Research, China; [4]Guangxi Citrus Research Institute (GCRI), Citrus Breeding, China; [5]State Key Laboratory of Subtropical Agro-Bioresources Conservation and Utilization (SKLSACU), Citrus Research, China; and [6]Horticulture Research Institute of Guangxi Academy of Agricultural Sciences (HRIGAAS), Citrus Research Group, China. liaohuihong2001@gmail.com

Primers based on the 23S/5S rDNA conserved sequences of "*Candidatus* Liberibacter asiaticus" (Las), "*Candidatus* Liberibacter africanus" (Laf) and "*Candidatus* Liberibacter americanus" (Lam) were designed to

amplify different geographical Huanglongbing affected citrus samples. The PCR products were sequenced and the diversity of different strains was studied. The results showed that the amplified size is 1654 bp including 5S rRNA gene for six strains from Guangxi, Fujian, Hunan, Taiwan, 1648-1650 bp including 5S rRNA gene for four strains from South Africa, 1635 bp including *glp*K gene and 5S rRNA gene for six strains from Brazil, respectively. The strains from Brazil shared 94.1%-94.3% of sequence identity with Las, 94.0-94.2% with Laf and 99.8%-100% with Lam. The strains from South Africa shared 97.4%-97.5% of sequence identity with Las, 99.9-100% with Laf and 94.1%-94.2% with Lam. The strains from China mainland, Taiwan and Florida shared 99.8%-100% of sequence identity with Las, 97.3-97.5% with Laf and 94.2%-94.4% with Lam. Total 4 bps of *glp*K sequence were mutant among three strains from Brazil, but only two amino acids were changed. A phylogenetic tree was constructed for the 16 strains and other proteobacterias based on the 23S/5S rDNA sequences. The results showed that the strains from Brazil were clustered with Lam, those from South Africa clustered with Laf, and those from China mainland, Taiwan and Florida clustered with Las.

S12P06

Phloem anatomy of citrus trees: healthy *vs* HLB affected

Etxeberria E., and Narciso C.

University of Florida, Citrus Research and Education Center, USA. eetxeber@ufl.edu

Phloem cells of HLB-affected trees become obstructed with callose, P-protein plugs, and in some instances, starch grains. The presence of these plugs is believed to hinder the transport of photoassimilates (nitrogenous and reduced carbon compounds) to the root system. However, even with a seemingly collapsed phloem tissue, citrus trees remain viable and produce fruit for some time, suggesting either incomplete plugging of phloem elements or the existence of alternative routes for photoassimilate transport. In this study, we examined the basic structure of phloem tissue from HLB-unaffected and HLB-affected trees under light and scanning electron microscopy. To avoid any possible interference with callose induced by injury during sampling, we employed freeze substitution technique. Sieve elements from HLB-unaffected trees show sizable lateral pit fields to phloem and ray parenchyma. The cells have very angular sieve plates and appear relatively clean from cellular components. Sieve pores are of approximately 0.1-0.5 µm in diameter. HLB-affected phloem cells contain massive amounts of amorphous material clearly traversing sieve plates and lateral pit fields. Eventually, the walls thicken and cells totally collapse into an almost solid cell wall barrier. Occasionally, wound phloem appears along the petiole cortex in HLB-affected trees. Most notable is the large number of wall perforations all along the cortex parenchyma with abundant pit fields. These anatomical features of the parenchyma cells provide an alternative route that would allow movement of photassimilates from photosynthetic cells through cortex and ray parenchyma.

S12P07

Anatomical comparison of HLB-affected sweet orange and rough lemon

Fan J.[1], Chen C.[2], Gmitter Jr. F.G.[2], Achor D.S.[2], Brlansky R.H.[2], and Li Z.G.[1]

[1]Chongqing University, Key Laboratory of Biorheological Science and Technology, China; and [2]University of Florida - Citrus Research and Education Center, USA. fgmitter@ufl.edu

Citrus Huanglongbing (HLB) is considered the most serious problem for citrus production. There is no known resistant germplasm although some citrus types appear to be more tolerant and/or insensitive to the disease, exhibiting slow decline and rejuvenating capability. To understand the underlying mechanisms, symptomatic, asymptomatic, and healthy leaf, stem and root tissues from sensitive sweet orange and tolerant rough lemon were compared using light and transmission electron microscopy. Phloem collapse, plugged sieve elements and accumulation of starch were observed in leaf petioles of symptomatic leaves from both HLB-diseased rough lemon and sweet orange, compared with their own mock-inoculated controls. In symptomless leaves, significant anatomical changes (e.g. phloem cell collapse and starch accumulation) were found in HLB-diseased sweet orange, but not in rough lemon. Furthermore, starch depletion and phloem fiber degradation were observed in secondary roots of diseased sweet orange, but not in rough lemon. In young green stems, a few plugged sieve elements were seen in both diseased rough lemon and sweet orange, but starch deposition

only occurred in the latter. Taken together at the whole plant level, HLB infection induces fewer and less severe anatomical changes in rough lemon than in sweet orange. In particular, the absence of obvious changes in the rough lemon root system is suggested to be critical for sustaining plant growth after infection, and should greatly contribute to its HLB tolerance.

S12P08

The significance of early root infection in Huanglongbing disease development and crop loss

Johnson E.G., Bright D.B., and Graham J.H.

University of Florida (UF-CREC), Citrus Research and Education Center, USA. egjohnson@ufl.edu

Huanglongbing (HLB) is a systemic disease of citrus caused by the phloem-inhabiting bacterium "*Candidatus* Liberibacter asiaticus" (*Las*). Disease in grove trees is initially identified by foliar symptoms, most commonly blotchy mottle. Detection of *Las* in leaf tissue by qPCR early in disease development is usually limited to symptomatic leaves and proximal young leaves. Over multiple years, disease symptoms and detectable Las spread to the rest of the canopy. A negative exponential relationship observed between canopy disease severity and yield decline indicates larger than expected crop loss when symptoms are limited to one or a few branches. Observations in potted and grove trees revealed that *Las* is frequently detectable in roots months before detection of *Las* in leaves and foliar symptom development. Asymptomatic and recently symptomatic grove trees with *Las* detected in root tissue have from 26-46% lower root density compared to apparently healthy trees. These results suggest that early infection of roots by *Las* causes root damage before the appearance of foliar symptoms and is associated with the larger than expected yield reduction on trees with initial foliar symptoms. Further understanding of the early effects of *Las* infection on roots and HLB tree decline is needed.

S12P09

Monitoring "*Candidatus* Liberibacter asiaticus" in a commercial orchard of sweet orange and 'Tahiti' lime in the northwest of Paraná State

Sauer A.V.[1], Nocchi P.T.R.[1], Zanutto C.A.[1], Rinald D.A.M.F.[2], and Nunes W.M.C.[1]

[1]Universidade Estadual de Maringá (UEM), Nucleo de Pesquisa em Biotecnologia Aplicada (NBA) - Centro de Ciências Agrárias (CCA), Brazil; and [2]Secretaria de Estado da Agricultura e Abastecimento (SEAB), Brazil. alinevanessasauer@hotmail.com

Huanglongbing (HLB) stands out as one of the most devastating citrus diseases worldwide, affecting several species of citrus. The symptoms are characterized by branches with yellow leaves, usually isolated in plants. The objective of this study was to evaluate the population behavior of the bacterium "*Candidatus* Liberibacter asiaticus" in a commercial orchard. Plants of sweet orange and 'Tahiti' lime naturally infected with HLB were protected with aphid-proof screens to prevent the spread of disease in the area, after confirmation of diagnosis by conventional PCR. The bacterial population monitoring was performed by real-time PCR after total DNA extraction from main veins of symptomatic leaves collected monthly, for twenty months. In each plant, four samples were collected and analyzed from the quadrants of the tree. In the species studied erratic behavior of the bacteria were observed. Even after detection in one quadrant of the plant, after a few months it was not possible to verify the presence of the etiologic agent in the same place. For the sweet oranges, the quadrants that expressed more severe symptoms, showed the greater amount of bacteria, with the Ct between 18 and 33. For the 'Tahiti' lime similar results to those described for sweet oranges were found. These results contribute to the study of the epidemiology of HLB in this crop and helps to the search for effective control measures.

S12P10

Multiplication of "*Candidatus* Liberibacter asiaticus" in *Citrus sinensis, Citrus sunki, Poncirus trifoliata* and hybrids obtained from crosses between *Citrus sunki* and *Poncirus trifoliata*

Boava L.P., Cristofani-Yaly M., Coletta Filho H.D., Bastianel M., and Machado M.A.

Centro de Citricultura Sylvio Moreira, Instituto Agronômico, Brazil. mbastianel@centrodecitricultura.br

Huanglongbing (HLB) is caused by a phloem-limited bacterium and can be transmitted by both psyllid vector and graft propagation. "*Ca.* Liberibacter asiaticus" (Las) is the most widespread specie in Brazil and

affects all known citrus species and relatives. However, there are reports that some genotypes are slower to develop HLB symptoms. In this work, we examined the Las multiplication in 3 genotypes (*C. sinensis, C. sunki* and *P. trifoliata)* and 10 hybrids obtained from crosses between *C. sunki* and *P. trifoliata.* All genotypes (3 biological replicates) were graft inoculated with budwood from HLB-infected plants and kept under controlled conditions. At 60, 90 and 120 days after grafting, leaves were collected for DNA extraction from leaf midrib. qPCR reactions were carried out using TaqMan® PCR system with different primers-probes previously described (16S rDNA, elongation factor Ts and prophage region *hyvI* and *hyvII*) and 18S and cytochrome oxidase were used as internal control. Although Las was able to multiply in all of the plants, a wide range of responses was observed among different hosts. The infection rate was higher in *C. sinensis* and *C. sunki* in relation to *P. trifoliata.* Variability was observed in relative Las concentration among the 10 hybrids tested with some of them showing high Las concentration and others significantly lower levels of Las. These tolerant hybrids have been valuable for ongoing work in functional genomics, genetic mapping and eQTL studies.

S12P11

Low titers of "*Candidatus* Liberibacter asiaticus" in citrus plants exposed to high temperatures result in low acquisition rates of the bacterium by the insect vector *Diaphorina citri*

Lopes S.A., Luiz F.Q.B.Q., Martins E.C., Fassini C.G., and Sousa M.C.
Fundo de Defesa da Citricultura (Fundecitrus), Científico, Brazil. slopes@fundecitrus.com.br

In Brazil, HLB is associated to "*Ca.* Liberibacter americanus" (Lam) and "*Ca.* Liberibacter asiaticus" (Las), with Las being now the most prevalent species. First found in 2004 in the center of São Paulo state HLB progressed faster and reached higher incidences towards the south. Since in the south summer temperatures are relatively milder and water deficit lower than those in the north, the environment could be playing a role on HLB spread. In fact, exposing infected trees to daily regimen of 24-38°C led to elimination of Lam and to a significant reduction in Las titer in the exposed trees (Plant Dis. 93:257-262). To gain more information on this, Las-infected graft-inoculated 'Valencia' plants at early, medium or late infection stages were pruned. One group was kept in the greenhouse (GH), an environment favoring Las infection, and the other in a growth chamber (GC) programmed to 24-38°C daily regimen. After 15, 30 and 60 days groups of 7 plants each were removed from GH and GC. Non-infective adult *D. citri* were caged at the tip of the new developing flushes. Two days later insects and flushes were processed and analyzed by qPCR. Incidence of Las-positive flushes and Las titer (in log of Las genome/g tissue) were lower on the GC- (64.9%; 4.9log) than on the GH-incubated (89.1%; 5.9log) plants. Similarly, the incidence of Las-carrying insects and Las titer (in log of Las genome/ lot 5 insects) were lower on those caged on GC-incubated (29.3%; 3.1log) than on those caged on the GH-incubated (72.8%; 3.5log) plants. Data will be presented and their implication on the HLB epidemic discussed.

S12P12

Pre-inoculation with virus and citrus viroids in "*Candidatus* Liberibacter" infection and HLB symptoms

Carvalho S.A., Silva L.F.C., Souza M.C.S., Francisco C.S., and Coletta-Filho H.D.
Instituto Agronômico (IAC), Centro de Citricultura Sylvio Moreira, Brazil. sergio@centrodecitricultura.br

Synergistic or antagonistic interactions among microorganisms may affect its development in many plant hosts, including suppression or increase in diseases expression. The objective of this research was to evaluate the effect of pre-inoculation with five isolates of different severity of Citrus Tristeza Virus (CTV) in the infection of "*Candidatus* Liberibacter asiaticus" (Las) and symptoms of Huanglongbing (HLB). Additionally were included 3 treatments with CTV isolates of medium severity, infected also with Citrus Psorosis Complex Virus (Psorosis), Citrus Exocortis Viroid (CEVd) or Citrus Cachexia Viroid (CCaVd). The sources of viruses and viroids were plants of the IAC-Citrus Active Germplasm Bank, previously characterized by biological indexing. The experiment was conducted in greenhouse using nine months old nursery-trees of healthy 'Pera' sweet orange on 'Rangpur' lime. Viruses were bud inoculated in the canopy trunk using 12 plants per treatment, and seven months later, after confirmation of CTV infection by ELISA, Las was bud inoculated in 10 of them. It was evaluated the incidence and intensity of symptoms of HLB in leaves, being the presence of bacteria confirmed by PCR. In asymptomatic plants Las was also monitored by qPCR. Results obtained at 7, 14 and 21 months after inoculation with Las does

not indicate effect of the previous presence of CTV in Las infection and HLB symptoms, with similar proportion in treatment with viruses (68%) and without viruses infection (60%). On the other hand, mild ("PIAC") or of medium severity ("CV 154") isolates of CTV, showed the lowest rate of occurrence of HLB (30-40%), which reached 70-80% when plants were pre-inoculated with the severe CTV isolates "Capao Bonito" "CV 93" and "Barao B". The pre-inoculation of isolates of medium severity of CTV: "CV 104", "CV 155" and "CV 27", also carrying respectively CCaVd, Psorosis and CEVd, induced 70, 80 and 100% of HLB symptoms.

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S12P13

The efficient transmission of "*Candidatus* Liberibacter americanus" from periwinkle (*Catharanthus roseus*) to different citrus genotypes by dodder

Francisco C.S., Locali E.C., Machado M.A., and Coletta-Filho H.D.

Centro APTA Citros Sylvio Moreira (IAC), Laboratório Biotecnologia, Brazil. carolinaf@centrodecitricultura.br

In Sao Paulo State, Brazil, both bacteria "*Candidatus* Liberibacter asiaticus" (Las) and "*Ca.* L. americanus" (Lam) are associated with Huanglongbing - HLB or Greening disease in citrus. Nowadays Lam has been observed in low prevalence in HLB-disease plants, contrary to its high prevalence in the first 2 years of disease outbreak, 2005 and 2006. But despite of this drastic swift on prevalence of "*Ca.* Liberibacter" species scientific interests remain on Lam bacteria. Based on our experience and supported by the Lopes & Frare (2008) data, Lam grafting transmission is randomly and not efficient for most citrus species, which increases difficulties in experiments under controlled conditions aiming to study plant-pathogen interaction. But, similarly to Asiatic species of *Ca.* Liberibacter, Lam reached high titers in periwinkle plants (*Cataranthus roseus*). The main objective of this study is to analyze the transmission of Lam from periwinkle to citrus by dodder (*Cuscuta spp.*) as an alternative to improve the transmission efficiency of that pathogen. Periwinkle seedlings infected with Lam were used as source for bacterial acquisition by dodder. After a period of 15 days parasitizing periwinkle plants, the dodder were keep connected to citrus plants by 30 days for the transmission. The citrus plant tests were analyzed by end-point PCR and qPCR. The results show that 'Ponkan' mandarin (*Citrus reticulata*) and 'Dourada' lime (*Citrus limettioides*) were infected at rate of 100%, while 'Hamlin' sweet orange (*Citrus sinensis*) of 75%, and 'Key' lime (*Citrus aurantifolia*) of 50%. However, the back transmission of Lam from periwinkle to citrus by dodder is an efficient strategy to be used for overcoming the low rate of transmission of this pathogen by grafting.

S12P14

Population dynamics of *Diaphorina citri* (Hemiptera: Psyllidae) in orchards of 'Valencia' sweet orange, 'Ponkan' mandarin and 'Murcott' tangor trees

Beloti V.H.[1], Felippe M.R.[2], Rugno G.R.[1], do Carmo-Uehara A.[2], Garbim L.F.[2], Godoy W.A.C.[1], and Yamamoto P.T.[1]

[1]Escola Superior de Agricultura Luiz de Queiroz (ESALQ), Entomologia e Acarologia, Brazil; and [2]Fundo de Defesa da Citricultura (Fundecitrus), Científico, Brazil. pedro.yamamoto@usp.br

Huanglongbing (HLB), a destructive disease of citrus, is caused by endogenous, sieve tube-restricted bacteria, named *Candidatus* Liberibacter species. *Diaphorina citri* (Hemiptera: Psyllidae) is a vector of the bacteria in Asia and America. The purpose of this work was to study the population dynamics of *D. citri* in orchards of 'Valencia' sweet orange, 'Ponkan' mandarin, and 'Murcott' tangor trees located at the São José farm in the municipality of Taquaritinga, SP, Brazil. Yellow sticky traps were used to monitor the vector. The largest number of *D. citri* was captured on the 'Ponkan' trees followed by the 'Murcott' and 'Valencia' sweet orange. Chemical pest control and environmental factors are likely to be responsible for this result. Pearson's correlation analysis indicated that there is a weak correlation between the abundance of *D. citri* and the environmental factors of temperature and precipitation. An analysis of variance suggested that there was a significant difference in the abundance of *D. citri* among the three citrus tree varieties, and the autocorrelation was significant for the 'Valencia' orange orchard. Spectral analysis indicated that there were greater frequencies of peaks in the 'Ponkan' mandarin and 'Murcott' tangor trees, and the simulations performed using a stochastic logistic model suggested that the psyllid populations on orange trees are the most susceptible to local extinction; this result likely occurred because of the high levels of chemical control applied to orange trees.

S12P15

Systemic insecticides are effective in preventing the transmission of "*Candidatus* Liberibacter asiaticus" by *Diaphorina citri*?

Yamamoto P.T.[1], de Miranda M.P[2], and Felippe M.R.[2]

[1]Escola Superior de Agricultura Luiz de Queiroz (ESALQ), Entomologia e Acarologia, Brazil; and [2]Fundo de Defesa da Citricultura (Fundecitrus), Científico, Brazil. pedro.yamamoto@usp.br

The purpose of this study was to evaluate the effectiveness of soil-applied systemic insecticides in the control of Asian Citrus Psyllid (ACP) *Diaphorina citri* and thereby prevent the transmission of "*Candidatus* Liberibacter" spp. in citrus bearing trees. The experiments were conducted in farms located in the region of Araraquara, SP, Brazil. The first experiment was carried out in 7-year-old 'Valencia' sweet orange on 'Swingle' citrumelo, with application in Aug/2009. The second and third were conducted in 8-year-old 'Pera' sweet orange on 'Rangpur' lime, in sandy and clay soil, with application in Nov/2009 and in Feb/2010. In the first experiment, the insecticides were effective in controlling ACP. Thiamethoxam at doses of 0.31 and 0.62 g ai/m of plant height and imidacloprid at a dose of 1.2 g ai/m were effective. Application with a volume of 1 liter/plant and applied to the soil at the base of the trunk was more efficient compared with 0.5 L and application in the projection of the canopy. For applications done in Nov/2009 and Feb/2010 systemic insecticides were not effective in controlling ACP. The concentration of thiamethoxam (0.31 g/m) reached 1000 ppb in the first experiment; however, when applied in the other two experiments, was less than 200 ppb. The answer to the question is yes, because the systemic insecticides are efficient to control ACP. However, in some conditions they are not absorbed and translocated into the citrus trees, showing low efficiency.

S12P16

Vector control and foliar nutrition for management of Huanglongbing in Florida citrus

Stansly P.A., Arevalo H.A., Qureshi J.A., Jones M., Hendricks K., Roberts P.D., and Roka F.M.

University of Florida (IFAS), SW Florida Research and Education Center, USA. pstansly@ufl.edu

Vector control and foliar nutrition are widely employed in Florida citrus to respectively slow the spread of HLB and mitigate effects of the disease on citrus trees. A replicated field study was conducted in a 13-acre commercial block of young 'Valencia' through four harvests employing a factorial design to evaluate individual and compound effects of a popular nutrient/SARs program and threshold-based vector management. Asian Citrus Psyllid populations were maintained at contrasting levels in insecticide-treated and untreated plots despite proximity. Nevertheless, incidence of HLB, estimated by PCR at near 30% at the beginning of the study, rose to over 90% early in the third year without significant contribution from vector control. However, yields were significantly improved all four years by the combined effects of nutrition and vector control, with a significant contribution from nutrition in the fourth year when production in the combined treatment approached the pre-HLB regional average. Economic analysis showed that, while the insecticide nutritional treatment produced the highest gain in production, it would not have been profitable at current juice prices. Fine tuning by reducing costs of the nutrient package and/or the insecticide regime could have made the program profitable, as could higher margins of a fresh fruit market. Further research is necessary to establish economic thresholds for both insecticide and nutrient application under different market and environmental conditions.

S12P17

Foliar spray of nutrients and growth regulators on the management of citrus affected by HLB – Is it possible?

Medina C.L.[1], Bataglia O.C.[2], Furlani P.R.[2], Machado R.S.[2], and Creste A.[3]

[1]Grupo de Consultores em Citros (GCONCI), Research, Brazil; [2]Conplant - Consultoria, Treinamento, Pesquisa e Desenvolvimento Agrícola LTDA (Conplant), Research, Brazil; and [3]Fazenda São José (FSJ), Manager, Brazil. clmedina@conplant.com.br

The phloem of HLB affected plants are obstructed by callose that inhibit carbohydrates, minerals and water flow to the newer developing tissues. This causes damages to plant development, debilitates the plants and thus reduces the yield. The callose phloem obstruction may be related with the reaction defense

mechanism against "*Candidatus* Liberibacter asiaticus". In many tree species the variations on phloem callose concentrations are related to the natural alterations of hormonal cytokinins and auxins levels. In order to verify if plant growth regulator (PGR) could have any effects on the HLB incidence (I) and severity of HLB (S), orange trees were treated with a foliar nutrient solution containing macro and micronutrients plus dimethylammonium (2,4-dichlorophenoxy) acetate with auxinic function. This treatment (PhT) started in the spring and continued during the summer with six applications. At one tested site a block with 18 % of affected plants I was reduced to 1.0% and this level persisted during the following year, while control blocks treated by the conventional treatment (vector control and elimination of all symptomatic plants), CT, I increased to 4 %. In another treated site, I of PhT was 1.7% while in CT was 2.9% and S was 2.7 and 5.6 respectively. The results are very encouraging and open an attractive possibility to the development of treatments with nutrient and PGR to reduce HLB symptoms keeping normal function of citrus trees.

S12P18

Antibiotic therapy of citrus bud wood for the management of Huanglongbing disease

Yaqub M.S., and Khan I.A.

Institute of Horticultural Sciences, University of Agriculture (IHS UAF FSD PK), Pakistan. citsykk@yahoo.com

Huanglongbing is a serious graft transmissible disease of citrus. Control of this disease is very difficult. Efforts are going on to control this disease in Pakistan. Disease control plan includes antibiotic therapy of budwood. Tetracycline and penicillin antibiotics were used in this study to clean the budwood from the bacterium "*Candidatus* Liberibacter asiaticus" causing Huanglongbing disease. Tetracycline doses used were 250ppm, 500ppm, 750ppm, 1000ppm and 1250ppm. Whereas penicillin doses were 25ppm, 50ppm, 75ppm, 100ppm and 125ppm. 'Kinnow' mandarin (*Citrus reticulata*) was used as indicator plant. These plants were raised in the insect free screenhouse and found Huanglongbing negative before grafting. Budwood was treated with different doses of antibiotics and grafted on to the indicator plant. Conventional PCR was performed after seven months of grafting by using- 16s rDNA primers OI1/OI2C and rplKAJL-rpoBC operon primers A2/J5 for amplification and they show 1160bp and 703bp bands respectively in positive plants. Data regarding the treatments showing Huanglongbing negative and Huanglongbing positive results shall be presented.

S12P19

Reduction of Huanglongbing in the project of appropriate techniques expansion for the cultivation of 'King' mandarin (*Citrus nobilis*) in Southern Vietnam

Yuasa K.Y.[1], Nguyen V.H.N.[2], and Ichinose K.I.[3]

[1]Japan International Cooperation Agency (JICA), Vietnam office, Vietnam; [2]Southern Horticultural Research Institute (SOFRI), Vietnam; and [3]Japan International Research Center for Agricultural Sciences (JIRCAS), Tropical Agriculture Research Front, Japan. Kazuyoshi.Yuasa@gmail.com

Our project has been performed for expansion of appropriate cultivation techniques of 'King' mandarin (*Citrus nobilis*) to growers who have average-sized farms of 1,000 to 10,000m2 in southern Vietnam. In this region, most citrus trees are infected by Huanglongbing (HLB) two years after the planting. This paper reports the impact of the project on the reduction of HLB infection proportion. The HLB management techniques consisted principally of windbreak by 'Nippa' palms (*Nypa fruticans*) or 'Banana' (*Musa* spp.) on all sides of orchards, plantings of guavas (*Psidium guajava*) first and disease-free 'King' mandarin trees in one to two months later, and insecticide application. Its application was scheduled as 1) soil-drenching of neonicotinoid around 'King' mandarin trees 10 days before the planting and every two months for one year thereafter; 2) leaf- spray was performed every month from the second year on in 11 orchards. In seven of these orchards, 0.3 - 2.6 % King mandarin trees were infected by HLB in the first year and 3.0 - 21.9 % in the second year. On the other hand, the infection proportion in the first year reached at 21.1 - 39.3 % in four orchards where the insecticide was not correctly applied. The results indicate that the appropriate use of neonicotinoid evidently curtailed the infection of 'King' mandarin trees by HLB. The success of our project thus depends on how precisely the techniques are transferred and extended to the growers.

S12P20

Heat treatment of Huanglongbing –affected citrus trees in field for reduction of "*Candidatus* Liberibacter asiaticus"

Deng X.L.[1], Guan L.[1], Liang M.D.[1], Xu M.R.[1], Xia Y.L.[2], Sequeira R.[3], and Chen J.C.[4]

[1]South China Agricultural University, China; [2]North Carolina State University, USA; [4]USDA-APHIS, USA; and [5]USDA-ARS, USA. xldeng@scau.edu.cn

Huanglongbing (HLB, yellow shoot disease) is a devastating citrus disease worldwide. Research conducted by Lin Kung-Hisang et al. in 1960s China suggested that heat treatments were effective at eliminating HLB pathogen in scions. We tested the effect of high temperatures on the reduction of "*Candidatus* Liberibacter asiaticus" (Las) titers in HLB-affected citrus trees in Guangdong, China. Heat treatments were delivered via covering a tree with a temporary enclosed tent of plastic sheeting, which used natural sunlight to raise ambient temperature. Twenty-four sweet orange trees with HLB symptoms in an orchard were selected and divided into six blocks with three blocks of four trees, each receiving heat treatments, and the other three blocks used as controls. Heat treatments were performed three times in August with temperature exceeding 38º C for three hours each time. Leaf samples were collected in the following months and real-time PCRs with primer set HLBasf/HLBasr and TaqMan probe HLBp were used to monitor Las titer changes.

S12P21

Gas exchanged and water relations of 'Valencia' oranges trees infected with Huanglongbing (HLB, ex greening)

Saccini V.A.V.[1], Medina C.L.[2], Santos D.M.M.[3], Machado R.S.[4], and Silva J.[1]

[1]Universidade Estadual Paulista - Faculdade de Ciências Agrárias e Veterinárias (UNESP-FCAV), Produção Vegetal, Brazil; [2]Grupo de Consultores em Citros (GCONCI), Research, Brazil; [3]Universidade Estadual Paulista - Faculdade de Ciências Agrárias e Veterinárias (Unesp-FCAV), Biologia Aplicada a Agropecuária, Brazil; and [4]Conplant - Consultoria, Treinamento, Pesquisa e Desenvolvimento LTDA (Conplant), Research, Brazil. clmedina@conplant.com.br

Symptoms of plants infected with HLB (causal agent "*Candidatus* Liberibacter "spp.) as part of the leaves yellowing and mottling, indicate that their photosynthetic capacity may have changed. Obstructions in the phloem, common in the infectious process, may also affect photosynthesis and water relations. In this study we verified whether HLB affects gas exchange and water relations in two phases of development of symptoms in 'Valencia' oranges trees, grafted in Rangpur, with 12 years of age. The treatments consisted of (a) leaves of symptomatic patients, (b) leaves of asymptomatic patients (both PCR) and (c) leaves of plants PCR -. Evaluations were performed in December 2011 (onset of symptoms) and March 2012 (overt symptoms). We investigated the rate of CO2 assimilation (*Pn*), transpiration (*E*), stomatal conductance (gs), water use efficiency (*WUE*) and the instantaneous efficiency in the use of CO2 (*Pn/Ci*) three times (8:00, 12:00 and 15:00) using a Li-IRGA 6400. The water potential (ψ), measured on a PMS pressure chamber, was observed at 6:00 and 14:00 hours. The results showed that *Pn* and the *WUE* were not affected, but there was a reduction of *E*, during the hottest hours of the day, probably related to lower gs and ψ. The *Pn/Ci* was somewhat reduced in infected plants, only in March 2012. The maintenance of photosynthetic capacity and changes in water balance are possibly related to the obstruction of the phloem and to the damage of the plant root system.

S12P22

Assessing plant health risk in relation to *Xanthomonas citri* strains causing citrus bacterial canker and evaluating measures for managing this risk

Holeva M.[1], Olivier V.[2], Hostachy B.[2], Smith J.[3], MacLeod A.[3], and Pruvost O.[4]

[1]BPI, Department of Phytopathology, Laboratory of Bacteriology, Greece; [2]ANSES, LSV, France; [3]FERA, UK; and [4]CIRAD, UMR PVBMT, Réunion, France. olivier.pruvost@cirad.fr

In the frame of a project funded by the European Food Safety Authority (Prima Phacie), effort was put into identifying and testing qualitative plant-pest risk assessment schemes for their suitability in supporting risk management decisions for the European Union. Five schemes were tested, two largely based on the EPPO

scheme and three adapted from schemes used in non-European countries. We report the results from the application of these schemes as applied to *Xanthomonas citri* strains causing Citrus Bacterial Canker, in regard to the risk of its entry, establishment and spread, as well as its potential impact. For this pathogen, three entry pathways into the EU risk assessment area were considered: a) import of fresh citrus fruits, b) import of ornamental rutaceous plants or plant parts, and c) illegal entry of plant propagative material. With the current EU measures in place, of the five schemes tested, two indicated path (c) as that of the highest risk, whereas the other three suggested path (a) as such. This discrepancy is due to the different level of details the components of the risk of entry are considered in each scheme. Most schemes suggested that the establishment potential lay around the mid-range of possible values. All schemes indicated a high rate for potential spread (primarily through human activities) and a medium to high rate for impact potential. The effectiveness of risk management measures was evaluated by comparing results of assessments with and without management measures in place.

S12P23

Characterization of a new *Xanthomonas citri* subsp. *citri* isolate which triggers a host-specific response

Roeschlin R.A.[1], Chiesa M.A.[1], Enrique R.[2], Favaro M.A.[1], Torres P.S.[3], Filippone M.P.[2], Gmitter Jr. F.G.[4], Vojnov A.A.[3], Castagnaro A.P.[2], Marano M.R.[1], and Gadea J.[5]

[1]Instituto de Biología Molecular de Rosario-CONICET (IBR), Argentina; [2]Estación Experimental Obispo Colombres (EEAOC-Tucuman), Argentina; [3]Fundación Pablo Cassará-Buenos Aires, Argentina; [4]University of Florida (IFAS), Citrus Research and Education Center Lake Alfred, U.S.A.; and [5]Instituto de Biología Molecular y Celular de Plantas (IBMCP), Spain. jgadeav@ibmcp.upv.es

Citrus is an economically important fruit crop that is severely afflicted by Asiatic Citrus Bacterial Canker (CBC), a disease caused by *Xanthomonas citri* subsp. *citri* (XC). Recently, we have identified a XC strain, named XCT44, which induces a hypersensitive-like response in *Citrus limon* leaves. To gain insight into the dynamics of these host-specific responses, we have investigated biofilm formation and accumulation of defense markers by XCT44 on different citrus species. Biofilm formation was monitored by confocal laser scanning microscopy (CLSM) over a 10-day period. XCT44 strain was unable to form structured biofilm after 6 days post-inoculation. This observation is associated with early production of reactive oxygen species by the plant. Transcriptomic analysis of *C. limon* leaves in response to the non pathogenic (XCT44) and pathogenic (XC) strains showed that XCT44 induces specific genes that points towards the involvement of salicylic acid and glucosinolates metabolism in the onset of the defense responses. Further characterization of these genes with a role in this HR-like response will be important for the development of new strategies to manage the disease.

S12P24

Analysis of genetic diversity of *Xanthomonas citri* subsp *citri* strains. Characterization of a new isolate which triggers a host-specific response

Siciliano M.F.[1], Chiesa M.A.[1], Ornella L.[2], Pino Delgado N.[1], Favaro M.A.[1], Sendín L.N.[3], Roeschlin R.A.[1], Orce I.G.[3], Ploper L.D.[3], Vojnov A.A.[4], Filippone M.P.[3], Castagnaro A.P.[3], and Marano M.R.[1]

[1]Instituto de Biologia Molecular y Celular de Rosario, Consejo Nacional de Investigaciones Cientificas y Tecnologicas, Universidad Nacional de Rosario, Facultad de Ciencias Bioquímicas y Farmacéuticas, (IBR), Area Virologia, Argentina; [2]Centro Internacional Franco Argentino de Ciencias de la Información y de Sistemas (CIFACIS), Argentina; [3]Estación Experimental Agroindustrial Obispo Colombres (EEAOC), Sección Biotecnología, Argentina; and [4]Instituto de Ciencia y Tecnologia Dr. Cesar Milstein, Fundacion Pablo Cassara-CONICET, Argentina. fragarina@yahoo.com.ar

Citrus is an economically important fruit crop that is severely afflicted by Asiatic Citrus Bacterial Canker (CBC), a disease caused by *Xanthomonas citri* subsp. *citri* (XC). Argentina is one of the world´s largest citrus producers, accounting for 17% of global lemon supply, with the majority of production concentrated in the northwestern province of Tucuman. To gain insight into the genetic diversity of *Xanthomonas* causing CBC in Tucuman, a total of 42 *Xanthomonas* isolates were collected from different citrus species across seventeen different orchards and subjected to molecular, biochemical and pathogenical tests. Analysis of genome-specific XC markers and DNA polymorphism based on rep-PCRs showed that all isolates belonged to XC. Thirty-eight haplotypes with relatively high genomic inter-relationship compared to XC reference strains were discriminated. Pathogenicity tests showed that one isolate, named XCT44, which shares 91% genetic similarity to the reference strain XCT, has specific host range. XCT44, induces a hypersensitive-like response in *Citrus limon* and *Citrus paradisi* leaves and weak canker lesions in *Citrus aurantifolia* and *Citrus clementina*

leaves. Although XCT44 produces less exopolysaccharides than the reference strain XCT, this deficiency is not involved in the phenotype observed in *C. limon* leaves. This study constitutes the first report on the characterization of XC in the northwest of Argentina.

S12P25

The causal agent of Citrus Canker, *Xanthomonas citri* subsp. *citri*, enters in a reversible viable but non culturable state induced by copper, that can be reverted by citrus leaf extract

Golmohammadi M.[1], Llop P.[2], Cubero J. [3], and López M.M.[2]

[1]Iran Citrus Research Institute (ICRI), Dept. Plant Protection, Iran; [2]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain; and [3]Instituto Nacional de Investigación y Tecnología Agraria (INIA), Dpto. Protección Vegetal, Spain. mlopez@ivia.es

Xanthomonas citri subsp. *citri* (Xcc) is a quarantine organism in the European Union, that causes Citrus Canker, a severe bacterial disease that affects most commercial citrus species, but not present in the Mediterranean countries. Copper compounds are widely used in the integrated management of this disease and have been described as inducers of the viable but non culturable state (VBNC) in several plant pathogenic bacteria, including Xcc. VBNC may be considered as a state previous to bacterial death or a survival strategy adopted by bacteria in adverse conditions. To demonstrate the biological significance of VBNC in Xcc, experiments have been performed in order to clarify two essential characteristics to consider this state as a survival strategy: reversibility and durability. Entrance of Xcc into the VBNC state has been observed in low nutrient medium, or in presence of copper at concentrations employed in field treatments and persistence of Xcc in VBNC condition has been demonstrated until five months under copper exposure. Reversibility was demonstrated after addition of culture media, or citrus leaf extract, both acting as chelating agents of copper ions. Culturability was evaluated by colony counting and viability by membrane integrity, a respiratory test and a real-time RT-PCR system targeting mRNA from *gumD* gene. This report demonstrates that this state is a reversible long term survival strategy that may occur in the field after copper treatments, giving false negative results when checking for their efficiency.

S12P26

Effect of N-acetyl-L-cysteine (NAC) on the epiphytic fitness of *Xanthomonas citri* subsp. *citri* (Xcc) in sweet orange

Picchi S.C., Inui-Kishi R.N., Takita M.A., Machado M.A., and de Souza A.A.

Centro APTA Citros Sylvio Moreira (IAC), Laboratório de Biotecnologia, Brazil. rosangela@centrodecitricultura.br

The leaf surface harbors bacterial epiphytes that are capable of influencing the cell-to-cell signaling system of the plant pathogen Xcc, causal agent of Citrus Canker. Here, we have evaluated the role of bacterial attachment and biofilm formation in leaf colonization during canker development on a susceptible variety of sweet orange. NAC is a mucolytic agent that has anti-bacterial properties and also decreases biofilm formation by a variety of bacteria, and copper-based products are routinely used as a standard control measure for Citrus Canker. We investigated if it is possible to use NAC as an antimicrobial compound together with copper and the effect of the NAC on the epiphytic fitness of bacteria. The Xcc GFP-labeled bacteria were inoculated by spraying on the surface of sweet orange leaves. The bacterial cell multiplication and diffusion processes were observed directly under fluorescence microscope at different intervals after inoculation. The results showed that NAC had a detachment effect and that the leaves that sheets copper application, showed reduced lesions. CFU/mL from leaves were counted, showing a reduction in Xcc concentration.

S12P27

Disruption of ParB alters the virulence of *Xanthomonas citri* subsp. *citri*

Ucci A.P.U., and Ferreira H.F.

[1]Universidade Estadual Paulista (UNESP), Biological Sciences, Brazil. mandapiovesan@gmail.com

The Gram-negative bacterium *Xanthomonas citri* subsp. *citri* (Xcc) is the etiological agent of Citrus Canker, a severe disease that affects all species and cultivars of citrus worldwide. Since there is not an effective control

for this disease, we started the characterization of some essential biological processes of Xcc, among them chromosome segregation, in an attempt to identify targets to which we could develop antimicrobials. Here, we describe the effects of ParB disruption in Xcc, a well conserved factor in bacteria that has no homologues in derived eukaryotes. ParB, together with its partner ParA, is responsible in several other organisms for the proper segregation of the genetic material. Using phase contrast microscopy we show that Xcc mutants disrupted for *parB* (Xcc *parB*::pAPU2) form filaments, which is consistent with a chromosome segregation/ cell division defect. Surprisingly, we found that these cells also lost the ability to colonize the susceptible host Rangpur lime. ParB has been implicated with bacterial motility and divisional septum placement; however, our results are the first to report on the involvement of the chromosome segregation protein ParB with virulence.

S12P28

Analysis of microRNAs involved in defense response signaling against *Xanthomonas* spp. in *Citrus limon*

Chiesa M.A.[1], Torres P.S.[2], Campos L.[3], Roeschlin R.A.[1], Gerhardt N.[1], Filippone M.P.[4], Conejero V.[3], Castagnaro A.P.[4], Vojnov A.A.[2], Gmitter Jr. F.G.[5], Gadea J.[3], and Marano M.R.[1]

[1]Instituto de Biologia Molecular de Rosario-CONICET (IBR), Argentina; [2]Fundación Pablo Cassará-Buenos Aires, Argentina; [3]Instituto de Biologia Molecular y Celular de Plantas (IBMCP), Universidad Politecnica de Valencia-CSIC, Spain; [4]Estacion Experimental Obispo Colombres (EEAOC-Tucuman), Argentina; and [5]University of Florida (IFAS), Citrus Research and Education Center Lake Alfred, U.S.A.. jgadeav@ibmcp.upv.es

MicroRNAs (miRNAs) are small conserved noncoding RNAs of ~ 22 nt long that are present in most eukaryotic genomes. They play a fundamental role as negative regulators of target genes, which are involved in different signaling pathways of biological processes, such as development, hormone signaling and response to biotic and abiotic stresses. *Citrus* spp. is severely afflicted by Asiatic Citrus Bacterial Canker (CBC), a disease caused by *Xanthomonas citri* subsp. *citri* (XC). To get insight into the plant defense responses that could ameliorate canker development, we studied *C. limon* miRNAs expression in the interaction with a highly virulent strain (XC), a non-pathogen strain of citrus (*X. campestris* pv. *campestris*, Xcc) and a strain that generates a hypersensitive response (*X. fuscans* pv *aurantiifoli* strain C, Xfa-C). The obtained results revealed interesting expression patterns for some of the miRNAs involved in hormone signaling pathways (miR159, miR160, miR167, miR393), oxidative stress (miR398) and cell-wall strengthening (miR408). These miRNAs are presumably regulating target genes involved in signalling pathways important for *C. limon* resistance. The biological significance of the differential expression of the assayed miRNAs in compatible, incompatible and non-pathogenic interactions is discussed.

S12P29

Influence of different copper hydroxide spray volumes and doses to control Citrus Canker in lemon in Tucumán, Argentina

Salas López H.[1], Mansilla C.[2], Stein B.[1], Rojas A.[1], and Figueroa D.[1]

[1]Estación Experimental Obispo Colombres (EEAOC), Fruticultura, Argentina; and [2]CITRUSVIL, Argentina. hsalas@eeaoc.org.ar

Citrus Canker caused by *Xanthomonas citri* subsp. *citri* is one of the most important citrus diseases. Three volumes (high, medium and low: 10000, 5000 and 2000l/ha, respectively) and two doses (15 and 7.5 Kg/ha of commercial product) of copper hydroxide (53.8%) bactericides were evaluated in lemons. A trial was conducted throughout three consecutive crop seasons in a commercial grove (Limoneira 8 A Lisbon/Swingle citrumelo) planted with 250 trees/ha density in 1997. A randomized complete block design with four replicates and six treatments was considered: 1) high volume-high dose (traditionally used); 2) medium volume-high dose; 3) low volume-high dose; 4) medium volume-low dose; 5) low volume-low dose and 6) untreated. Monthly sprays from October to January were performed in each season and canker incidence on fruit was estimated at harvest. Regardless of the volume and dose, copper significantly decreased canker incidence on fruit with a considerable decrease as the year elapsed. The highest incidence was recorded in untreated trees (91% of infested fruit) and the lowest (7%) in copper-treated trees. Differences among treatments were not significant in years with low

incidence of canker. Only in the year with 91% of incidence in untreated trees, a high dose of copper sprayed in medium and low volumes significantly increased canker control, compared to the traditional high volume treatment. The latter was in turn more effective than low dose sprayed in medium and low volumes.

S12P30

Control of Citrus Canker(*Xanthomonas citri* subsp. *citri*) mediated by neonicotinoids in combination with acibenzolar-*S*-methyl and copper

Barreto T.P.[1], Pozzan M.[2], and Leite R.[1]

[1]Instituto Agronomico do Parana (IAPAR), Area de Proteção de Plantas, Brazil; and [2]Syngenta Proteção de Cultivares Ltda. (Syngenta), Proteção de Cultivos, Brazil. ruileite@iapar.br

Asiatic Citrus Canker, caused by *Xanthomonas citri* subsp. *citri*, is a serious disease for commercial citrus production. Multiple sprays of copper-based bactericides during the growth season are standard measure for Citrus Canker control worldwide. Disadvantages for long-term use of copper bactericides include the selection for copper resistance in *Xanthomonas* populations and accumulation of copper in the soil. Systemic acquires resistance (SAR) is an innate plant defense that may confer long-lasting protection against a broad spectrum of microorganisms. SAR may be activated by treatment of plants with chemical inducers. The purpose of this research was to evaluate the efficacy of applications of SARs neonicotinoids in combination with acibenzolar-*S*-methyl and copper oxychloride (COC) to control Citrus Canker. Plants of sweet orange 'Pera' (*Citrus sinensis*) were treated by drench with neonicotinoid insecticides imidacloprid (IMI) and thiamethoxam (TMX), and acibenzolar-S-methyl (ASM), and by foliar applications of ASM and COC. ASM and COC were applied during the entire growth season, in a six-spray program. IMI and TMX were applied in the first and third sprays of the six-spray program in an attempt to reduce early canker disease on foliage. ASM or TMX in combination with COC were the most effective treatments to reduce Citrus Canker incidence on foliage as compared to the untreated plants, COC alone and the other SARs treatments.

S12P31

Activity of antimicrobial peptides (AMPs) against *Xanthomonas citri* subsp. *citri* (Xcc) in *Citrus sinensis* var. ´Baía´

Inui-Kishi R.N.[1], Freitas-Astua J.[2], Picchi S.C.[1], Brand G.D.[3], Bloch JR C.[3], Falcao A.O.[4], Stach-Machado D.[4], Cilli E.M.[5], and Machado M.A.[1]

[1]Centro APTA Citros Sylvio Moreira (IAC), Laboratorio de Biotecnologia, Brazil; [2]Embrapa Cassava and Fruits (EMBRAPA), Cruz das Almas, Brazil; [3]EMBRAPA CENARGEM (EMBRAPA), Brazil; [4]Universidade Estadual De Campinas (UNICAMP), Biologia, Brazil; and [5]Universidade Estadual Paulista Julio De Mesquita Filho (UNESP-ARARAQUARA), Departamento de Bioquimica e Tecnologia Quimica, Brazil. rosangela@centrodecitricultura.br

The control of plant pathogens such as Xcc requires continuous use of chemicals, favoring the selection of resistant isolates and increasing the risk of contamination to different organisms and environment. Thus, it becomes important to search for feasible alternatives as AMPs. The aim of this study was to evaluate the activity of six potential AMPs found within the citrus genome, as well as in other organisms, in the control of Xcc. The *in vitro* growth inhibition of Xcc by the peptides was determined by MIC and MBC. The pC1 obtained from citrus efficiently controlled Xcc at 64 µg mL-1, while the MIC values for peptides pH2, pH3 and pH4 were 16, 4 and 8 µg mL-1 respectively. Experiments using detached *C. sinensis* leaves were conducted with the same conditions used for the *in vitro* tests. For these experiments, a strain of Xcc marked with GFP was used. The fluorescence obtained by GFP allowed monitoring the development of the bacteria within the leaves. To confirm if it is able to control bacteria growth, CFU/mL from lesion leaf was determined. The inhibition of Xcc growth was observed by five of the six peptides evaluated. Hemolysis activity from the selected peptides was determined as the main measurement of peptide toxicity toward higher eukaryotic cells. pC1 showed low percentage of hemolysis, while pH1, pH3 and pH4 exhibited higher hemolysis levels. Five of the peptides tested show clear effect on Xcc, and the pC1 obtained from citrus could be an interesting candidate.

S12P32

Bacteriostatic and bactericidal activity of different essential oils to alternative control *in vitro* of *Xanthomonas citri* subsp. *citri*

Sauer A.V.[1], Santos E.M.[1], Zuliani-Gonçalves A.M.O.[1], Nocchi P.T.R.[1], Nunes W.M.C.[1], and Bonato C.M.[2]

[1]Universidade Estadual de Maringá (UEM), Núcleo de Pesquisa em Biotecnologia Aplicada (NBA), Centro de Ciências Agrárias (CCA), Brazil; and [2]Universidade Estadual de Maringá (UEM), Centro de Ciências Agrárias (CCA), Brazil. alinevanessasauer@hotmail.com

Xanthomonas citri subsp. *citri* is the causal agent of Citrus Canker, which is easily disseminated and there has been no effective control until now. Excessive use of pesticides causes problems of human and environmental contamination, and the selection of resistant pathogens. Thus, the study of alternative control methods with a bactericidal action is critical to mitigate the adverse effects mentioned above. The evaluation of the chemical components of essential oils indicated that some of these may change their activity and have bactericidal action. The aim of this study was to evaluate the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of essential oils of *Citrus aurantium*, *Cymbopogon winterianus*, *Foeniculum vulgare*, *Pinus elliottii* and *Ocimum gratissimum*. The method of micro dilution plate was used to evaluate the MIC. Subsequently, an aliquot of 5µl (from the incubation used for determination of MIC) was used to analyze the MBC, using Petri plates containing Nutrient Agar (NA), as a growing medium. The results showed bactericidal activity of the essential oils, however *C. winterianus* had the lowest concentration among the oils tested (MIC = 0.007 µl/ml and MBC = 0.487 µl/ml). With the result of the MIC (0.007 µl/ml) *C. winterianus* is considered bacteriostatic, and only from 0.487 µl/ml it would be bactericidal.

S12P33

Canker control by the siderophore pyochelin from *Pseudomonas fluorescens*

Adler C.[1], Lami M.J.[1], de Cristobal R.E.[1], Filippone M.P.[2], Castagnaro A.P.[2], and Vincent P.A.[1]

[1]Instituto Superior de Investigaciones Biológicas (INSIBIO), Argentina; and [2]Estación Experimental Agroindustrial Obispo Colombres (EEAOC), Biotecnología, Argentina. conadler@uolsinectis.com.ar

This work shows the identification of a compound able to inhibit *Xanthomonas citri* pv. *citri* growth *in vitro* and *in vivo*. Firstly, we isolated from environmental samples, bacteria able to inhibit *X. citri* pv. *citri* (Citrus Canker agent) *in vitro*. Among the selected isolates, we focused in one showing a remarkable activity. The strain was characterized as *Pseudomonas fluorescens* after sequencing its 16S rDNA and analyzing the sequence with BLASTn. Purification and chemical analysis of the active compound allowed us to assign the inhibitory activity to pyochelin (Pch). Since this molecule is a siderophore, we wondered if the inhibition observed was a result of iron scavenging. Surprisingly, when we supplemented media with an excess of iron, we observed practically no change in the inhibition activity. In an attempt to identify the action mechanism of pyochelin, we evaluated the ability of pyochelin to generate reactive oxygen species (ROS) within a culture of *X. citri* pv. *citri* and its correlation with the inhibitory activity. In fact, we observed increased ROS levels when Pch was added. In addition, the reducer agent ascorbic acid, lowered ROS levels and the antibiotic activity implying that inhibition is probably caused by oxidative stress. Finally, we studied the use of Pch in a model of canker disease. Pch showed to reduce canker formation on leaves of 'Eureka' and 'Lisbon' variety plants. These results show Pch as a promising compound for Citrus Canker biocontrol.

S12P34

Genetic response of sweet orange treated with chitosan to the infection of *Xylella fastidiosa*

Coqueiro D.S.O.[1], Rodrigues C.M.[2], Souza A.A.[2], Takita M.A.[2], Kishi L.T.[2], and Machado M.A.[2]

[1]Universidade Federal da Bahia (UFBA), Biochemistry, Brazil; and [2]Centro de Citricultura Sylvio Moreira, Instituto Agronômico (IAC), Laboratory of Biotechnology, Brazil. marcos@centrodecitricultura.br

One alternative that has been widely studied of disease control is the use of chitosan. This study aimed to generate and evaluate the transcriptome of sweet orange (*Citrus sinensis*) plants treated with chitosan, challenged or not with *Xylella fastidiosa* (*Xf*). Seven months old sweet orange plants grafted on 'Rangpur'

lime were treated with chitosan (CHI) 4 mg.mL-1 in HCl 0.05 N pH 5.6. After 24 h, plants were artificially inoculated with suspension of *Xf* 9a5c strain (108 cells.mL-1) in PBS buffer or PBS buffer only. The total RNA of four replicates per treatment (CHI Xf, HCl Xf, CHI PBS and HCl PBS) was extracted and mixed forming a pool of RNAs which were sequenced in Illumina platform. After sequencing, the transcripts quantitation was used to calculate the level of differential expression between treatments and control (HCl PBS) and their significance in Cuffdiff software. We obtained 640 differentially expressed transcripts to CHI treatment, 1232 to CHI Xf and 1075 to HCl Xf. For genes of plant defense we found predominantly repression of genes of auxin pathway. For the induced genes, CHI treatment showed predominance of genes of flavonoid pathway and hypersensitivity response, for the CHI Xf were observed predominance of genes of ethylene and jasmonate pathway and for the HCl Xf genes of salicylic acid pathway, ethylene and jasmonate pathway were observed. Therefore, we suggest that chitosan induce jasmonate and ethylene-dependent defense-signaling pathway in sweet orange.

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S12P35

Genetic variation of California *Spiroplasma citri* populations revealed by two genetic loci

Wang X.[1], Doddapaneni H.[2], Chen J.C.[1], and Yokomi R.K.[1]

[1]USDA Agricultural Research Service, San Joaquin Valley Agricutural Sciences Center (USDA ARS SJVASC), Crop Diseases, Pests and Genetics, USA; and [2]Baylor College of Medicine (BCM), Human Genome Sequencing Center, USA. ray.yokomi@ars.usda.gov

Citrus Stubborn Disease (CSD), known to be present in California since 1915, was confirmed to be caused by *Spiroplasma citri* in 1972. Hosts of *S. citri* include citrus and a wide range of annual weeds, ornamentals and crops such as carrots and sesame. Genetic variation of *S. citri* in California was examined previously by RAPD-PCR with 20 primer pairs but no unique genetic signatures were found. Using partial chromosome and plasmid sequences of *S. citri* recently released in GenBank, a conserved hypothetic peptidyl-arginine deiminase protein (PADP) and a transmembrane protein (TMP) of plectrovirus spv1-r8a2b were selected *in silico* to evaluate genetic diversity of *S. citri* from different hosts and locations. Two specific PCR primer sets designed proved to be effective. The first was PADPf/PADPr which targeted a region with variable tandem repeat numbers (TRNs); the second was TMPf/TMPr which had multiple priming sites in the published genome of *S. citri* strain GII3-3X. A panel of 31 strains of *S. citri* from California, Illinois and the Mediterranean region was evaluated using the two primer sets by PCR. Electrophoretic profiles of PCR amplicons from the strains tested consistently showed three TRN patterns in the PADP locus and three in the TMP locus. Interestingly, strains which showed a different profile for PADP also showed a different profile for TMP and each pattern group constituted a set. Sequence analyses were conducted amongst strains in each pattern group and revealed at least three genotypes present in Californian *S. citri* populations. Further study of field strains from different geographical origins using the two loci described should provide insight on the biology and epidemiology of *S. citri*.

S12P36

Serological detection of *Spiroplasma citri* using a bacterial secreted protein as the detection marker

Shi J.S., Pagliaccia P.D., Morgan M.R., Ma W.M., and Vidalakis G.

University of California, Riverside (UCR), Plant Pathology and Microbiology, U.S.A. vidalg@ucr.edu

Citrus worldwide is facing major threats from insect-transmitted and phloem-limited bacterial diseases such as Huanglongbing (HLB, "*Candidatus* Liberibacter" spp.) and Stubborn (CSD, *Spiroplasma citri*). Management of such diseases is difficult, expensive, heavily based on tree eradication and insecticide treatments for vector control. The success of any disease management program is based on early pathogen detection. The most commonly used diagnostic or survey methods are PCR based and require the presence of bacterial cells or DNA in the tested sample for positive diagnosis. This can be problematic in the case of citrus since only a few leaves or stems from a whole tree and eventually a few mg of tissue are processed for DNA extraction while the titer and distribution of the pathogen within trees, types of tissue and disease progression are variable.

Here, we report a novel diagnostic method for CSD using a secreted protein of *S. citri* as the detection marker. Because *S. citri* resides in the phloem, secreted proteins could disperse through the plant transportation system, thereby facilitating detection. We identified *S. citri* proteins that are secreted in high levels in the presence of phloem extract by mass spectrometry. We then generated antibodies against one of these proteins (ScCCPP1) and used it in direct tissue imprint detection assays. Remarkably, ScCCPP1 antibody robustly detected *S. citri*-infected trees, in a manner that at certain cases was even more reliable than quantitative PCR. Furthermore, using an experimental protocol for mechanical inoculation of *S. citri*, we successfully traced *S. citri* during infection development using both PCR- and ScCCPP1 antibody-based methods. In conclusion, ScCCPP1 antibody holds the promise of developing robust immunoassay-based diagnostic methods for CSD. More importantly, the novel strategy of using secreted proteins as detection markers can be adapted for the diagnosis of ecologically similar diseases, such as HLB.

S12P37

Witches' Broom Disease of Lime, a serious disease of acid lime *(Citrus aurantifolia)* in the Arabian Peninsula, and viral-like diseases of citrus in Oman

Al-Sadi A.M., AL-Yahyai R.A., and Al-Said F.A.

Sultan Qaboos University (SQU), Crop Sciences, Oman. alsadi@squ.edu.om

Acid lime (*Citrus aurantifolia*) is among the top four fruit trees in terms of production and area of cultivation in Oman. In the 1970s, disease symptoms were observed on lime trees in the northern part of the country. The symptoms were characterized by clustering of leaves, which become small in size and light green to yellow in color. Affected trees are usually killed within 5 years after appearance of symptoms. The disease, which was called Witches' Broom Disease of Lime (WBDL), is caused by "*Candidatus* Phytoplasma aurantifolia". It spread to other areas of the country and was reported in the UAE in 1989, in Iran in 1997, in India in 1999 and in Saudi Arabia in 2009. Loss of area cultivated with lime trees in Oman is currently 50% of that in 1990 and the disease wiped out over half a million lime trees in the country. Population genetic analysis of acid limes in Oman provided evidence that the low level of genetic diversity of acid lime in Oman and frequent movement of acid lime planting material across districts are two main factors which contributed to the spread of the disease and high susceptibility of acid limes to WBDL. A survey in 2009 and 2010 of 20,000 lime trees in Oman showed that the disease is present in all regions and farms, with only 2 asymptomatic lime trees which are 25-45 years old. Although the two lime trees may appear to have tolerance or resistance to WBDL, studies are in progress to investigate factors suppressing disease symptoms in these trees. Empirical data provided evidence for a synergistic effect between phytoplasma and some viral pathogens (e.g. tristeza) in the decline of acid limes, a hypothesis which is currently under investigation. Other areas of research on WBDL include genetic diversity of the phytoplasma, physiological aspects of the phytoplasma-affected limes and management options.

S12P38

Portable chemical sensors for monitoring infection-specific volatiles in asymptomatic citrus

Fink R.L.[1], Aksenov A.A.[2], Thuesen L.H.[1], Pasamontes A.[2], Cheung W.H.K.[2], Peirano D.J.[2], and Davis C.E.[2]

[1]Applied Nanotech Inc. (ANI), USA; and [2]University of California, Davis (UC Davis), Mechanical and Aerospace Engineering, USA. dfink@appliednanotech.net

Volatile organic compounds (VOCs) are emitted from all plants, and there is mounting evidence these VOCs reflect internal health status and change in response to pathogen infection and other cues. Our group has developed a portable chemical sensing platform that can monitor for VOC emission changes that result from citrus bacterial and viral infections. To date, our VOC library includes putative signal fingerprints for Huanglongbing (HLB), Citrus Tristeza Virus (CTV) and Citrus Variegated Chlorosis (CVC). Our mobile platform is robust and capable of operating in field conditions. We have also developed customized data analysis methods to compare data from unknown samples to our database, and to determine the probability of infection for a newly sampled tree.

S12P39

Unforbidden fruits: Preventing citrus smuggling by introducing varieties culturally significant to ethnic communities

Karp D.[1], Siebert T.[1], Vidalakis G.[2], Krueger R.R.[3], Lee R.F.[3], and Kahn T.[1]

[1]University of California, Riverside, Department of Botany and Plant Sciences, United States; [2]University of California, Riverside, Department of Plant Pathology and Microbiology, United States; and [3]United States Department of Agriculture, National Clonal Germplasm Repository for Citrus and Dates, United States. dkarp@ucr.edu

Fruits and vegetative materials of citrus and citrus relatives are commonly smuggled into California, presenting a risk of introducing Huanglongbing ("*Candidatus* Liberibacter" spp.) and its insect vector, Asian Citrus Psyllid (*Diaphorina citri*), as well as other exotic citrus diseases and pests. Certain species and cultivars such as curry leaf (*Bergera koenigii*), bael (*Aegle marmelos*) and Etrog citrons (*Citrus medica*) are in demand from ethnic and religious communities, but supplies are limited because of quarantine restrictions. Orchards, nursery trees, and disease-tested propagating materials necessary for their production have not been available. The Unforbidden Fruits project aims to complement interdiction by making pathogen-tested sources of these trees and leaves available in California. The project has collected relevant germplasm, tested it for seed-transmissible diseases, and distributed seeds to California nurseries. Before starting seed distribution, it was necessary to modify state and federal regulations that required that all mother and increase plants in approved insect-resistant structures be derived from material that has undergone shoot-tip grafting or therotherapy, which is impractical for citrus relatives such as curry leaf and bael, for which no such protocols exist. Other citrus and citrus relatives of quarantine significance include wampee (*Clausena lansium*), satkara (*C. macroptera* var. *assamensis*), and Asian cultivars of pummelo (*C. maxima*).

S12P40

Introduction of control on main citrus diseases in P. R. China

Zhou C.Y.

Citrus Research Institute of Chinese Academy of Agricultural Sciences (CRIC), China. changyong@hotmail.com

Since 2001 ca. 100 modern virus-free citrus nurseries have been established in 13 provinces of China, which have the capacity of producing ca. 114 million nursery trees per year. Through practicing the virus-free propagation scheme, the losses caused by the graft-transmissible citrus diseases in inland area have been dramatically reduced. Severe stem-pitting tristeza is still a threat to susceptible sweet oranges and pummelos, a few mild isolates with potential protective capability screened from thousands of field isolates have been being applied in field experiments for mild strain cross protection. In order to control quarantine diseases and pests, Chongqing Citrus Quarantine Pest-free Zone has been being practically set up since 2007, as well as many efforts on setting up early warning system for citrus quarantine pathogens and pests have been made in Chongqing. However, Huanglongbing (HLB) is still the main threat to the coastal citrus areas, with high potential threat to those boundaries due to the northward movement of citrus psyllids. In recent years, the control of HLB is forcefully organized by the plant protection stations of the provincial governments. Three measures including strictly controlling the psyllid, removing diseased trees promptly and planting HLB-free nursery trees are still recommended. Guangxi province is an example of the successful control of HLB in the past few years, where the percentage of HLB has dropped down to now less than 1% from over 10%, whereas much attention has not been paid in other coastal area. The control of fungi diseases is focusing on Anthracnose, Brown Spot (*Alternaria alternata*), Black Spot (*Phyllosticta citricarpa*) and Fruit Rot. This poster will also introduce the progress on virus-free scheme, construction of quarantine pest-free zone, and a few state projects for public interest on controlling HLB, Citrus Canker, virus diseases of fruit trees and fruit rot.

S12P41

Gaining experience with exotic citrus pest and disease threats to Australia through collaborative work in Bhutan

Sanderson G.[1], Hardy S.[2], Donovan N.[3], Beattie A.[4], Holford P.[4], Dorjee D.[5], Wangdi P.[5], Thinlay T.[6], and Om N.[6]

[1]Dareton Primary Industries Institute (DPII), NSW Department of Primary Industries, Australia; [2]University of Newcastle, Ourimbah Campus (UN), NSW Department of Primary Industries, Australia; [3]Elizabeth Macarthur Agricultural Institute (EMAI), NSW Department of Primary Industries, Australia; [4]University of Western Sydney (UWS), School of Science and Health, Australia; [5]Horticulture Division (DoA), Department of Agriculture, Bhutan; and [6]National Plant Protection Centre (NPPC), Department of Agriculture, Bhutan.
graeme.sanderson@dpi.nsw.gov.au

The Australian Centre for International Agricultural Research (ACIAR) commissioned the NSW Department of Primary Industries (NSW DPI) to undertake a project to help improve mandarin production in Bhutan. An important aspect of the project has been research on pests and diseases that are exotic to Australia, including Huanglongbing (HLB), its vector the Asiatic Citrus Psyllid (ACP), Chinese Citrus Fruit Fly and Citrus Powdery Mildew. HLB and its vectors pose one of the greatest risks to Australia's citrus and nursery industries. HLB and ACP are not present in Australia, but occur in nearby Indonesia, East Timor and New Guinea. Movement of the pathogen and the psyllid eastward from Asia to Australia through cyclonic winds or illegal movement of infected plant material is highly likely. Chinese citrus fruit fly is a major pest of citrus in South-west China and North east India and Bhutan. In Bhutan, the fly infests mandarin fruits causing premature ripening and subsequent fruit drop. Yield losses due to this fly can go up to 80% in severely affected orchards. Citrus powdery mildew is a common problem in Asian citrus orchards, leading to premature leaf and fruit drop and twig dieback. It is particularly prevalent in nursery plantings, shady orchards and poorly ventilated orchards and can be a significant problem in recently pruned trees infecting all the new re-growth. Powdery mildew reduces tree productivity, fruit yield and quality. It is important that Australia has personnel with expertise in the identification of these exotic pests and diseases. Our main defense against these threats is through the maintenance of strict quarantine protocols and routine monitoring and surveillance programs. Working in Bhutan provides the Australian citrus and nursery industries with increased capabilities for the development of exotic pest and disease surveillance programs, contingency plans and the ability to quickly react to an incursion.

S12P42

Nucleotide sequence analysis of ribosomal protein gene sub-units (*rplA-rplJ*) of "*Candidatus* Liberibacter asiaticus" infecting different citrus cultivars in Maharastra, India

Ghosh D.K.[1], Bhose Sumit[1], and Gowda S.[2]

[1]Plant Virology Laboratory, National Research Center for Citrus, India; and [2]Citrus Research and Education Center, University of Florida, USA. ghoshdk@hotmail.com

Citrus greening disease (Huanglongbing, HLB) is one of the most serious and destructive citrus disease known in the world. The causative agent, "*Candidatus* Liberibacter asiaticus", a Gram negative alpha Proteobacterium associated with in the sieve tube elements of the phloem tissue of infected plants has not been cultured so far. The disease is transmitted by grafting and vectored by the insect, citrus psylla *Diaporina citri*. During a recent survey conducted in 2010 in Western Maharashtra of India, disease occurrence was recorded in different citrus cultivars viz. sweet orange cultivar 'Mosambi', 'Rangpur' lime, 'Nagpur' mandarin, acid lime and rough lemon. Maximum disease incidence up to 41.5% was observed in sweet orange cultivar 'Mosambi' in Ahmednagar district of Western Maharashtra and was confirmed by biological indexing and PCR as "*Ca.* Liberibacter asiaticus". Four different citrus cultivars viz. 'Mosambi', 'Rangpur' lime, 'Cleopatra' mandarin and acid lime that showed variable symptoms of suspected greening in the field were used in the present study. Presence of "*Ca.* Liberibacter spp." was confirmed by PCR amplifications using 16S rDNA primers. PCR amplifications and sequences comparison of another well conserved regions β operon's subunits *rplA-rplJ* revealed the pathogen as "*Ca.* Liberibacter asiaticus". There was variability in symptoms expression and sequences of HLB isolates from different citrus cultivars in this geographical region, but all isolates were closely related to "*Ca.* Liberibacter asiaticus" strains and no similarity was observed with the Africanus strain.

S12P43

Analysis of citrus huanglonbing spatial dynamics in non-intervened groves of Cuba by Spatial Analysis by Distance Indices (SADIE)

Batista L.[1], Peña I.[1], Luis M.[1], Acosta I.[2], Hernández L.[1], Llauger R.[1], Casín J.C.[3], and Laranjeira F.F.[4]

[1]Instituto de Investigaciones en Fruticultura Tropical (IIFT), Fitopatología, Cuba; [2]Empresa de Cítricos Victoria de Girón, Cuba; [3]Centro Nacional de Sanidad Vegetal, Cuba; and [4]Embrapa Cassava and Tropical Fruits, Cuba. lochy@infomed.sld.cu

Huanglongbing (HLB) citrus disease was first reported in Cuba in 2007 associated to the "*Candidatus* Liberibacter asiaticus" bacteria. Due to the presence of the pathogen and its vector *Diaphorina citri* in all citrus areas, it was essential to carry out epidemiological studies aiming at supporting more effective management strategies. To achieve this, the presence of symptomatic plants was evaluated bimonthly in non-intervened grapefruit and orange groves. Spatial patterns of aggregation were determined by calculating SADIE's Aggregation Index (Ia). Spatial association between plants with HLB symptoms and newly detected ones was evaluated using the Association Index (X). The spatial pattern was characterized by the presence of diseased plants clusters, which were larger in the highest disease incidence area. Ia values increased with time, showing values significantly higher than 1. Likewise, the size of the patch clusters increased, indicating an increase in aggregation with time. The largest patch clusters were located in the field edge adjoining an old grove with high HLB incidence. Significant spatial association was found in the clustering of existent diseased plants and newly detected ones 7 months afterwards. No significant aggregation was found in the area with lowest incidence of diseased plants. These results are currently being used to support decisions on HLB survey and management in Cuba.

S12P44

Identification of tolerance in citrus germplasm for Huanglongbing

Yaqub M.S., and Khan I.A.

Institute of Horticultural Sciences, University of Agriculture, (IHS UAF FSD PK), Pakistan.citsykk@yahoo.com

Huanglongbing is a serious threat to the citrus industry around the world. There is no claim of complete and long lasting control of the disease still. Identification of tolerance in citrus germplasm is a hope to save the industry. Research is going on in the University of Agriculture, Faisalabad, Pakistan to identify tolerance in citrus germplasm against Huanglongbing. In an experiment with the above mentioned objective twenty five germplasm accessions were raised in the insect proof screenhouse. These accessions were tested and found Huanglongbing disease free before inoculation. These accessions were graft inoculated with Huanglongbing positive budwood. The budwood was taken from PCR tested Huanglongbing positive plants. Conventional PCR was performed after three months of inoculation by using -16s rDNA primers OI1/OI2C and rplKAJL-rpoBC operon primers A2/J5 for amplification and they show 1160bp and 703bp bands respectively in positive plants. Data regarding the germplasm accessions found Huanglongbing negative and/or positive will be presented.

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Session 13

FRUIT FLIES

S13O01

Application of the Sterile Insect Technique: an effective biological control method against fruit fly pests and its contribution to food security, the environment and trade.

Hendrichs J., Pereira R., and Reyes J.

Joint Food and Agriculture Organization and International Atomic Energy Agency Division (Joint FAO/IAEA Division), Insect Pest Control Section, Austria. J.Hendrichs@iaea.org

Fruit flies are major pests destroying globally large portions of fruit and vegetable production. They require intensive insecticide applications, which lead to resistance problems and the surge of secondary pests. Many subtropical and tropical countries cannot export these high-value crops because of the presence of fruit flies pests. The sterile insect technique (SIT) is a type of insect "birth control" that has been applied against tephritid fruit flies on all continents. It is very environment-friendly as it is non-polluting, species-specific and does not introduce fertile exotic insects. Effective control is achieved when the sterile insects are systematically released as part of an area-wide integrated pest management (AW-IPM) approach. It has a good track record for the suppression, containment, prevention or eradication of populations of different pest fruit flies, achieving very significant socioeconomic impacts in terms of reducing losses and insecticide use, leading to a more sustainable pest management and environment for rural communities, and healthier commodities for consumers. Furthermore, it has been successfully used to establish pest-free areas and areas of low pest prevalence, thereby opening access to lucrative export markets and creating thousands of jobs in rural areas. As important, the SIT has been effectively used to eliminate invasive pest outbreaks, thereby avoiding the establishment of new fruit fly pests.

S13O02

Assessing the effectiveness of sterile males in Mediterranean fruit fly population reduction by molecular techniques.

Juan-Blasco M.[1], Sabater-Muñoz B.[1], Argiles R.[2], Jacas J.A.[3], Castañera P.[4], and Urbaneja A.[1]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Unidad Asociada de Entomología UJI-IVIA-CIB CSIC. Centro de Protección Vegetal y Biotecnología. Spain; [2]Transformaciones Agrarias SA (TRAGSA), Dpto. de Plagas, Spain; [3]Universitat Jaume I (UJI), Unidad Asociada de Entomología UJI-IVIA-CIB CSIC. Departament de CiènciesAgràries i del Medi Natural. Spain; and [4]Centro de Investigaciones Biológicas del Consejo Superior de Investigaciones Científicas (CIB CSIC), Unidad Asociada de Entomología UJI-IVIA-CIB CSIC. Departamento de Biología Ambiental. Spain. sabater_bea@gva.es

The success of an area-wide program integrating the Sterile Insect Technique (SIT) against the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) relies on the mating success of the released sterile males in the field. However, little is known about the effectiveness of sterile males to mate with wild females in the field and how these matings contribute to reduce wild populations of *C. capitata* in Spain. As a first step to answer these questions, wild females, wild males and Vienna-8 (V8) sterile males were exposed to three different host fruits (apples, clementine mandarins and sweet oranges) under five sterile males release ratios (1:0, 1:1, 1:5,1:10 and1:20) under laboratory conditions. By means of sterile sperm ID method, V8 sperm was positively detected in those females exposed to the male ratios 1:5, 1:10 and 1:20 in the three host fruits.The highest number of sterile mates was detected on clementine mandarins, although it was on apple where more *C. capitata* progeny were found. Secondly, the same release ratios were assayed under field conditions on caged-clementine trees, using sentinel apples as the most susceptible host fruit to be attacked. Sterile V8 sperm in wild females was positively correlated to the ratio of V8 males released and more importantly, the *C. capitata* progeny was also positively correlated to the percentage of sterile mates. These results confirm the usefulness of molecular detection of V8 sperm to predict *C. capitata* population reduction under field conditions. Implications of these results on the efficacy measurement of a SIT program are discussed.

S13O03

Multiple insecticide resistance traits in a field derived population of the Mediterranean fruit fly, *Ceratitis capitata.*

Ortego F., Couso-Ferrer F., Arouri R., Huertas-Rosales O., Beroiz B., Perera N., Cervera A., Hernández-Crespo P., and Castañera P.

Centro de Investigaciones Biológicas (CIB - CSIC), Departamento de Biología Medioambiental, Spain. ortego@cib.csic.es

Resistance to malathion is widely spread in Spanish field populations of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). A malathion resistant strain (W-4Km), derived from a field population collected in Castellón in 2004, was obtained by laboratory selection. The W-4Km strain was 176-fold more resistant to malathion than a susceptible C strain, established from wild *C. capitata* collected at non-treated experimental fields in Valencia in 2001, and maintained in our laboratory without exposure to insecticides. The resistance mechanism of the W-4Km strain was found to be associated with a single point mutation in the target acetylcholinesterase (AChE). Further laboratory selection of this strain with malathion or other insecticides led to a highly malathion resistant strain (W-10Km, about 500-fold resistance), a lambda-cyhalothrin-resistant strain (W-1Kl, 205-fold), and a spinosad resistant strain (Xabia-W-100s, resulting from the cross of the W-4Km strain with a field derived population, over 200-fold). A novel resistance mechanism associated with the AChE gene has been discovered in the W-10Km strain. The synergistic activity of PBO and DEF in the W-1Kl strain suggests that metabolic resistance mediated by both P450s and esterases may be involved in the development of resistance to lambda-cyhalothrin. However, none of the mechanisms previously described appear to be implicated in the resistance to spinosad. These results indicate that multiple resistance traits are present in the field derived population. Moreover, we have shown that resistance to lambda-cyhalothrin and spinosad can rapidly evolve to levels that may compromise their effectiveness in the field.

S13O04

Citrus fruits and the Mediterranean fruit fly.

Papadopoulos N.T.[1], Papachristos D.P.[2], and Ioannou C.S.[1]

[1]University of Thessaly, Laboratory of Entomology and Agricultural Zoology, Greece; and [2]Benaki Phytopathological Institute, Entomology and Agricultural Zoology, Greece. nikopap@uth.gr

The Mediterranean fruit fly (medfly), *Ceratitis capitata* (Diptera: Tephritidae) is considered one of the most important pests of citrus fruits. Recent and older studies demonstrate a variable degree of susceptibility among different citrus species to medfly infestations. They indicate that the chemical properties of the citrus fruit rind are the most important barrier prohibiting survival and development of medfly immature stages. The toxic properties of rind vary considerable among different citrus species as a result of quantitative and qualitative (composition) aspects of citrus essential oils. Effects of fruit flesh on biological traits of larvae do not vary much among different citrus species. On the other hand, citrus essential oils affect, in a variable manner among different fruit species, a range of adult behavioral traits. For example, male medflies are attracted to citrus essential oils while exposure to them increases mating competitiveness over non-exposed males. Likewise, citrus oils regulate female ovipositional decisions. The current paper provides a comprehensive account of the complex interactions between medfly and citrus plants. Practical and theoretical implications are discussed.

S13O05

Looking inside the chemosensory system of the medfly *Ceratitis capitata*

Malacrida A.R., Gomulski L.M., Scolari F., Falchetto M., Siciliano P., Manni M., and Gasperi G.

University of Pavia (UNIPV), Department of Biology and Biotechnology, Italy. malacrid@unipv.it

The Mediterranean fruit fly, *Ceratitis capitata* is an important pest of citrus crops and this has led to the development of extensive control programmes. Apart from traditional insecticide-based control methods that are often associated with mass-trapping, it has become a model organism in Sterile Insect Technique

(SIT) application. Both mass-trapping and the SIT are dependent on the availability of efficient species-specific attractants. For the SIT the availability of attractants is critical for monitoring the size and composition of the target and the sterile populations. The efficiency of an artificial attractant depends on its ability to deceive the exquisite sensitivity and selectivity of an insect's chemosensory system. Little is known about the genes, receptors and the processes that mediate chemosensory behaviours in the medfly. We are characterizing the medfly chemosensory system using functional molecular, protein, behavioral and electrophysiological approaches.

The study of the molecular basis of the perception of odours and pheromones in *C. capitata*, apart from providing new information on the reproductive biology of this invasive species, also represents the basis for future developments. The identification of the specific odours that bind to proteins involved in chemoreception could lead to the development of new attractants and repellents that could have an important role in environmentally friendly biological control methods. This would form the basis for the extension of such biotechnological approaches to other species of agricultural and medical importance. As such this represents yet another example of the key role of the medfly as a model organism for innovative control programmes.

S13O06

Field infestation and suppression of the invasive fruit flies *Bactrocera invadens* on citrus in Kenya.

Ekesi S.

International Centre of Insect Physiology and Ecology (ICIPE), Plant Health, Nairobi, Kenya. sekesi@icipe.org

Field infestation rates of the invasive fruit fly species, *Bactrocera invadens* on *Citrus* spp. was determined at different localities in Kenya. The level of infestation varied with location ranging from 3.0 to 36%. At some of the locations and especially at high elevations, *B. invadens* frequently shared the same fruit with the indigenous fruit fly species *Ceratitis capitata* but often occurred at higher numbers than *C. capitata*. Among the five *Citrus* species sampled, sweet oranges (*Citrus sinensis*) recorded the highest level of infestation compared with the other species. There was a significant inverse relationship between numbers of flies per kg of fruits and elevation at which citrus fruits were collected. Field trail were conducted on sweet oranges to compare catches of *B. invadens* in Multilure trap baited with 4 commercial food-based attractants namely Mazoferm®, Torula® yeast, Hymlure®, and Nulure®. Mazoferm and Torula yeast were the most effective attractants and captured between 2.4-2.8 times and 3.2-4.1 times more flies, respectively than the standard Nulure. In field suppression trials using Mazoferm-Spinosad bait spray, % reduction in *B. invadens* population relative to the control was 82% at 4-weeks after bait spray and 94% at 8-weeks after treatment application. At harvest, percentage fruit infestation was significantly lower in the treated orchards (5%) compared with the control orchards (39%) and demonstrates the efficacy of bait spray for the management of *B. invadens* on citrus.

S13O07

Quarantine mitigation for Tephritid fruit fly pests in *citrus.*

Liquido N. J.[1], and Griffin R. L.[2]

[1]United States Department of Agriculture (USDA-APHIS-PPQ-CPHST), Plant Epidemiology and Risk Analysis Laboratory, USA; and [2]United States Department of Agriculture (USDA-APHIS-PPQ-CPHST), Plant Epidemiology and Risk Analysis Laboratory, USA.
Nicanor.J.Liquido@aphis.usda.gov

The fruit flies of the family Tephritidae impose enormous constraints on the diversification of agricultural production and expansion of agricultural trade around the world. Their polyphagous feeding habits and persistent ecological adaptiveness distinguish them among the worst invasive pest species requiring vigilant detection, effective suppression, and regimented area-wide eradication. Among the suitable host plants of quarantine-significant fruit flies belonging to genera *Anastrepha*, *Bactrocera*, and *Ceratitis* are *Citrus* spp. Rigorous quarantine procedures and regulatory safeguards are required and enforced to prevent the spread of these fruit flies through legitimate international and domestic movement of citrus.

With abundant production worldwide, *Citrus* spp. rank among fruit crops with the highest commercial value. *Citrus* spp. have the distinction of encompassing extensive basic research and validation studies aimed at developing acceptable and efficacious Probit 9 quarantine mitigations, including fumigation, high-temperature forced-air, vapor heat, cold, and irradiation treatments. We will review and discuss conventional Probit 9 treatment schedules and alternative risk-based (e.g., conditional non-host, less than Probit 9) mitigation approaches in achieving quarantine security for fruit flies in citrus.

S13P01

The transcriptome and protein baits of *Bactrocera dorsalis* (Diptera: Tephritidae).

Zheng W., Zhang W., and Zhang H.

Huazhong Agricultural University (HZAU), College of Plant Science and Technology, China. hongyu.zhang@mail.hzau.edu.cn

Bactrocera dorsalis is a destructive polyphagous pest feeding on more than 250 fruits and vegetables. To understand molecular mechanisms of *B. dorsalis*'s behavior, to finally effectively control it, its extensive transcriptome was produced using the Roche 454-FLX platform, and a new bait based on protein X was developed. We obtained over 350 million bases of cDNA derived from the whole body of *B. dorsalis* at different developmental stages. In a single run, 747,206 sequencing reads with a mean read length of 382 bp were obtained. These reads were assembled into 28,782 contigs and 169,966 singletons. The mean contig size was of 750 bp. Additionally, we identified a great number of genes that are involved in reproduction and development, as well as genes that represent nearly all major conserved metazoan signal transduction pathways. Furthermore, transcriptome changes during development were analyzed. A new bait based on protein X was developed. Among 12 proteins tested, protein X was most attractive to both females and males of *B. dorsalis*. After a series of lab bioassays, a mixture of protein X, brown sugar and white vinegar was chosen as the bait to be used in the field bioassays. Methyleugenol (ME) and a sugar/vinegar mixture were designed as the two controls. The results suggested that the protein X bait had the highest attractant rate, and the number of trapped flies (83.5 males and 61 females) by this bait was 4.1 and 9.2 times of that in the control of ME (35.5 males and 0 females)and sugar/vinegar mixture (5.7 males and 10 females), respectively.

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S13P02

The potential benefits of using engineered Medfly to improve the efficacy and to reduce the cost of the Sterile Insect Technique: RIDL strain OX3864A

Slade G.[1], Koukidou M.[1], Leftwich P.T.[1], Rempoulakis P.[1], Economopoulos A.[2], Vontas J.[2], and Alphey L.[1]

[1]Oxitec Limited (Oxitec), United Kingdom; and [2]University of Crete (UOC), Department of Biology, Greece. glen.slade@oxitec.com

The Mediterranean fruit fly (Medfly, *Ceratitis capitata* Wiedemann) is the most destructive insect pest worldwide, infesting over 300 types of fruits, vegetables and nuts. Current control measures include insecticides, lures and the Sterile Insect Technique (SIT). Traditional SIT involves releasing large numbers of laboratory-reared, radiation-sterilized males into infested areas where they mate with wild females who then produce non-viable progeny. The efficacy of SIT depends upon factors including the overall fitness and mating competitiveness of the insects; cost drivers include production methods and required release numbers. The engineered Medfly strain OX3864A incorporates a repressible female-specific lethality system enabling population suppression without the cost or fitness penalty of irradiation. The female-specific nature of the trait enables simple production of male-only release cohorts, while its heritability means that a given suppression level can be achieved with fewer insects, compared to conventional SIT. The strain also has heritable genetic fluorescence, making field monitoring more reliable. Laboratory and field trials with OX3864A showed 100% efficacy in separating sexes, mating competitiveness comparable to wild type and the ability to suppress and then eradicate a stable wild-type Medfly population in less than three months. Hence OX3864A can improve the efficacy and financial viability of using SIT to control field populations of Medfly.

S13P03

An early step toward the development of a method to predict Mediterranean fruit fly adult emergence under different soil moisture and temperature regimes

Garrido-Jurado I., Valverde-García P., and Quesada-Moraga E.

University of Córdoba (UCO), Agricultural and Forestry Sciences, Spain. g72gajui@uco.es

The most important insect pest of citrus crops in the Mediterranean Basin is the medfly *Ceratitis capitata* (Wiedemann). The fly lays its eggs in citrus fruit and the larvae feed and grow in the mesocarp. During development, third instar larvae drop from fruits to the ground, burrow into the soil, and form a puparium overwintering as pupa several cm below the soil. To this end, entomapathogenic fungi that have the advantage of contact action, may be used for medfly microbial control either targeting adults or pupating larvae and pupae in the soil beneath the tree canopy. However, optimizing the timing of soil fungal application for maximum pupa and adult control at the onset of adult emergence from the soil would need a better understanding of the effect of soil temperature and humidity on pre-imaginal development. An approach based on a lognormal parametric survival model was used to define the effects of soil moisture and temperature on pre-imaginal medfly mortality and to understand the relationship between both factors and their interaction as influences on the development of pre-imaginal medflies in the soil. The number of days required for the immature flies to complete their development and reach the adult stage (DT) were studied at five temperatures (15, 20, 25, 30 and 35ºC) and under five soil moisture regimes 1, 5.0, 9.0, 13.0 and 17.0% (wt:wt).The model was significant for *C. capitata* pre-imaginal development, with DT_{50} (the development time for 50% of the pre-imaginal *C. capitata* to reach the adult stage) ranging from 12.8 to 32.4 days. The average development time of the medfly pre-imaginals reaching the adult stage was inversely related to temperature and ranged from 7.4 to 26.1 days. This model could allow the monitoring of medfly pre-imaginal natural mortality in the soil, and therefore, the identification of suitable application times in the medfly lifecycle to achieve the maximum degree of adult and pre-imaginal control

S13P04

Fruit flies in orange plantations of TicoFrut - Costa Rica

Camacho H.

TicoFrut. University of Costa Rica: Fabio Baudrit Agricultural Research Station, Costa Rica. hcamachov@hotmail.com

TicoFrut Company, Central America's largest industrial fruit production and processing enterprise, operates its plants and plantations in the northern region of Costa Rica. In order to best ascertain how to select the most effective and suitable strategies for tephritid control, the diverse tephritid populations were studied. The study was carried out at six orange plantations (5,604 hectares) during the harvest periods of 2007 - 2009. Multilure traps baited with Nu Lure were used, with checks each week. Weekly sampling of 60 ripe oranges took place in the same lots where the traps had been placed. Larvae and pupae were collected on a weekly basis to identify them. In a total of 1093 traps were set in the plantations, 9,009 dipterous flies were collected: among these 69 were Tephritidae, 803 Lonchaeidae, and 907 Ulididae. There were only 2 *Anastrepha ludens* and 2 *Ceratitis capitata* captured. Other flies trapped of the *Anastrepha* genus: 14 *A. striata*, 33 *A. obliqua*, 5 *A serpentina* and, *2 A. fraterculus*. None of these specimens were collected in or on fruits, but rather only in traps. In the orange crop sampling, 22,920 fruits were analyzed (a total of 382 samples) and the following flies were obtained: 1,154 Lonchaeidae, 380 Muscidae, but no Tephritidae or Ulididae flies. An unforeseen outcome was that the Lonchaeidae *Neosilba batesi* was caught in traps and in the fruit sampling and the number of the Ulididae *Xanthacrona bipustulata* captured in traps. The largest number and widest diversity of tephritids were captured in the area of the industrial plant facilities. In 2007, 112 traps were set and 1,360 Diptera were collected: 152 were tephritids (6 *A. ludens*, 1 *C. capitata*, 116 *A. striata*, 18 *A. obliqua* and 4 *A. fraterculus*) and 85 lonchaeids. In 2008-2009, 75 traps were set, and these caught 383 flies: 28 were tephritids (2 *A. ludens*, 2 *C. capitata*, 18 *A. striata* and 6 *A. obliqua*). This observed greater diversity was due to the large quantity of fruit stored at this locale which were originating from many sites around the country.

S13P05

Role of phytosanitary surveillance of *Anastrepha* spp fruit flies (Diptera: Tephritidae) in the context of the citrus industry of Cuba.

Borges Soto M.[1], Beltrán Castillo A.[1], Avalos Rodriguez Y.[1], Hernandez D.[1], Sabater-Muñoz B.[2], and Rodriguez Rubial M.[1]

[1]Research Institute of Tropical Fruit (IIFT), Entomology, Cuba; and [2]Instituto Valenciano de Investigaciones Agrarias (IVIA), Entomology, Spain. sabater_bea@gva.es

The phytosanitary surveillance plays an important role in preventing harmful pests of economic and quarantine importance. The presence of the fruit flies *Ceratitis capitata*, *Anastrepha ludens, Bactrocera spp* and *Anastrepha spp* (Diptera: Tephritidae) on the American continent constitute a potential risk of introduction threatening the citrus production of countries in the region. In Cuba, the presence of these fruit flies is not registered, however, surveillance of these quarantine pests and for other species of national interest continues. Results have been obtained from research on preferred host fruits located in the surroundings of citrus plots, revealing the presence and potential risk to citrus of *A. suspensa*, identified as of quarantine importance in countries such as the United States and others. During the period 2010-2012 case studies were conducted to characterize, diagnose and train for the strengthening of phytosanitary surveillance of *Anastrepha* fruit flies in citrus production areas in: Ceiba (Artemisa), Victoria de Giron (Matanzas), Arimao (Cienfuegos) and Ciego de Avila Citrus Enterprises. To fulfill this objective, the composition of host fruit presence in production areas with respect to *A. suspensa* were evaluated. Host preferences of the main *Anastrepha* species detected and the effectiveness of traps and attractants were determined, and the monitorin system was relocated. The information obtained allowed designing the monitoring strategy in production areas. Training, one of the most important tools, was developed through seminars and workshops. This result provides inputs to the monitoring programs and the procedures that are essential in the development and implementation of measures for the phytosanitary surveillance and integrated management of fruit flies in Cuban citrus production.

S13P06

DECIS TRAP MedFly®: innovative platform technology for the sustainable management of the Mediterranean fruit fly *(Ceratitis capitata)*.

Wirtz K.[1], Ramos E.[1], and Fullana J.[2]

[1]Bayer CropScience AG (BCS), Development, Germany; and [2]Bayer CropScience S.L. (BCS S.L.), Marketing, Spain. kai.wirtz@bayer.com

How does a farmer ensure that his crops meet the multidimensional requirements of the market? Consumers become more and more conscious of top-quality, healthy and safe food, the food value chain increasingly demands high crop quality and quantity standards and the public awareness on environmental protection and sustainable use of natural resources continuously grows. Thus, current production practices and pest management solutions of citrus cultivation require a fundamental rethinking. With Decis Trap MedFly® Bayer CropScience has developed a novel platform technology for the control of Mediterranean fruit flies. Decis Trap MedFly® will be one cornerstone of a new sustainable crop solution system based on IPM-principles combining biorationals, innovative selective chemical crop protection products and advanced services. The novel ready-to-use “attract & kill”-system, which contains the control-agent Deltamethrin and a long-lasting insect attractant, enables growers to replace high-risk chemistry and minimize required inputs through less chemical treatments while providing a season-long, convenient control of fruit flies - using 50 traps/ha for citrus (at costs/ha of around 200-250 EUR/ha). Bayer CropScience and its partner SEDQ (Sociedad Española de Desarrollos Químicos) – a provider of efficient and environmentally friendly solutions for pest control based on pheromones and attractants – will expand the Decis Trap® platform technology to other crops and pests. With annual sales of about EUR 7 billion Bayer CropScience is one of the world’s leading innovative cropscience companies in the area of crop protection (Crop Protection), non-agricultural pest-control (Environmental Science), seeds and plant biotechnology (BioScience).

S13P07

Potential of secondary metabolites secreted by the entomopathogenic mitosporic ascomycetes *Beauveria* sp. and *Metarhizium* spp. for medfly *Ceratitis capitata* adult control.

Lozano-Tovar M.D., Garrido-Jurado I., and Quesada-Moraga E.

University of Cordoba (UCO), Agricultural and Forestry Science, Spain. z82lotom@uco.es

The Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) is a cosmopolitan pest that feeds on a wide range of plants. In citrus crops, it causes important direct and indirect annual costs. Medfly management is mainly based on treatments with chemical insecticides, but there are increasing concerns on the effect of such compounds on environment and humans. Besides, the new European Directive for the sustainable use of pesticides promotes the non-chemical control methods with emphasis on biopesticides. This work was performed to search for new insecticidal compounds of natural origin secreted by entomopathogenic fungi for medfly control. Crude extracts of several *B. bassiana*, *M. anisopliae* and *M. brunneum* isolates and their dialyzed and adialyzed fractions were tested on *C. capitata* newly emerged adults. Crude extracts from four isolates were highly toxic, with mortalities ranging between 87 and 100% at 48 hours. In most cases, the toxic fraction of the crude extracts was retained after dialysis 3500 da cut-off membranes. The most active fraction was submitted to organic extraction, with methanol and buthanol fractions being highly toxic per os against *C. capitata*.

S13P08

Comparison of different food attractants for fruit fly capture (Diptera: Tephritidae) in citrus orchards in the state of São Paulo, Brazil

Rodrigues M.D.A.[1], Raga A.[2], Maldonado Jr W.[1], and Barbosa J.C.[3]

[1]Universidade Estadual de Campinas (UNICAMP), Brazil; [2]Instituto Biológico (IB), Centro Experimental, Brazil; and [3]Universidade Estadual Paulista (UNESP), Brazil. adalton@biologico.sp.gov.br

Anastrepha fraterculus (Wiedemann) and *Ceratitis capitata* (Wiedemann) are the most important fruit fly species in Brazil. Both species are responsible for significant losses in citrus production in the State of São Paulo. The aim of this study was to compare the efficacy of different food bait attractants for monitoring fruit flies in IPM programmes. The experiment was conducted under completely randomized design with seven treatments (yellow plastic McPhail traps) and four replications. The treatments were: BioAnastrepha (3% v/v), Isca Mosca (3% v/v), Samaritá® (3% v/v), Torula® (3 tablets per trap), Milhocina® plus borax (5% v/v 3% p/v), sugar cane molasses (7% v/v) and grape juice Aurora brand (25% v/v). The experiments were performed in two seasons, from March until April (season 1) and from November until December 2008 (season 2), in two orange orchards (organic and conventional systems) located in Mogi-Guaçu, SP. The number of flies captured by each attractant was evaluated weekly during seven weeks. In total 4,327 adults of Tephritidae were captured: 3,073 specimens of *A. fraterculus* and 1,258 specimens of *C. capitata*. Milhocina plus borax and Torula during season 1, and Torula during season 2 were more effective to capture fruit flies. These differences were sorted out by Tukey´s test between treatments. In the organic orchard, Torula and BioAnastrepha captured significantly more tephritids during season 2. In general, Torula attracted more females than males.

S13P09

Evaluation of protein bait laced with various insecticides on the Queensland fruit fly (Diptera: Tephritidae): attraction, feeding, mortality and bait persistence

Mahat K.[1], and Drew R.A.I.[2]

[1]National Plant Protection Centre (NPPC), Department of Agriculture, Thimphu, Bhutan; and [2]International Centre for the Management of Pest Fruit Flies (ICMPFF), Griffith School of Environment, Environment, Science, Engineering and Technology, Griffith University, Nathan Campus, Queensland 4111, Australia. kiranmahat@gmail.com

Use of malathion in fruit fly protein bait sprays has raised serious concerns due to its adverse effects on non-target organisms. This has necessitated the need to evaluate novel, reduced risk compounds. This study evaluated the effects of spinosad, fipronil, malathion and chlorpyrifos mixed with fruit fly protein bait

(Mauri Pinnacle protein®) on attraction, feeding and mortality of the Queensland fruit fly, *Bactrocera tryoni* (Froggatt). The effects of outdoor weathering of these mixtures on fly mortality were also determined. In field-cage experiment, protein-starved flies showed the same level of attraction to baits containing spinosad, fipronil, malathion, chlorpyrifos and protein alone used as control. Female protein-starved flies were deterred from feeding on baits containing malathion and chlorpyrifos compared to baits containing spinosad, fipronil and protein alone. Baits containing malathion and chlorpyrifos caused higher fly mortality and rapid fly knock down than spinosad and fipronil. However, spinosad acted slowly and caused an increase in fly mortality over time, causing up to 90% fly mortality after 72-hr. Baits containing malathion and chlorpyrifos, applied on citrus leaves and weathered outdoors, had longer residual effectiveness in killing flies than spinosad and fipronil. Residual effectiveness of the spinosad bait mixture waned significantly after 3 days of outdoor weathering. Results suggest that spinosad and fipronil can be potential alternatives for malathion in protein bait sprays.

S13P10

Effectiveness evaluation of two attractants, CeraTrap® and Tripack®, for the mass trapping of *Ceratitis capitata* (Diptera: Tephritidae) in *citrus* orchards in different regions of Tunisia

Hafsi A., Rahmouni R., Ben Jannet M., Harbi A., and Chermiti B.

Institut Supérieur Agronomique de Chott-Meriem (ISA-CM), Département des Sciences Biologiques et de la Protection des Végétaux, Tunisia. harbi.ahlem@hotmail.fr

Ceratitis capitata Wiedemann (Diptera: Tephritidae) is a key pest on citrus at worldwide range, being of quarantine importance for export issues for countries on which this pest is present. In Tunisia *C. capitata* is of economic importance due to importance of citrus sector. Damages of *C. capitata* in Tunisia may cause losses of about 1.06 millions Dollars (US) per year on *Citrus*. By this factor, mass trapping is being evaluated for an effective control of this pest in citrus. Nowaday, the area covered by mass trapping is of about 300 ha. The trials were conducted during 2 years (2010, 2011) with two types of Mediterranean fruit fly mass trapping systems CeraTrap® and Tripack® in *Citrus* orchards in three different geographical regions of Tunisia, under different management systems: strictly organic (biotope of Chott-Meriem), semi organic (biotope of Takelssa) and finally the conventional (biotope of Mornag). The obtained results showed that the mass trapping of *C. capitata* used under: a) different management systems; b) trees densities, c) *Citrus* varieties (Maltaise, Washington Navel) and d) with or without aerial treatments and localized applications (tasks treatments); is able to protect fruits against the Mediterranean fruit fly. The CeraTrap® system showed a similar or even a higher effectiveness than the Tripack® system in controlling the Med fly population and both mass trapping systems were selective with the non-target fauna.

S13P11

Field releases of the larval parasitoid *Diachasmimorpha longicaudata* in Spain: first results on dispersal pattern

Harbi A.[1], Beitia F.[2], Tur C.[3], Chermiti B.[1], Verdú M.J.[2], and Sabater-Muñoz B.[2]

[1]Institut Supérieur Agronomique de Chott-Meriem (ISA-CM), Département des Sciences Biologiques et de la Protection des Végétaux, Tunisia; [2]Instituto Valenciano de Investigaciones Agrarias (IVIA), Entomología, Centro de Protección Vegetal y Biotecnología (PVyB), Spain; and [3]Transformaciones Agrarias SA (TRAGSA), Departamento de Plagas, Spain. harbi.ahlem@hotmail.fr

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is a key pest on citrus and other fruit trees in the Valencian Community (Spain). Currently it is being controlled by means of chemical applications, mass-trapping and by the Sterile Insect Technique. As more environmentally safe techniques are being demanded by the European Union, a biological control program with parasitoids is also under study at the IVIA research station in Valencia. The braconid *Diachasmimorpha longicaudata* (Ashmead) is a larval parasitoid of several Tephritid species, which is being used as biological control agent in several countries (Argentina, Australia, Guatemala, Hawaii (USA), Mexico). This species was imported to Spain from Mexico in 2009, and after its stay in the quarantine station, it has been adapted to laboratory rearing using medfly as host. The objective of this work is to understand parasitoid movement in the open field and its parasitism success under Mediterranean

climatic conditions, including Tunisian conditions. In this work we show the results of our release assays at different citrus areas and under different releases and parasitoid movement and parasitism assessment systems. The use of artificially infested sentinel apples arose as the best method to establish the dispersal pattern and parasitism assessment of released parasitoids. Application of host odour clues as pre-release treatment is discussed.

S13P12

Magnet® MED: A new long-life, ready-to-use "Attract and kill" system to control Mediterranean fruit fly, *Ceratitis capitata* Wiedemann.

Colás C., Dominguez M., Marti S., and Alfaro C.

Suterra Europe Biocontrol SL (Suterra), Biocontrol, Spain. mdominguez@suterra.com

The goal of Magnet Med® is to control the pest bringing the insect to the poison instead to bring the poison to the insect. Magnet Med® combines a good stable long-life deltamethrine formulation with a long-life female attractant, Unipack®. Unipack® is female lure based on USDA patent and is being applied in more than 20.000 ha for mass trapping in Spain citrus area, achieving very good results attracting ratio of 70/30 females/males. The effectiveness of Magnet Med® as "A&K" system to control *C. capitata* has been demonstrated in many field trials conducted since 2006, in citrus as well as in stone fruits, pome fruits and table grapes. In 2011 and 2012, laboratory tests demonstrate that the new deltamethrine formulation, exposed under filed conditions, keep affecting the flies during at least 6 months. The attraction power of Unipack® was also evaluated under field conditions, showing very high female attraction even after 6 months of being placed on the field. Those results prove the 6 months lasting of Magnet Med®. It allows the grower not only to protect the crop but also to keep affecting the *C. capitata* population and achieving a population decrease year after year.

This new effective period of the product give the possibility to change the strategy control achieving a long-season Medfly control. Moreover, Magnet Med® is shown as ready-to-use product, environmental friendly, residues free, very easy to be combined with other fruit fly management strategies and permitted in European organic orchards (EU Directive 2092/91).

S13P13

Assessment of the efficacy of lure and kill devices for the control of Mediterranean fruit fly in citrus orchards.

Navarro-Llopis V., Primo Millo J., Vacas S.

Instituto Agroforestal del Mediterráneo (CEQA), Universitat Politècncia de València, Spain. vinallo@ceqa.upv.es

Due to new restrictions in the use of insecticides by governments and the increasing social awareness in its use, the development of new control methods has become essential to manage this pest. Bait sprays with spinosad, mass trapping and lure and kill techniques have been the base for new integrated pest management programs. In this work, three-years field trials were conducted in three citrus areas to test the efficacy of attract and kill devices against mass trapping and spinosad or organophosphate plus bait treatments. In the three trials, Magnet® MED attract and kill device, Spintor® treatments and mass trapping achieved good control of C. capitata populations, as confirmed by low percentages of damaged fruit in the assessments performed during the harvest period. On the contrary, fly population levels on plots untreated or treated with a low efficacy attract and kill prototype device increased more than three times, regarding to the populations recorded in the rest of treated plots. The same effect was observed on fruit damage, obtaining from six to eight times less damage with Magnet® MED and spinosad treatments respectively, regarding the attract and kill prototype devices. Untreated plots registered 20 fold fruit damage than treated plots. As a conclusion, using an effective attractant, conventional trapping systems can be replaced with cheaper and easier to handle attract and kill devices. The advantages of using lure and kill devices with respect to conventional mass trapping systems are stressed.

S13P14

Evaluation of mass trapping technique for control of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) in citrus orchards of Northern Iran.

Mafi Pashakolaei S.

Agricultural and Natural Resources Research Centre of Mazandaran, Plant Protection, Iran. mafiali@hotmail.com

The Mediterranean fruit fly (Medfly), *Ceratitis capitata* is one of the most destructive pests of citrus fruits in Mazandaran province of Iran. The mass trapping technique based on the use of female and male-targeted attractants was carried out for the first time in two citrus groves (0.5 ha) using Tephri-traps (baited with Cera Trap as a food attractant for females) and Jackson trap (baited with Trimedlure as a sexual attractant for males) in 2008. In total 50 traps were installed, distributed on one-third of citrus trees in treated plots. All traps were placed 1.5-1.8 m above the ground, slightly inside of tree canopy. For each Jackson trap, three Tephri-traps were set. Monitoring of adult population of Medfly in the control plot was performed by using two Jackson traps. Tephri-traps were serviced once every two weeks and trimedlure dispensers of Jackson traps were replaced every month. The effect of mass trapping technique was based on randomly examining 500 fruits every week from September until harvesting time (i.e. November), for production of viable adults. At the end of experiment, approximately 8939 female and 2656 male flies were recorded in the treated plot [what about the control plot]. Data analysis indicated significant differences ($P<0.0001$, $df=11$, $t=32.22$) between fruit infestation in treated and control plots. The pest control was fully satisfactory and fruit damage by Medfly was less than 0.5%, compared to the control plot (more than 40%). Thus, according to the results of this study the mass trapping technique based on the use of female and male-targeted attractants could be an appropriate strategy for the control of Medfly in infested regions.

S13P15

The seasonal population dynamics of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) and fruit damage on horticultural crops in Northern Iran

Mafi Pashakolaei S.

Agricultural and Natural Resources Research Centre of Mazandaran, Plant Protection, Iran. mafiali@hotmail.com

The Mediterranean fruit fly (Medfly), *Ceratitis capitata* was detected for the first time in September 2006, in several citrus orchards of Mazandaran province, Iran. In the following years different host plants of Medfly were detected. Among citrus, the most important host was the mandarin (*Citrus reticulate*). The other host plants were: *Japanese medlar* L., *Prunus persica* L., *Prunus prunus* L., *Feijoa sellowiana* L., *Actinidia deliciosa* L., *Ficus carica* L., *Zizyphus sativa* L., *Diospyros virginiana* L., *Punica granatum* L., *Malus domestica* L., *Citrus sinensis* cv. Thompson Navel, *C. sinensis* cv. Blood Orange, *C. reticulate* Blanco, *C. aurantium* L., *C. sinensis* cv. Valencia, *Fortunella spp*. Swing and *C. paradise* Macf. The seasonal fluctuation of Medfly was studied in fruit orchards of Mazandaran province during 2007-2010 by using the Tephri-trap with the food attractant Cera Trap. Monitoring covering the entire province at the end of 2009, revealed the presence of *C. capitata* almost throughout the region. Population density varied at different localities and years. In 2007, first flies were caught in the middle of September and the population peak observed in early November. In 2008, first flies were trapped in middle of June and two main peaks were observed at the end of October and early December. In 2009, first flies were caught in middle of June and the peak occurred at the middle of November. In 2010, first flies were captured in early June and the most important peak occurred in the middle of December. During 2007-2009, last flies were captured in mid December, but in 2010, it happened in early January. During these four year studies, first infestations were observed on unharvested citrus fruits from the preceding season and then on fig and kaki fruits. Fruit damage by *C. capitata* in northern Iran on the different horticulture crops without any treatment was determined to be approximately 40-50%.

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Session 14

VIRUS AND VIRUS LIKE DISEASES

S14O01

Recent developments on *Citrus tristeza virus* research

Folimonova S.

University of Florida, Citrus Research and Education center, Plant Pathology, USA. svetlana@ufl.edu

Citrus tristeza virus (CTV) causes two citrus diseases that cause economic losses in citrus production globally: quick decline on sour orange rootstocks and stem pitting. Recent research results from several labs around the world provided valuable contributions that greatly increase our understanding of the virus-citrus host interactions as well as generate a necessary foundation for development of effective procedures to manage CTV diseases. Our lab demonstrated that p33, p13, and p18 proteins are required for systemic infection of some citrus varieties, suggesting that genes of these nonconserved proteins were acquired during virus evolution to extend its host range. Another interesting discovery was demonstration that stem pitting does not necessarily result from a specific sequence or protein, but from a balance between the expression levels of different viral genes. Several important results were obtained by IVIA researchers in Valencia, Spain. One of them was the development of a long-awaited successful strategy for obtaining transgenic resistance against CTV by expression of untranslatable versions of genes encoding the three silencing suppressors of CTV, *p25*, *p20*, and *p23*. Cross-protection has been an effective management procedure that has allowed economic production of citrus in some regions in which endemic severe isolates of CTV made citrus production unprofitable. However, finding protecting isolates has been empirical and rarely successful. Research results obtained by our lab has advanced our understanding of how CTV cross-protection works, showing that exclusion works only for isolates within a strain, and, thus, provided a "recipe" for selection of protective isolates. A next frontier of virus biology is developing an understanding of how viral populations form, induce symptoms, and react to cross protect. Some progress in this area has been done. These and other recent contributions will be discussed.

S14O02

Large scale survey of *Citrus tristeza virus* (CTV) and its aphid vectors in Morocco

Afechtal M.[1], Djelouah K.[2], Cocuzza G.[3], and D'Onghia A.M.[2]

[1]National Institute of Agricultural Research (INRA), Plant Protection, Morocco; [2]Mediterranean Agronomic Institute (IAMB), Integrated Pest Management of Mediterranean Fruit Crops, Italy; and [3]University of Catania (UNICT), Science and Phytosanitary Technologies, Italy. mohamedafechtal.inra@gmail.com

A survey of *Citrus tristeza virus* (CTV) was carried out in the main Moroccan citrus growing areas (Souss, Gharb, Moulouya, Tadla, Haouz, Loukkos). A total of 3,208 plants from commercial groves, nurseries, plots of budwood sources and varietal collections were analyzed by DTBIA. About 26% of the tested trees proved to be CTV-infected in the groves, mainly in Loukkos region with a 60% infection rate. On the contrary, just a few infected trees were detected in Souss and Gharb regions. No CTV infections were found in the remaining investigated sites. CTV vectors were monitored in Gharb and Loukkos regions. *Aphis spiraecola* (56%) and *A. gossypii* (33%) were the most abundant aphid species, while no evidence of *Toxoptera citricidus* was reported. Four out of six selected Moroccan CTV isolates showed, by coat protein gene sequencing, the highest nucleotide homology with the Spanish mild isolate T385. The remaining isolates, from Loukkos, clustered close to the Portuguese 19-21 reference strain; one of these induced a mild stem pitting on Duncan grapefruit by biological indexing. This study confirmed CTV genetic diversity in some Moroccan orchards. The finding of the stem pitting form represents a serious threat to the Moroccan citrus industry, mainly if *T. citricidus* is introduced in the country.

S14O03

Capillary electrophoresis-single–strand conformation polymorphism and multiple molecular marker genotyping allow a rapid differentiation of CTV isolates

Licciardello G.[1], Russo M.[1], Daden M.[2], Bar-Joseph M.[3], and Catara A.[1]

[1]Parco Scientifico e Tecnologico della Sicilia (PSTS), Italia; [2]International Plant Analysis and Diagnostics S.r.l. (IPADLAB), Italia; and [3]GimlaoTec, Israel. acatara@pstsicilia.it

Despite the noticeable sensitivity to *Citrus tristeza virus* (CTV), the sour orange rootstock remains popular in some Mediterranean areas because of its horticultural advantages. Since some trees remain symptomless,

even if infected, an investigation was developed to differentiate between decline and non-decline CTV isolates in view of potential management strategies of the disease. Furthermore, the importance of genetic similarities between the protecting and challenging isolates in cross protection point out the importance of molecular characterization of CTV isolates. In this study we have evaluated the recently developed capillary electrophoresis-single–strand conformation polymorphism (CE-SSCP) method of differentiation associated with multiple molecular markers (MMM) assay to screen CTV isolates collected from surviving trees in areas severely affected by CTV decline on sour orange rootstock. Analysis of the population structure based on the PCR products of coat protein (*p25*), *p23* and *p27* genes and genotyping by MMM allowed the selection of nine mild and seven severe isolates. Interestingly, only two of the selected mild isolates have shown a T30 single genotype whereas seven reacted also with the T3 and/or T36 and/or VT markers. However, phylogenetic analysis on *p23* or *p25* nucleotide sequences revealed that all of them showed a close similarity with the T30 and T385 CTV isolates. Evaluation of cross protecting ability of mild isolates is still in progress on sweet orange/sour orange, sour orange and Duncan grapefruit growing in a growth chamber.

S14O04

Genetic and biological stability of *Citrus tristeza virus* (CTV) infecting the non-natural host *Nicotiana benthamiana*

Navarro-López J.[1], Ruiz-Ruiz S.[2], Moreno P.[1], and Ambrós S.[1]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Moncada, Valencia, Spain; and [2]Instituto de Biología Molecular y Celular de Plantas (IBMCP), Valencia, Spain. sambros@ivia.es

Although citrus is the only natural host of *Citrus tristeza virus* (CTV), agroinoculation of *Nicotiana benthamiana* (NB) plants with an infectious cDNA clone of CTV-T36 results in systemic infection of this non-natural host. Since replication in NB might affect CTV biological and molecular characteristics, we analyzed viral populations of CTV-T36 and T36-GFP (expressing the *gfp* gene) after successive passages in NB. First we optimized a NB-to-NB graft-transmission procedure that enabled systemic infection of receptor plants in 1-2 months. The consensus full-genome sequence of the first CTV-NB populations was identical to that of the cognate cDNA clones. The CTV populations of passage 5 (P5) showed just 1 (CTV-T36-GFP) or 6 (CTV-T36) aminoacid (aa) changes in ORF1a regions with no functional relevance, whereas the P11 populations had 12 (CTV-T36-GFP) or 8 (CTV-T36) aa substitutions in ORFs1a, 1b, p33, p61, p18, p20 and/or p23, with 5 of these changes being common to both lineages. Two of the p23 substitutions in CTV-T36 were in the Zinc finger domain without affecting its structure. The infection rate in successive NB passages was similar, albeit viral titer increased and the time for systemic infection and symptom appearance was shorter. Virions from P5 and P11 NB plants infected 100% of the receptor alemow plants as did the parental citrus virions. Symptoms incited by citrus and NB virions in Mexican lime, Pineapple sweet orange, Duncan grapefruit and sour orange were also similar.

S14O05

Citrus tristeza virus enhances the titer of *Citrus dwarfing viroid* in co-infected Mexican lime plants through p23 expression

Serra P.[1], Bani Hashemian S.M.[1], Fagoaga C.[1], Romero J.[1], Bertolini E.[1], Ruiz-Ruiz S.[2], Gorris M.T.[1], and Durán-Vila N.[1]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain; and [2]Instituto de Biología Molecular y Celular de Plantas (IBMCP), Valencia, Spain. duran_nur@gva.es

Several virus and virus-like pathogens have been reported naturally infecting citrus. Citrus viroids are spread worldwide, usually as latent infections, whereas *Citrus tristeza virus* (CTV), which is the causal agent of the Tristeza disease, affects many citrus growing areas. Although many citrus trees are co-infected with CTV and viroids, there is no information regarding interaction between these two types of citrus pathogens. Northern hybridisation analysis of Etrog citron (*Citrus medica*), the experimental host for viroid bioassay, singly infected with *Citrus dwarfing viroid* (CDVd) or co-inoculated with CDVd and CTV revealed that the viroid titer is not affected by the presence of CTV. Unexpectedly, a similar assay using the CTV sensitive indicator host Mexican lime (*C. aurantifolia*), showed that the viroid titer was significantly enhanced by the presence of CTV. Since CTV encodes three distinct proteins (p25, p23 and p20) that have been characterized as silencing suppressors, an

assay was performed to find out if the increased viroid titer was related to suppression of post-transcriptional silencing. Transgenic Mexican limes expressing each one of these proteins were inoculated with CDVd. Northern hybridisation analysis showed that, in plants expressing p23, viroid accumulation was enhanced at similar levels as in plants co-infected with CTV. These results indicate that the expression of p23 via CTV infection or via transgenic plants alters the silencing mechanism implicated in host defence, supporting the hypothesis that viroids are targets of the RNA silencing machinery of their hosts.

S14O06

The p23 protein encoded by *Citrus tristeza virus:* fine dissection of its determinants for nucleolar localization and for suppression of RNA silencing and pathogenesis

Ruiz Ruiz S.[1], Soler N.[2], Sánchez-Navarro J.[1], Fagoaga C.[2], López C.[3], Navarro L.[2], Moreno P.[2], Peña L.[2], and Flores R.[1]

[1]Instituto de Biología Molecular y Celular de Plantas (IBMCP), Valencia, Spain ; [2]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Moncada, Valencia, Spain; and [3]Instituto de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV), Valencia, Spain. suruirui@upvnet.upv.es

One of the three RNA silencing suppressors (RSS) encoded by *Citrus tristeza virus* (CTV) is p23, a RNA-binding protein of 209 amino acids that also is a pathogenic determinant when expressed ectopically in citrus. Confocal microscopy of *Nicotiana benthamiana* in which the fusion p23-GFP was transiently expressed revealed its accumulation in nucleolus, Cajal bodies and plasmodesmata, thus being the first closterovirus protein with a nucleolar localization signal (NoLS). Assay of seven truncated versions of p23 showed that regions 50-86 and 100-157 (excluding fragment 106-114), both with basic motifs and the first with a Zn-finger domain, contain a NoLS bipartite. Further examination of ten point-mutants delimited this signal to three Cys of the Zn-finger and some basic amino acids within and preceding it, and to fragment 143-155 with six basic amino acids. Essentially all deletions and substitutions annulled RSS activity of p23 (in transgenic *N. benthamiana* expressing constitutively GFP), implicating most p23 regions in this activity. Expression in *N. benthamiana* of p23 as a subgenomic RNA of *Potato virus X* induced necrosis. This phenotype only was preserved in deletion mutant 158-209 and in one point-mutant, showing that the Zn-finger and flanking basic motifs are part of the pathogenic determinant. Ectopic expression of p23 and some deletion mutants in transgenic Mexican lime delimited a similar determinant, suggesting that p23 affects related pathways in citrus and *N. benthamiana.*

S14O07

Viruliferous *Brevipalpus phoenicis* gradient from Citrus Leprosis affected trees

Bassanezi R.B.[1], Montesino L.H.[1], and Novelli V.M.[2]

[1]Fundo de Defesa da Citricultura (Fundecitrus), Departamento Científico, Brazil; and [2]Centro APTA Citros Sylvio Moreira - Instituto Agronômico de Campinas (Centro APTA Citros Sylvio Moreira - IAC), Brazil. rbbassanezi@fundecitrus.com.br

Citrus leprosis, caused by *Citrus leprosis virus* (CiLV-C) transmitted by *Brevipalpus phoenicis* mite, is important in Brazil and its control is mainly based on mitecide sprays. A weak relation between spatial distribution of leprosis symptomatic trees and mite infested trees was observed in previous studies. Diseased trees were much more aggregated than mite infested trees. This could be explained by a limited movement of mites and the high dependence of viruliferous mites on symptomatic citrus trees. The aim of this work was to assess the viruliferous mite gradient from citrus leprosis affected trees. Surveys were conducted in sweet orange citrus blocks in which all trees were inspected for the presence of symptoms and the mite vector. Symptomatic trees and mite infested trees were spatially located and 5-mite samples from each infested tree were analyzed by RT-PCR for CiLV-C. From 163 samples collected on healthy trees, only 37.4% were CiLV-C positive, and even from 337 samples from symptomatic trees, only 37.7% were positive, confirming that most of the mite population was non-viruliferous. Out of 178 CiLV-C positive samples, 71.3% were from symptomatic trees, 95% were found within 7 m around a symptomatic tree, and no positive sample was found more than 23.5 m away from a diseased tree. The frequency of viruliferous mite samples (y, in %) as a function of minimum distance from a leprosis symptomatic tree (x, in m) was fitted to a negative exponential model y=71.exp(-0.43 x).

S14O08

New generation sequencing platforms for detection and characterization of viruses and viroids in citrus

Olmos A.[1], Bertolini E.[1], Varveri C.[2], Candresse T.[3], Martínez M.C.[1], Pina J.A.[4], and Cambra M.[1]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain; [2]Benaki Phytopathological Institute (BENAKI), Laboratory of Virology, Greece; [3]Institut National de la Recherche Agronomique (INRA) and Université de Bordeaux, Virology, France; and [4]Generalidad Valenciana, Consellería de Agricultura, Alimentación y Agua, Servicio de Sanidad Vegetal, Spain. aolmos@ivia.es

The accumulation of virus-derived small interfering (si) RNAs (21-24 nucleotides) induced by Dicer recognition of dsRNA formed during viral replication can be analyzed by next generation sequencing technologies, namely Ion Torrent and Illumina. This strategy was compared with biological indexing for the analysis of several citrus samples. Large contigs corresponding to virus species, such as *Citrus tristeza virus*, allowed their detection and full genome reconstruction through subsequent mapping of the specific siRNAs against several reference isolates. At the same time, bioinformatic analysis allowed reconstruction of whole genomes of viroids present, such as *Hop stunt viroid*, *Citrus exocortis viroid*, *Citrus viroid III* (CVdIII) and *Citrus viroid IV* (CVdIV). Results from both platforms not only were in agreement with indexing results but in addition facilitated identification of CVdIII and CVdIV, which cause inconspicuous symptoms in indicator plants. Both Ion Torrent (300.000-800.000 sequences) and Illumina (4.000.000-5.000.000 sequences) platforms could further generate contigs for viruses for which no sequence information was available, allowing reconstruction of genomes *de novo*. Next generation sequencing is a very powerful technology, much faster (15 days) and less costly (currently ~600€/sample) than biological indexing, that could greatly simplify routine diagnosis and characterization of known plant pathogens. This molecular tool has also the potential to identify poorly or uncharacterized graft-transmissible agents.

S14O09

Deep sequencing of viroid-derived small RNAs from citron

Cao M.J., Su H.N., Yang F.Y., Chen H.M., and Zhou C.Y.

National Engineering Research Center for Citrus, Citrus Research Institute, Southwest University; College of Plant Protection, Southwest University, Chongqing, 40072, People's Republic of China. mengjic@ucr.edu

Citrus are natural hosts of seven viroid species of the family *Pospiviroidae*. Although there is little information about the pathogenesis of citrus viroids, several experiments indicated that viroid derived small RNAs (sRNAs) are key effectors of viroid pathogenesis. To understand the host RNA silencing defence induced by citrus viroids, we examined by deep sequencing (Solexa-Illumina) the sRNAs of leaves from three viroid-infected and one healthy citron. Each infected citron plant contained 4 to 6 citrus viroids and displayed typical viroid symptoms. Our data show that: (a) Citrus viroid sRNAs represent about 7% of the total sRNAs. (b) Citrus viroid sRNAs are predominantly of 21- and 22-nt, with different viroids yielding a distinct biased distribution of their 5' nucleotide and different accumulation of both RNA polarities. (c) Citrus viroid sRNAs derive mostly from specific regions (hot spots) of their RNAs. Most of the (-) polarity viroid-derived sRNAs of CEVd, CBCVd, CVd-V, CVd-VI and CVd-I-LSS were from downside of the TR region. CBLVd, HSVd and CDVd have the same hot spot region in different viroid combinations. (d)The plant sRNA profile, dominated by the 24-nt sRNAs in the mock-inoculated control, exhibited a significant reduction of the 24-nt sRNAs and increase of the 21-nt sRNAs in two viroid-infected citrons. We have also determined how viroid influences 21-nt miRNAs accumulation and 24-nt rasiRNAs reduction in citron.

S14O10

The complete genome sequence of *Citrus vein enation virus* (CVEV) obtained through deep sequencing of small RNAs

Vives M.C., Velázquez K., Pina J.A., Moreno P., Guerri J., and Navarro L.

Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain. cvives@ivia.es

Citrus Vein Enation (VE), a graft-transmissible disease naturally spread by several aphid species in a persistent mode, has been reported in many citrus growing areas. It causes vein enations on leaves and woody galls

on stems and trunks of sensitive citrus species such as Mexican lime, rough lemon and *Citrus volkameriana*. The disease is currently diagnosed by biological indexing on sensitive indicator plants, an expensive and time-consuming method. In order to identify its causal agent and develop specific and reliable molecular detection methods, we analyzed small RNAs (sRNAs) from healthy and VE-infected Etrog citron plants by deep sequencing using the Illumina Solexa platform. Assembly of VE-associated sRNAs yielded several contigs that showed sequence homology with *Pea enation mosaic virus* 1 (PEMV-1), the type species of genus *Enamovirus*, family *Luteoviridae*. The gaps between adjacent contigs were filled by RT-PCR amplification, cloning and sequencing in order to obtain the complete genome sequence of a new virus, *Citrus vein enation virus* (CVEV). The CVEV genomic RNA has 5983 nt organized in five open reading frames, resembling that of PEMV-1, and phylogenetic comparison of amino acid signatures in RNA-dependent RNA polymerases of the family *Luteoviridae* clearly grouped CVEV with PEMV-1. Therefore, we propose that CVEV should be included in the genus *Enamovirus*. A rapid and specific detection procedure was developed based on RT-PCR with CVEV-specific primers.

S14O11

Deep sequencing of citrus affected by graft-transmissible diseases of unknown aetiology leads to discovery of two novel viruses

Loconsole G.[1], Giampetruzzi A.[1], Saldarelli P.[2], Onelge N.[3], Yokomi R.K.[4], and Saponari M.[2]

[1]Università di Bari "Aldo Moro", Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti (DiSSPA), Italy; [2]Consiglio Nazionale delle Ricerche (CNR), Instituto di Virologia Vegetale (UOS), Italy; [3]Çukurova University Agriculture Faculty, Plant Protection Department (PPD), Turkey; and [4]United States Department of Agriculture-ARS (USDA-ARS), USA. m.saponari@ba.ivv.cnr.it

Citrus are susceptible to a number of graft- and arthropod-transmitted pathogens. Citrus cultivars vary in susceptibility or tolerance to pathogens or strains and may remain symptomless when infected. Established tests for known disease agents are used by certification and disease management programs. However, diseases with unknown aetiology remain difficult or impossible to diagnose. Illumina next generation sequencing (NGS) technology was used to generate sequence datasets from citrus affected by two such diseases: Citrus Chlorotic Dwarf (CCDD) (whitefly-transmitted) and Yellow Vein Clearing disease (YVCD) (aphid-transmitted). CCDD is the most serious citrus disease in Turkey; YVCD is a graft-transmissible disorder observed in lemon and sour orange in Pakistan and India and later in Turkey (2000) and China (2010). Contigs from small interfering RNAs were assembled and used to screen sequence homologies against the virus database in GenBank. DNA fragments from CCDD-affected plants were used to re-construct a circular single-stranded DNA viral genome with homologies to those of geminiviruses. The genome size and organization of the provisionally named *Citrus chlorotic dwarf-associated* virus (CCDaV) was determined and indicated it was a highly divergent member of the family *Geminiviridae*. Similarly, the whole genome of a putative filamentous virus associated with CYVCD-affected lemon was reconstructed. The genome structure was found to be typical of flexiviruses and led to new serological, biological and molecular investigations on the provisionally named *Citrus yellow vein clearing virus* (CYVCV). The recovered data suggested that CYVCV is a new species in the genus *Mandarivirus*. PCR-based assays for CCDaV and CYVCV were developed and serve as important new diagnostic tools for citrus disease management programs in Turkey and other citrus-producing regions.

S14P01

Genetic variation of *Citrus tristeza virus* (CTV) isolates from Calabria, Italy

Fontana A.[1], Debreczeni D.[2], Albanese G.[1], Davino S.[3], Flores R.[4], and Rubio L.[2]

[1]Università degli studi Mediterranea di Reggio Calabria, Gestione Sostenibile dei Sistemi Agrari e Forestali (GESAF), Italy; [2]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Proteccion Vegetal y Biotecnología, Valencia, Spain; [3]Università degli Studi di Palermo (UNIPA), DEMETRA, Italy; [5]Instituto de Biología Molecular y Celular de Plantas (IBMCP), Valencia, Spain.
anna.fontana@unirc.it

Citrustristeza virus(CTV) isolates differing in pathogenicity have been reported worldwide. Although some isolates are asymptomatic, most of them cluster in two groups: i) mild, causing only decline and death of citrus species propagated on sour orange rootstocks, and ii) severe, additionally causing stem pitting,

stunting and low fruit yield and quality in some varieties regardless of the rootstock used. Characterization of CTV isolates and estimation of their genetic variation can provide epidemiological information and be useful for disease control. The first CTV outbreaks in Italy appeared in 2002: mild and severe isolates in Sicily and mild isolates in the Apulia region (South-Eastern peninsular Italy). In this work, the genetic variation of 12 mild and 18 severe CTV isolates from recent outbreaks occurred in the Calabria region (South-Western peninsular Italy) was studied. Phylogenetic analysis of CTV *p20* gene showed that the Calabrian severe isolates clustered with severe isolates from other countries (including Egypt, Argentina and India), whereas the Sicilian severe isolates were in other cluster together with severe isolates from other countries (including Brazil, Spain, USA and China). This finding suggests distinct introductions for the severe isolates from Calabria and Sicily. The nucleotide diversity was very low (0.002), suggesting a rapid spread of CTV in Calabria. In another respect, the Calabrian mild isolates clustered with mild isolates from California, Florida, Spain and Italy, some of them being identical to the Sicilian mild isolates and suggesting that the Calabrian mild isolates could come from Sicily. Their very low nucleotide diversity (0.001) supports also a rapid spread. However, the mild isolates from Apulia formed a subgroup with higher nucleotide diversity (0.011) probably due to their earlier introduction.

S14P02

Incidence, distribution and first identification of *Citrus tristeza virus* by RT-PCR in citrus orchards in South Western Nigeria

Adediji A.O.[1], Atiri G.I.[1], and Kumar P.L.[2]

[1]Department of Crop Protection and Environmental Biology (CPEB), University of Ibadan, Nigeria; and [2]Virology and Molecular Diagnostics Unit (VMD), International Institute of Tropical Agriculture, Ibadan, Nigeria. adedapo.adediji@yahoo.com

In order to ascertain the presence, incidence, and distribution of *Citrus tristeza virus* (CTV), extensive surveys were conducted in citrus growing areas of South-Western Nigeria during 2011-12. A total of 565 citrus trees were sampled in three states. Leaf samples from citrus trees were collected and symptom severity was recorded. Symptoms observed included stem pitting, stunting, vein clearing and leaf curling. Molecular analyses by RT-PCR of nucleic acid extracted from diseased samples were used to detect virus presence using CTV-specific primers PIN1 and PIN2 directed to the conserved 3' untranslated region. Results show the presence of CTV in all the three states surveyed while symptom severity differed from one location to another. Disease incidence varied from 85.2% in Oyo State to 48.4% in Ogun State. Citrus trees were infected regardless of scion-rootstock combinations and were detected in sweet orange (87.0%), tangerine (77.6%), grapefruit (73.8%) and lemon (69.5%). This is the first report of molecular detection of CTV in Nigeria.

S14P03

A rapid procedure to evaluate the protecting ability of *Citrus tristeza virus* mild isolates against severe isolates

Ruiz Ruiz S.[1], Navarro-López J.[2], Moreno P.[2], and Ambrós S.[2]

[1]Instituto de Biología Molecular y Celular de Plantas (IBMCP), Valencia, Spain; and [2]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Moncada, Valencia, Spain. suruirui@upvnet.upv.es

Measures to control damage caused by severe stem pitting isolates (SP) of *Citrus tristeza virus* (CTV) include selective eradication and cross protection with mild strains (MS). Implementation of these measures needs sensitive and specific methods to discriminate between SP and MS variants in the viral populations. We developed a quantitative real-time RT-PCR method using TaqMan locked nucleic acid (LNA) probes that enabled quantification of SP and MS variants in natural CTV populations. Here we used this method to monitor the evolution of the CTV population in Pineapple sweet orange plants pre-inoculated with a MS (T32) or a SP (T318) isolate, and then challenge-inoculated with budsticks infected with a SP or a MS isolate, respectively. While control plants singly inoculated with T32 or T318 only contained the cognate CTV variant in successive flushes, the plants doubly inoculated contained both types of sequence variants in tissues derived from either the receptor plant or the inoculum budstick, with no strain impairing systemic

invasion by the other. The relative amount of T32 and T318 variants in the viral population of co-infected plants varied between flushes although T32 appears to be prevalent. The co-infected plants showed stem pitting symptoms similar to those of plants inoculated only with T318, whereas control plants inoculated only with T32 or non-inoculated remained symptomless. This procedure enables rapid evaluation of MS for cross protecting ability.

S14P04

Expression of different proteins in sweet orange induced by severe and mild *Citrus tristeza virus* (CTV) isolates

Yang F.Y., Li Z.A., Zhou C.Y., and Zhou Y.

Citrus Research Institute of CAAS (CRIC), National Citrus Engineering Research Center, P.R. China. yfangyun@163.com

A comparative proteomics analysis was performed to identify the molecular response of sweet orange plants to the infection of severe and mild *Citrus tristeza virus* (CTV) isolates. Two-dimensional difference gel electrophoresis (2D-DIGE) was adopted to compare and evaluate total proteins extracted from leaves. A total of 82 protein spots showed differences in their relative abundance with a one-way analysis of variance [1-ANOVA] value of ≤0.001. Fifty differentially expressed protein spots were identified by MALDI-TOF/MS, and searched in protein databases. According to the putative functions, the identified proteins were classified into four groups such as carbohydrate metabolism, energy metabolism, nucleotide metabolism, and folding, sorting and degradation. The identified proteins were involved in various physiological responses such as gluconeogenesis, citrate cycle, ascorbate and aldarate metabolism, glyoxylate and dicarboxylate metabolism, carbon fixation in photosynthetic organisms, purine metabolism, glutathione metabolism, protein processing in endoplasmic reticulum and RNA degradation. The results showed that the phospho-D-glycerate hydrolase, iron superoxide dismutase, xyloglucan endotransglycosylase, adenylate kinase and major latex-like protein were likely associated to severe CTV pathogenesis, whereas NADP-isocitrate dehydrogenase, heat-shock protein, miraculin-like protein, thioredoxin, L-ascorbate peroxidase, enolase, isocitrate dehydrogenase and HSP20 family protein might be related to mild CTV effect.

S14P05

Screening of *Citrus tristeza virus* in sweet oranges and limes of the Colombian Citrus Collection (Corpoica - Meta)

Guzmán-Barney M.M.[1], Rodríguez P.[1], Ordúz J.[2], and Martínez J.[1]

[1]Universidad Nacional de Colombia - Instituto de Biotecnologia (UN-IBUN), Bogotá, D.C., Colombia; and [2]Corporación Colombiana de Investigación Agropecuaria (Corpoica), Meta, Colombia. mmguzmanb@unal.edu.co

Citrus tristeza virus (CTV), a phloem-limited virus with a ssRNA genome, is transmitted by aphids including *Toxoptera citricida*. The main symptoms incited by CTV are Quick Decline (QD) and Stem Pitting (SP). CTV is deleterious for citriculture and endemic in Colombia. Being important to distinguish CTV variants for disease management, we characterized CTV isolates infecting sweet oranges (*Citrus sinensis*, SO) and Tahiti limes (*C. latifolia*, TL) grafted on different rootstocks. Three groups of ten year old trees were studied: 10 SO and 22 TL grafted on Cleopatra mandarin and 60 TL grafted on different rootstocks (Sunki x English, Sunki x Jacobsen, Kryder, volkamer lemon, Cleopatra mandarin, Rubidoux and Pomeroy trifoliate orange and Carrizo citrange). Antibody MCA13 and 8 CTV-specific reported probes were used. Dried tissue infected with CTV isolates B272, B274 (mild), B128 (SP), T385 (mild) and T346 (common) were used as control. Probes labeled with Dig-UTP were: ABC (CTV) B3A (severe SP Madeira) B1 (T36 QD strain) B2 (K strain) B4 (T3 SP-QD, Florida) B5 (severe SP SO B249, Venezuela) B8 (mild) III (VT Israel-28C). MCA13 positive reactions were observed with all trees suggesting severe isolate infections. Probes B3A, B5 and B1, yielded optical density values (OD>1). The probes B1, CTV III and mild B8 hybridized showing OD<1. No differences were detected among TL trees on different rootstocks. Trees propagated on Sunki hybrids showed highest yield and longevity. This is the first study about the complexity of CTV in Colombian citriculture looking trees and rootstocks.

S14P06

Molecular diversity of *Citrus tristeza virus* isolates collected over the past 50 years and maintained *in planta* collections in California

Wang J.W.[1], Bozan O.B.[2], Kwon S.J.[1], Rucker T.R.[1], Thomas C.T.[3], Yokomi R.K.[4], Lee R.F.[5], Folimonova S.[6], and Vidalakis G.[1]

[1]University of California, Riverside (UCR), Plant Pathology and Microbiology, U.S.A.; [2]University Of Çukurova (CU), Department of Plant Protection, Turkey; [3]Central California Tristeza Eradication Agency (CCTEA), U.S.A.; [4]United States Department of Agriculture-Agricultural Research Service (USDA-ARS), U.S.A.; [5]United States Department of Agriculture-Agricultural Research Service, National Clonal Germplasm Repository for Citrus and Dates (USDA-ARS, NCGRCD), U.S.A.; and [6]University of Florida/Citrus Research and Education Center (CREC), Plant Protection, U.S.A. vidalg@ucr.edu

Tristeza, caused by *Citrus tristeza virus* (CTV), is a serious citrus disease worldwide. Because of the economic damage that severe isolates of CTV can induce on fruit production and quality, CTV has been a regulated pathogen in California. CTV has been eliminated from all citrus germplasm sources and infected field trees have been eradicated in quarantine zones. Until recently, a low rate of natural spread and the eradication program limited CTV genetic diversity in Central California to primarily T30-like genotypes. These isolates cause no significant damage on citrus grafted on CTV resistant or tolerant rootstocks, under California conditions. Recently, severe non-T30 genotypes of CTV have been detected in California orchards and tristeza has become a re-emerging disease with potential to cause economic damage. To elucidate the origin of recent CTV isolates, genetic diversity of more than 300 CTV isolates collected from 1960-2010 across all major citrus-growing regions in California and maintained *in planta* collections at the University of California, Riverside, Citrus Clonal Protection Program and the Central California Tristeza Eradication Agency at Tulare were evaluated. These collections provided a valuable historical sample of past CTV occurrence in California. Full-length sequencing of the major coat protein gene and/or PCR amplification with molecular markers targeting different CTV genome regions showed T30 genotypes were abundant in California but a few T36-, VT- and B165-like strains were also present at some point in time. Several nonstandard CTV genotypes were also identified. The genetic diversity documented in this study provides a basis for development of CTV management strategies such as cross-protection and provides insight into interactions amongst CTV isolates as well as interactions that might occur with the introduction of virulent CTV isolates.

S14P07

Implication of *Toxoptera citricida* on temporal and spatial dispersion of *Citrus tristeza virus* in Southern Mexico

Domínguez Monge S.[1], Mora Aguilera G.[1], Loeza Kuk E.[2], Flores Sánchez J.L.[1], Acevedo Sánchez G.[1], and Robles García P.[3]

[1]Colegio de Postgraduados-Campus Montecillo (COLPOS), Fitopatología, México; [2]Campo Experimental Mococha, Yucatán México (INIFAP), Fitopatología, México; and [3]Dirección General de Sanidad Vegetal (DGSV), Dirección de Protección Fitosanitaria, México. dominguez.santiago@colpos.mx

At 11 years after *Toxoptera citricida* (TC) introduction into Mexico, this study was carried out to evaluate the epidemiological impact of TC on spreading *Citrus tristeza virus* (CTV) in the Yucatán Peninsula. Data from 1999-2008 were integrated with disease and vector assessments from 2010-2011 in 34 orchards from Yucatán, Campeche and Quintana Roo. Citrus variety, historical positive trees, age, planting density, rootstock, irrigation and symptom intensity were evaluated per orchard. TC adults were collected from 4 flushes of 10 trees/orchard from January to March 2011. Additionally, 7 orchards were examined for infection by analyzing 1672 samples by tissue print-ELISA. TC was restricted to the spring period, mainly on sweet orange trees and it was most prevalent in Yucatán and Campeche (5922 and 8810 aphids/orchard/year, respectively) suggesting that TC establishment was restricted and it neither influenced the regional spreading of CTV nor the occurrence of stem pitting and decline. In 2008, at the Peninsula level the cumulative incidence was 0.00005% relative to the estimated population of trees in the Yucatán Peninsula with the highest number of positive trees in Yucatán (1791) and Campeche (1139). At the orchard level the incidence was 0.6-36%, Yucatán being more affected, with Weibull epidemic rates of $1/b$=0.017-0.030, AUDPC=40-52, and final incidence of 27-36%, indicating that CTV spread continues. GIS risk maps were developed and validated with historical CTV dispersion using a multivariate index based on host and vector density and minimum temperature providing a tool for epidemiological surveillance.

S14P08

Population variation of *Citrus tristeza virus*

Qing L.[1], Ruan T.[1], Xiong Y.[1], Zhou Y.[2], Song Z.[2], Sun X.C.[1], Li Z.A.[2], and Zhou C.Y.[2]

[1]Southwest University (SWU), College of Plant Protection, China; and [2]Chinese Academy of Agricultural Sciences (CAAS), Citrus Research Institute, China. qling@swu.edu.cn

Using the specific primer pair P23f(5'-TAAGAGTCTGCGAGTTACGATG-3') and P23r(5'-TCCGTCCACTTCAATCAG-3'), the *p23* gene of severe (CT3 and CT14) and mild (CT11 and CT18) isolates of *Citrus tristeza virus* (CTV) from grapefruit (CT3 and CT18) and sweet orange (CT11 and CT14) were amplified by RT-PCR and cloned. Eighty six clones of this gene were chosen randomly for sequencing, which composed four CTV populations. Genetic structure and variability of these populations were analyzed. The results demonstrated that all CTV populations were heterogeneous, consisting of sequences that were not identical but closely related to the consensus sequence. The percentage of mutated clones (41.7%) and mutation frequency (8.8×10-4) of the CT11 population were highest among these four populations, whereas those of the CT3 population, with only 13.0% percent of mutated clones and 2.0×10-4 mutation frequency were lowest. The CT14 and CT18 populations shared the same mutation frequency (5.0×10-4), but the percentage of mutated clones of CT14 (26.7%) was lower than that of CT18 (33.3%). Comparison of the variability of CTV mild isolates in different hosts showed that the percentage of mutated clones and mutation frequency of CT18 from grapefruit were lower than those of CT11 from sweet orange. Similarly, the percentage of mutated clones and the mutation frequency of the severe isolate CT3 from grapefruit were lower than that of CT14 from sweet orange, suggesting that variability of CTV is higher in sweet orange than in grapefruit. The consensus nucleotide sequence of CT3 has the highest identity (97%) with CT18 and the lowest (89.7%) with CT14, whereas highest identity of CT11 (95.5%) is with CT14 and the lowest (91.4%) with CT18. Substitution and deletion mutations were detected in CTV populations with the dominant mutation type being base transition from G to A.

S14P09

Nucleotide sequence of three genes of *Citrus tristeza virus* from selected isolates in a program of preimmunization.

Zanutto C.A., Müller G.W., Corazza M.J., and Nunes W.M.C.

Universidade Estadual de Maringa (UEM), Nucleo de Pesquisa em Biotecnologia Aplicada, Brazil. william.nunes@pq.cnpq.br

Two capsid proteins (p25 and p27) are required for the correct assembly of *Citrus tristeza virus* (CTV) virions and for cell-to-cell movement. Protein p25 is also a suppressor of post-transcriptional gene silencing, as also is the p23 protein. To further advance in the knowledge on CTV isolates present in the Paraná State, Brazil, variability of the *p23*, *p25* and *p27* genes of two CTV isolates was analyzed. The isolates chosen for this study were the mild isolate CS-1, selected as protecting isolate for preimmunization in the Paraná State, Brazil, and the severe isolate called Rolândia, that has caused severe symptoms in many orchards in the North of the State and has been used as control in this program. The *p23*, *p25* and *p27* products RT-PCR amplified from these isolates were cloned, and multiple clones for each gene and isolate were sequenced. From the Rolândia isolate, five haplotypes were obtained for the *p23* gene, five for the *p27* and seven for the *p25* (out of 10 clones), which showed identity values ranging from 82.5 to 97.5%, 95.1 to 98 3% and 92.3% to 99.2%, respectively. From the CS-1 mild isolate four haplotypes of the *p25* gene and three haplotypes of the *p27* gene were obtained, which showed identity values ranging from 96.2 to 99.3% and 98.3 to 99.1%, respectively.

S14P10

A comparative study and vector transmissibility of relevant *Citrus tristeza virus* (CTV) populations from Italy indicates a risk for further epidemics

Yahiaoui D.[1], Djelouah K.[1], D'Onghia A.M.[1], and Catara A.[2]

[1]CIHEAM-Mediterranean Agronomique Institute of Bari (CIHEAM-MAIB), Integrated Pest Management, Italy; and [2]Università degli Studi di Catania, Dipartimento di Science e Tecnologie Fitosanitarie (DISTEF), Italy. djelouah@iamb.it

In Italy, Tristeza declining trees on sour orange rootstock trace back to 2002. A mild and a seedling yellows *Citrus tristeza virus* (CTV) population were the causes of heavy losses on Navelina and Tarocco sweet oranges

along the Ionian coast and the South-Eastern part of Sicily, respectively. For a better understanding of their genetic features and spread dynamics, representative CTV isolates from each variant were used for molecular characterization and transmission trials with local aphid biotypes. Sequencing of fragments over the replication-related 5'UTR, and the *p20*, *p23*, *p25* and *p18* genomic regions have been performed, in order to assess the genetic divergence between the two isolates and eventual alterations after aphid passage. The latter represents one of the most significant data in virus–vector interactions. High nucleotide identity was shown between the Apulian isolate and the Spanish mild T385 strain, and between the Sicilian isolate and the Spanish T318A strain, respectively. Moreover, only minor variations were observed in the *p18* region of sub-isolates obtained after *A. gossypii* transmission of the Sicilian isolate. The two divergent CTV populations investigated in the present work appeared to be efficiently transmitted by *A. gossypii* (50% of efficiency) but they were prone to get altered after aphid transmission. These results may give indications on virus epidemiology in the Italian citrus scenario.

S14P11

Prevalence and Epidemiology of *Citrus tristeza virus* in Andalucía and Murcia regions of Spain

Gorris M.T.[1], Muñoz C.[2], Cano A.[3], Hermoso de Mendoza A.[1], Martínez M.C.[1], Hermosilla A.[3], Fuentes F.[3], Bertolini E.[1], Collado C.[1], López A.[4], and Cambra M.[1]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Moncada, Valencia, Spain; [2]Consejería de Agricultura y Pesca (Junta de Andalucía), Laboratorio de Sanidad Vegetal, Montequinto, Sevilla, Spain; [3]Consejeria de Agricultura y Pesca (Región de Murcia), Laboratorio de Sanidad Vegetal, La Alberca, Murcia, Spain; and [4]Universidad de Valencia (UV), Estadística e Investigación Operativa, Burjassot, Valencia, Spain. ebertoli@ivia.es

The Spanish citrus industry is based on about 310,000 ha cultivation. Andalucía (74,000 ha), mainly growing sweet oranges, and Murcia (34,000 ha), growing lemons and mandarins, are the main citrus producing areas after Valencia. The spread of *Citrus tristeza virus* (CTV) was monitored by Tissue print-ELISA (Plant Print Diagnostics) since 1982 in Andalucía and since 1996 in Murcia to 2011 in different citrus orchards. Surveys in different municipalities of Andalucía suggest that the disease spreads slowly (i.e. CTV prevalence increased in a sweet orange orchard in Lora del Río/Sevilla from 0.25% in 1982 to 2.90% in 2011). In Alhama de Murcia CTV prevalence increased in a mandarin orchard from 1.00% in 1996 to 70.00% in 2011. The aphid species landing on the trees were monitored by the sticky shoot method during the same period. *Toxoptera aurantii* and *Aphis spiraecola* (inefficient CTV vector species) are predominant in both regions in contrast with Valencia where *A. gossypii* (efficient CTV vector) is the predominant aphid species. The different aphid species predominant in Andalucía-Murcia and in Valencia, could justify differences in the temporal spread of CTV among these areas: logistic model in Andalucía-Murcia versus Gompertz model in Valencia. The epidemiological data allowed specific recommendations to the growers in order to successfully reconvert the citrus industry (already done in almost 90% in both regions).

S14P12

Epidemiology of *Citrus tristeza virus* in experimental nursery blocks of citrus rootstock species in Spain

Gorris M.T.[1], Rodríguez A.[1], Martínez C.M.[1], Bertolini E.[1], Collado C.[1], Botella P.[2], López A.[3], and Cambra M.[1]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Moncada, Valencia, Spain; [2]Universidad CEU-Cardenal Herrera (CEU), Ciencias Físicas, Matemáticas y Computación, Alfara del Patriarca, Valencia, Spain; and [3]Universidad de Valencia (UV), Estadística e Investigación Operativa, Burjassot, Valencia, Spain. mcambra@ivia.es

The susceptibility to *Citrus tristeza virus* (CTV) natural infection of the most commonly used citrus rootstocks in Spain (Carrizo citrange, Cleopatra mandarin, *Citrus volkameriana*, *C. macrophylla*, sour orange and citrumelo), was studied by Tissue print-ELISA (Plant Print Diagnostics) in the Peñíscola area. The most susceptible rootstock species was *C. macrophylla* followed by *C. volkameriana*. Cleopatra mandarin showed an intermediate susceptibility and Carrizo citrange and sour orange very low susceptibility. Citrumelo resulted non-susceptible. Three experimental blocks of 10,000 plants each of *C. macrophylla,* Cleopatra mandarin and Carrizo citrange were established to elucidate the reasons of different susceptibility to natural CTV-infection.

Spatial analysis showed that small clusters of infected plants were only detected in the *C. macrophylla* block (4.8% CTV prevalence). CTV prevalence was 1.9% in Cleopatra and 0.7% in citrange experimental nursery blocks. Aphid species visiting rootstock seedlings were monitored by the sticky shoot method. *Aphis gossyppi* was the predominant vector species visiting nursery plants followed by *A. spiraecola*. May was the month with highest aphid populations and *C. macrophylla* was the most visited citrus rootstock species (about 1,700 *A. gossypii* individuals visited each plant). This fact justifies the relatively high CTV prevalence found in this species. Seedlings of the most susceptible citrus species must be permanently protected against natural CTV infection. In addition, individual testing of nursery plants by Tissue print-ELISA could guarantee the CTV-free status in certified citrus plants.

S14P13

Monitoring *Citrus tristeza virus* (CTV) and characterization of local isolates in Algeria

Larbi D.[1], Belkahla H.[1], Djelouah K.[2], and D'Onghia A.M.[2]

[1]Univesity Saad Dahleb Soumaa Blida (UniB), Agronomy, Algeria; and [2]Mediterranean Agronomic Institute of Bari (MAIB), Integrated Pest Management, Italy. lardjamila@yahoo.fr

During the period 2009-2012, the main Algerian citrus growing areas located in Mitidja region were surveyed to assess the presence of *Citrus tristeza virus* (CTV). The main citrus varieties from ten selected sites were sampled and serologically analyzed. The hierarchical sampling method was carried out in each selected grove, with flowers, explants, and leaves being collected and analyzed by Direct Tissue Blot Immunoassay (DTBIA) using the commercial kit from Plantprint (Valencia, Spain). Among the 1903 samples tested, 468 (25.16%) CTV-infected trees were identified. The highest CTV infection rate was on common mandarin (70%), followed by Thomson navel orange and Washington navel orange, with infection rates of 46% and 8% respectively, whereas lower CTV prevalence was found in satsuma, Taroco, lemon and clementine (6%). Clear Quick Decline associated to the citrus tristeza disease were observed in some trees during the survey. A few samples that were CTV positive by DTBIA were also positive by biological indexing on Mexican lime (*Citrus aurantifolia*) and immunocapture-reverse transcription-PCR. SSCP analysis and subsequent sequencing of the coat protein gene from five Algerian CTV isolates showed 99% identity with the mild T30 CTV reference isolate.

S14P14

Evaluation of the sampling method of *Citrus tristeza virus* (CTV) in Apulia region, Italy

D'Onghia A.M., Al Naasan Y., Santoro F., Figorito B., and Gualano S.

Centre International de Hautes Etudes Agronomiques Méditerranéennes/Mediterranean Agronomic Institute of Bari (CIHEAM/MAIB), Integrated Pest Management, Italy. donghia@iamb.it

Citrus Tristeza is the most destructive virus disease of citrus worldwide, mainly inducing decline of trees grafted onto the sour orange rootstock. The causal agent, *Citrus tristeza virus* (CTV), is widely present in the Mediterranean basin where it is efficiently transmitted by several aphid species, mainly by *Aphis gossypii*. Spain and Israel were forced to cope with this disease long time ago, whereas disease outbreaks in Italy, Greece and Morocco were reported in the last years. In Apulia region, Southern Italy, CTV monitoring and eradication programs are totally dependent on assessing the disease incidence in citrus groves, and according to the Apulian phytosanitary regulation the whole infected plot must be removed when a threshold of 30% CTV incidence is exceeded. The CTV monitoring programs adopted in Apulia have been implemented using systematic sampling method fundamentally based on sample group testing. This method differs from the Gottwald's hierarchical sampling (HS) method by testing all sampled trees individually using DTBIA instead of testing the groups by ELISA. A detailed comparison between the Gottwald's HS method and the one adopted in Apulia region is presented. The adopted sampling method was evaluated in 15 commercial groves with different infection rates. Also, we evaluated the estimation errors for the two sampling methods using statistical simulation tools. The results showed no difference between them.

S14P15

Integrated *Citrus Tristeza Virus* complex management in Sicily

Tumminelli R., Rotolo M.C., Saraceno F., Patti M.R., Saitta R., Conti F., Fisicaro R., Cavallaro A., Cavallaro G., Cutuli A., Greco G., Di Natale A., Garozzo M., Gullotta S., Privitera S., Quattrocchi S., Ricca G., Bertolami E., Calderone G., Maimone F., Schillaci G., Trifiletti A., Corno G., Bono G., Federico R., Lo Grasso F., Vicari M., Lo Presti P., Truncali S., Sicilia L., Giacalone A., Adragna V., Vecchio S., Filiddani R., Fascetto G.T., Marano G., Spadafora A., D'Anna R., Carta Cerella D., and Sinatra V.

Servizio Fitosanitario Regionale Siciliano (SFR SICILY), Quarantine and Crop Protection Service, Italy.
riccardo.tumminelli@regione.sicilia.it

Citrus tristeza virus (CTV) complex causes one of the most destructive diseases of citrus in the world. In Sicily (Italy) it was first detected in 1956. Recently the virus has spread widely in citrus areas where trees are grown almost exclusively on sour orange, a very susceptible rootstock. In 2012, in Sicily, a European Union aid (12 million euros) was issued for the management of this complex. In the period 2008-12, in the framework of the official controls, monitoring was carried out in Sicily for the detection of CTV in citrus trees cultivated in nursery and field conditions. Aphids were also surveyed in order to exclude the presence of *Toxoptera citricidus*, detected in 2005 in the North-Western area of the Iberian Peninsula. This aphid is known as a very efficient vector of CTV. In the survey period nursery trees and mature orchards were checked utilizing DAS-ELISA test, and nursery trees were sampled for the identification of aphid species present on new shouts. A protected foundation block for budwood production was indexed, but CTV was never detected. The quarantine aphid *T. citricidus* was not observed. In spring and fall samples the presence of *Aphis gossypii*, *A. spiraecola* and *T. aurantii* was recorded in decreasing frequency order.

S14P16

Biological and molecular characterization of a *Citrus tristeza virus* isolate inducing seedling yellows and stem pitting in Hunan province, China

Licciardello G.[1], Russo M.[1], Daden M.[2], Dai S.[3], Xiao C.[3], Deng Z.[3], and Catara A.[1]

[1]Parco Scientifico e Tecnologico della Sicilia (PSTS), Italy; [2]International Plant Analysis and Diagnostics S.r.l. (IPADLAB), Italy; and [3]National Center for Citrus Improvement, Hunan, China. acatara@pstsicilia.it

Although *Citrus tristeza virus* (CTV) has been present in China for a long time, symptoms of decline are not apparent since the rootstocks commonly used are tolerant or immune. Nevertheless, recently the disease has become a problem in many provinces, including Hunan, with dwarfing, short internodes, leaf curl and stem pitting associated with small fruits and poor yield. Hence characterization of the genetic structure of the virus population in the province has been undertaken. Here we report biological and molecular characterization of a severe isolate inducing symptoms of seedling yellows and stem pitting on Mexican lime, sour orange, grapefruit and sweet orange. The CE-SSCP profiles of genes *p18*, *p25*, *p20*, *p23* and *p27* revealed a complex pattern with multiple peaks, suggesting the presence of mixed infections. Indeed, after molecular cloning of the coat protein gene, three different CE-SSCP profiles were obtained with relative frequencies of 10% (profile A), 70% (profile B) and 20% (profile C). Phylogenetic analyses showed that the major and minor variants clustered with isolates from Thailand, the Indian decline Kpg3 and the Hawaiian isolates HA18-9 and HA16-5. The third sequence variant grouped with severe seedling yellows and stem pitting isolates (VT, SY568, T318 and NUagA). Analysis with multiple molecular markers (MMM) showed the presence of T36-, VT- and T30-like genotypes, differing from other isolates collected during the survey.

S14P17

Evaluation of productive and physiological parameters of Tarocco orange infected by a severe isolate of *Citrus tristeza virus*.

Sorrentino G.[1], Guardo M.[1], Russo M.P.[1], Davino S.[2], and Caruso A.[1]

[1]CRA - Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee (CRA-ACM), Italy; and [2]University of Palermo (DEMETRA), Italy.
guido.sorrentino@entecra.it

In this paper the effect of *Citrus tristeza virus* (CTV) on yield, fruit quality and chlorophyll content was investigated on a commercial field growing 30-yr-old Tarocco sweet orange grafted on sour orange. In 2002 32% of the trees were infected with a severe CTV isolate (CTV-DS2), easily transmitted by aphids. Analysis

was carried out for five years on groups of 50 plants showing different degrees of decline: severe decline (1), initial decline symptoms (2), symptomless infection (3) and CTV free (4). On each tree, fruit yield, fruit size, total soluble solids (T), acidity (A), T/A ratio, peel thickness, flesh and juice colour, and leaf chlorophyll content as estimated by SPAD 502 plus Konica Minolta, were measured. Simple variance and discriminant analysis showed that all parameters were influenced by the infection level. We postulated that SPAD index can be considered a first indicator to discriminate between healthy and CTV infected trees in well managed farms.

S14P18

Eradicating *Citrus tristeza virus* and *Citrus tatter leaf virus* from double infected citrus plants

Qiao Q., and Jiang J.

Huazhong Agricultural University (HZAU), College of the Department of Horticulture and Forestry, China. jiangling@mail.hzau.edu.cn

Because of their long-term agamogenesis in the production process, citrus species are often susceptible to one or several viruses such as *Citrus tatter leaf virus* (CTLV) and *Citrus tristeza virus* (CTV), which are key limiting factors for commercial citrus development. This study focuses on finding rapid and efficient methods for eliminating these two virus pathogens of infected citrus plants. Botanical agents A extracted from *Isatis indigotica* and B extracted from *Scutellaria baicalensis* were used in an experiment combined with traditional virus eliminating methods (shoot-tip grafting and thermotherapy). For shoot-tip grafting, the triangle method was implemented. Buds used were terminal buds with three leaf primordia and the rootstock used was Bingtang orange cultivated in dark for about 14 days. The heat treatment was performed as follows, first we adopted constant temperature (37 ºC) for 50 days, then 40 ºC and 10 h light and 25 ºC and 14 h dark for 30 days. Heat treatment and phytotherapy were operated at the same time. The reverse transcriptase and PCR amplification (RT-PCR) was used to test whether plants subjected to these treatment were virus-free. Results showed that adopting the agents from Chinese medicine combined with the heat treatment in which the highest temperature was 40ºC during the day (6 h/ d) and the lowest temperature was 25ºC in the evening to keep the vegetation for fifty days in the greenhouse provided the best results. This protocol allowed to completely eliminate CTV and CTLV from double infected plants. Furthermore, the combination of shoot-tip grafting and the antiviral Chinese medical treatment allowed 85.7% eradication of both viruses from infected plants.

S14P19

Construction of an infectious cDNA clone of *Citrus tatter leaf virus* from an infected sweet orange

Song Z., Zhou C.Y., Liu K.H., and Li Z.A.

Chinese Academy of Agricultural Science, Citrus Research Institute, China. songzhen168@163.com

A full-length cDNA clone of *Citrus tatter leaf virus* (CTLV-XHC) isolated from *Citrus sinensis* 'Xinhuicheng' was constructed after sequencing the virus. The complete genome sequence of CTLV-XHC was determined to be 6,496 nucleotides in length, excluding the 3'-terminal poly(A) tract, and contained two putative overlapping open reading frames (ORFs). ORF1 (positions 36-6351) encodes a potential polyprotein of 242 kDa. ORF2 (positions 4787-5747) codes for a 36 kDa protein. Nucleotide sequence comparison showed 80.9% to 97.6% identity to other CTLV or *Apple stem grooving virus* (ASGV) sequences. The cDNA clone of this CTLV genomic RNA was fused to a binary vector (pCAMBIA1301) containing the *Cauliflower mosaic virus* 35S RNA promoter and the *nopaline synthase* gene polyadenylation signal. Agro-inoculation of this plasmid to leaves of *Chenopodium quinoa* and *Nicotiana benthamiana* resulted in systemic infection, which was confirmed by RT-PCR and symptom expression. The potential use of the infectious clone as a vector for virus-induced gene silencing (VIGS) in citrus is discussed.

S14P20

The psorosis B syndrome in citrus is associated with a sequence variant of the *Citrus psorosis virus* RNA2

Velázquez K., Alba L., Pina J.A., Navarro L., Moreno P., and Guerri J.

Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Moncada, Valencia, Spain. velazquez_kar@gva.es

Psorosis disease of citrus is characterized by bark scaling in the stem and main branches of trees at least 10 years old. Graft-inoculation of sweet orange seedlings in the greenhouse with non-scaled bark pieces from an

infected tree incites the psorosis A syndrome (PsA), including a shock reaction of the first flush and transient chlorotic flecking in young leaves of the following flushes, whereas inoculation with scaled bark pieces incites the psorosis B syndrome (PsB), that in addition to PsA includes gummy pustules in old leaves and branches. Psorosis disease is caused by *Citrus psorosis virus* (CPsV), an ophiovirus with three negative-stranded genomic RNAs. While comparison of RNA1 or RNA3 fragments by single-strand conformation polymorphism (SSCP) analysis did not show any difference between plants affected by the PsA or the PsB syndromes, a fragment of RNA2 enabled discriminating both syndromes by their SSCP profile. Some plants inoculated with scaled bark did not show PsB at 6 months post-inoculation (mpi) and their RNA2 gave a PsA-type SSCP profile. At 12 mpi these plants showed pustules in the trunk but not in the leaves. SSCP analysis of RNA2 showed only PsA-type variants in the leaves, whereas the pustuled trunk areas contained both PsA and PsB variants, the latter being predominant. These results suggest that psorosis-affected trees contain the two sequence variants and that the PsB variant is associated with pustules and tends to accumulate in the trunk bark.

S14P21

Sensitivity to *Citrus psorosis virus* of species and hybrids of the genus *Citrus* and relatives

Velázquez K., Alba L., Zarza O., Vives M.C., Pina J.A., Juárez J., Navarro L., Moreno P., and Guerri J.

Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Moncada, Valencia, Spain.
velazquez_kar@gva.es

Citrus psorosis virus (CPsV), genus *Ophiovirus,* causes an important disease in many citrus growing regions. Virus damage can be controlled using resistant or tolerant cultivars, but sensitivity of many species and hybrids of *Citrus* and related genera is unknown. To find potential sources of CPsV resístance we first propagated 61 species (or cultivars) and hybrids of *Citrus* and related genera [*Citrus* (37), *Microcitrus* (5), *Fortunella* (6), *Eremocitrus* (1), *Pleiospermium* (1), *Atalantia* (1), *Severinia* (1), *Clausena* (1), *Poncirus* (1) and hybrids (7)] on rough lemon seedlings inoculated with the CPsV isolate PB-143. Out of the 61 accessions tested 54 showed symptoms and positive ELISA reaction, 2 (*M. inodora* y *F. hindsii*) were symptomless but gave high ELISA values, suggesting tolerance to CPsV, 5 (*C. depresa*, *C. reshni*, *C. excavata*, Carrizo citrange (CC) and citrumelo CPB 4475) showed symptoms in the first flush but negative ELISA reaction, and 1 (*P. trifoliata*) was ELISA negative and symptomless. We then examined infection by quantitative real time RT-PCR in *C. reshni*, *P. trifoliata* and CC seedlings inoculated with CPsV isolates P-121, PB-102 and PB-143. While P-121 was detected in all *C. reshni*, and CC, and in 75% of *P. trifoliata* plants, the other isolates infected only 32-82% (PB-143) or 20-91% (PB-102) of the inoculated plants. These three accessions showed necrosis around the inoculum patch that impaired or delayed infection, suggesting isolate-dependent partial resistance.

S14P22

Detection and quantitation of *Citrus psorosis virus* by real time RT-PCR

Velázquez K., Alba L., Guerri J., Moreno P., Navarro L., and Vives M.C.

Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Moncada, Valencia, Spain.
cvives@ivia.es

Citrus Psorosis, an important disease in many citrus growing regions, is caused by *Citrus psorosis virus* (CPsV), an ophiovirus transmitted through infected buds and naturally spread in areas of South America. The CPsV genome consists of three single-stranded, negative-sense RNAs. Psorosis has been diagnosed by biological indexing on indicator plants, and more recently by ELISA, tissue-print hybridization or RT-PCR. ELISA enables fast and specific detection of CPsV, albeit with low sensitivity, and the other procedures do not allow virus quantitation in different varieties, tissues and seasons. To develop a highly sensitive and reliable technique for CPsV detection and quantitation we set up a real-time RT-PCR assay (qrt-RT-PCR) using SYBR Green and the CPsV RNA3 as target. This protocol enables detection of 1000 CPsV RNA copies in plant extracts with a linear response along a dynamic range of 7 log units of concentration. Sensitivity of CPsV detection by qrt-RT-PCR is much higher than with ELISA or conventional RT-PCR, and it enables detection of distinct viral isolates infecting field trees of different citrus species all year around. Maximum accumulation of CPsV was observed in young leaves and bark, but it was also detected in old bark and leaves. The lowest CPsV accumulation was

detected in the main trunk and roots. The new assay is a valuable tool to screen for resistance and a major improvement for CPsV detection in sanitation, quarantine and certification programs.

S14P23

Improvement in diagnosis for Citrus Psorosis in Argentina by qRT-PCR

de Francesco A.[1], Reyes C.A.[1], Costa N.[2], and Garcia M.L.[1]

[1]Instituto de Biotecnología y Biología Molecular (IBBM), La Plata, Buenos Aires, Argentina; [2]Estación Experimental Agropecuaria Concordia (INTA EEA Concordia), Entre Rios, Argentina. agustinadefrancesco@hotmail.com

Citrus is one of the most important crops in Argentina, a leading export country of oranges and lemons. Several diseases affect citrus in Argentina, among them Psorosis is still a serious and widespread viral disease, making trees less productive and causing economic losses. In the field, symptoms are observed mainly in sweet orange, showing bark scaling restricted to the main trunk and limbs, and chlorotic flecks and spots in young leaves. *Citrus psorosis virus* (CPsV), the casual agent of the disease is the type member of genus *Ophiovirus*, family *Ophioviridae*. Virus particles have filamentous circular morphology, and its genome has three negative single-stranded RNAs encapsidated with a coat protein. CPsV can be detected by TAS-ELISA using polyclonal and monoclonal antibodies and by RT-PCR with specific primers. These procedures are faster and more reliable than biological indexing. Real-time RT-PCR (qRT-PCR) using Taqman probes has been applied successfully for detection of Mexican and European CPsV isolates. In this work, we applied a new qRT-PCR using SYBR Green and specific primers designed in a region of the coat protein gene that is conserved in Argentinian isolates in order to improve disease control in our country. We compared our results of qRT-PCR with TAS-ELISA and RT-PCR applied in field samples and will discuss reliability of these methods.

S14P24

Cloning and sequence analysis of the large coat protein of *Satsuma dwarf virus* Fengjie isolate

Sun X.C.[1], Qing L.[1], Yang F.Y.[2], and Zhou C.Y.[2]

[1]Chongqing Key Laboratory of Plant Disease Biology, College of Plant Protection, Southwest University, Chongqing, China; and [2]National Center of Citrus Engineering and Technology Research, Citrus Research Institute of Chinese Academy of Agricultural Sciences, Chongqing, China. xianchaosun@gmail.com

Satsuma dwarf virus (SDV) mainly infects satsuma mandarins, causing serious Satsuma Dwarf disease. In China, Satsuma Dwarf was found in Fengjie of Chongqing, Wu city of Jiangsu province and Huangyan of Zhejiang province since satsuma mandarins were introduced from Japan in the 1980s. However, it is unclear whether the virus in China and the S-58 isolate reported from Japan are the same strain or whether SDV has evolved and become adapted to the new environment and hosts. To answer this question, the large coat protein (CPL) gene of SDV isolated from Fengjie (SDV FJ) was amplified by RT-PCR and cloned into vector pGEM-T for sequencing. Sequence analysis showed that the SDV-FJ CPL gene had 1329 nt, encoding a protein of 443 amino acids. Comparison of this sequence with those of other viruses of the genus *Sadwavirus* showed high nucleotide and amino acid identity (98.1% and 98.6%) between SDV FJ and SDV S-58 from Japan. The SDV FJ CPL gene had 25 nucleotide differences with the SDV S-58 CPL, the most frequent being T and C transitions. Therefore, it is suggested that the SDV FJ and S-58 are the same strain of SDV.

S14P25

Characterization and incidence of *Citrus leaf blotch virus* (CLBV) in Southern Italy

Guardo M., Sorrentino G., and Caruso A.

CRA - Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee (CRA-ACM), Italy. guido.sorrentino@entecra.it

Citrus leaf blotch virus (CLBV), the type species of the new genus *Citrivirus* of the family *Flexiviridae*, has been associated with a bud union disorder of Nagami kumquat and calamondin scions grafted on trifoliate rootstocks. After the first report in Italy in 2007, surveys to monitor CLBV incidence were carried out in nurseries, private collections and commercial citrus orchards with bud union disorder problems from Sicily and Calabria using biological indexing and RT-PCR. Each positive sample was analysed by Single-Strand

Conformation Polymorphism (SSCP) and samples with different electrophoretic pattern were cloned and their nucleotide sequence compared by CLUSTALW. The results confirmed the presence of CLBV in Italy and three new sequences were found and deposited in GenBank. No positive samples were found in varieties other than Nagami kumquat, with 80% of infected trees, or calamondin, with 31% infected trees. This low CLBV incidence in symptomatic calamondin trees could be due to a low virus titer in calamondin, or to bud union crease being caused a different pathogen or by the interaction between biotic or abiotic factors.

S14P26

Citrus leaf blotch virus (CLBV) invades meristematic regions in *Nicotiana benthamiana* and citrus

Agüero J., Vives M.C., Velázquez K., Ruiz-Ruiz S., Juárez J., Navarro L., Moreno P., and Guerri J.

Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Moncada, Valencia, Spain.
jguerri@ivia.es

Systemic infection by plant viruses involves long distance movement through the phloem sieve tubes. Since the vascular system is not developed in the vicinity of apical meristems, most viruses are unable to reach this region, a phenomenon that has been exploited to recover virus-free citrus plants by shoot-tip grafting in vitro. However, *Citrus leaf blotch virus* (CLBV) is difficult to eliminate by this method and seed transmission has been observed, suggesting that this virus does invade meristematic regions. To examine this hypothesis we monitored CLBV movement and accumulation using and infectious cDNA clone of the CLBV genome engineered to express the green fluorescent protein (GFP) gene. For this purpose, the promoter inducing transcription of the ORF2 subgenomic RNA was conveniently reduced and duplicated in the 3' terminal untranslated region to obtain the infectious clone clbv3'pr-GFP. This extra gene does not affect virus functionality and produces GFP fluorescence in infected cells. *Nicotiana benthamiana* plants agroinoculated with clbv3'pr-GFP showed fluorescence in all tissues including meristematic regions, but in citrus plants infected with CLBV-GFP fluorescence was not visible and GFP had to be detected by western blot analysis. In lime plants inoculated with *Citrus tristeza virus* (CTV), which cannot infect meristematic regions, CLBV or CLBV plus CTV, hybridization and real time RT-PCR allowed detection of CLBV, but not of CTV, in shoot tips less than 0.2 mm long.

S14P27

Simultaneous detection of *Citrus exocortis viroid* and *Hop stunt viroid* in citrus plants by direct tissue-print duplex real-time RT-PCR

Bertolini E.[1], Martínez M.C.[1], Serra P.[2], Olmos A.[2], Carbó C.[1], Duran-Vila N.[1], and Cambra M.[1]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Moncada, Valencia, Spain; and [2]Instituto de Biología Molecular y Celular de Plantas (IBMCP), Departamento de Biología del Estrés, Valencia, Spain.
ebertoli@ivia.es

Citrus exocortis viroid (CEVd) and *Hop stunt viroid* (HSVd) are the causal agents of Exocortis and Cachexia, respectively, the most important viroid diseases of citrus trees. Traditionally the detection of citrus viroids has been performed by biological indexing and/or biochemical and molecular techniques. Biological indexing (based on the use of indicator citrus species) is time consuming and requires expensive infrastructures. Biochemical and molecular methods (based on sPAGE, tissue print-hybridization after graft-inoculation on *Citrus medica* or conventional RT-PCR), require extract preparation, and they are generally laborious and time consuming and present risk of contamination, often resulting inconvenient for routine diagnostics. The usefulness of real-time RT-PCR to detect viroids has been reported previously. A real-time RT-PCR method using two newly designed primer pairs and TaqMan probes was successfully developed and patented for the simultaneous detection of both viroid species. A direct tissue-print sample preparation method was tested and used to analyze samples from different citrus species and in different seasons. Results confirm that the real-time RT-PCR approach is as reliable as biological indexing and more sensitive than conventional sPAGE and molecular hybridization, enabling reliable detection of both citrus viroids at any season with the same accuracy. The developed methods open new possibilities for sensitive, simple and accurate detection of citrus viroids. A kit for the detection of both citrus pathogens has been validated and its use could facilitate the sanitary controls required in certification programs.

S14P28

Setting up and evaluation of new primer sets for the detection of *Spiroplasma citri* the causal agent of citrus stubborn disease

Abdel Fattah A., Valentini F., Frasheri D., D'Onghia A.M., and Djelouah K.

CIHEAM-Mediterranean Agronomic Institute of Bari (CIHEAM-MAIB), Integrated Pest Management, Italy. djelouah@iamb.it

Citrus Stubborn disease caused by *Spiroplasma citri* is a limiting factor for citrus production in countries with desert or semi arid conditions in the Mediterranean basin. Several polymerase chain reaction (PCR) assays has been previously developed but most of them lacked specificity or sensitivity for *Spiroplasma citri* detection. In order to develop a PCR assay specific and sensitive for detecting Mediterranean *S. citri* strains, three PCR primers were designed using the CLC Genomics Workbench 3.0 software, on the basis of repetitive sequences annotated "Hypothetical proteins"; a BLAST of the selected genes on the GeneBank showed no homology with genes of other organisms, spiroplasma plasmids, or known spiroplasma virus sequences. The primer pairs were annotated as ScX7, ScX8, and ScX9, their sensitivity was evaluated by PCR with serial dilutions of positive *S. citri* cultures and DNA extracted from *S.citri* infected plants, while the specificity was determined by PCR using the DNA extracted from cultures of other *Spiroplasma* species. Primer pairs were validated by comparing them with PCR primer pairs previously designed in other laboratories. The ScX7 (sequence derived from GenBank accession no. AM285309) was selected and used for further detection and surveys, owing to its high specificity and sensitivity when compared with all other primers.

S14P29

Biological characterization of a new lemon disease in China

Chen H.M., Li Z.A., Zhou Y., Wang X.F., Tang K.Z., and Zhou C.Y.

Citrus Research Institute, Chinese Academy of Agricultural Sciences (CRIC), National citrus Engineering Research Center, China. chenhongming_21@163.com

In 2009, a new disease was discovered on Eureka lemon (*Citrus limon*) in an orchard of the Yunnan province. Nearly 10% of the plants were infected and showed yellow vein clearing of lateral veins, shrinking, water-soaked, and boat-shaped leaves. The symptoms were more severe on spring and autumn leaves than in summer leaves. This disease was transmitted by grafting from infected Eureka lemon to six lemon (Femminello, Fino, Villafranca, Verna, Kutdiken and Monachello) and two sour orange (Daidai and Morocco) varieties inducing similar symptoms. Variable symptoms were observed in other hosts ranging from severe yellowing and vein clearing on Dweet tangor, slight vein clearing on Tongshui 72-1 sweet orange and Marsh grapefruit, to no symptoms on Etrog citron, Mexican lime and Ponkan seedlings. Eight herbaceous plants were tested for mechanical transmission of the disease. While *Vigna unguiculata* and *Capsicum frutescens* showed conspicuous symptoms, *Lycopersicum esculentum*, *Nicotiana benthamiana*, *Momordica charantia*, *Solanum melongena*, *Cucumis sativus* and *Sesamum indicum* remained symptomless. Symptom expression was influenced by temperature, being intense between 18 and 24ºC but disappearing over 32ºC. Furthermore, crude extracts from young lemon leaves with yellow vein clearing were examined by transmission electron microscopy and showed filamentous virus particles measuring 13-15×400-1000 nm. Based on the above results, this disease is likely to result from *Citrus yellow vein clearing virus* (CYVCV).

S14P30

Virus and virus-like diseases of citrus in Oman

Al-Sadi A.M.[1], and Al-Harthi S.A.[2]

[1]Sultan Qaboos University (SQU), Crop Sciences, Oman; and [2]Royal Court Affairs (RCA), Royal Gardens and Farms, Oman. alsadi@squ.edu.om

A study was conducted to investigate virus and virus-like diseases of citrus in Oman. A survey over 2009 to 2011 showed that *Citrus tristeza virus* (CTV), *Citrus exocortis viroid* (CEVd) and *Hop stunt viroid* (HSVd) are common in various regions of the country. These agents were detected in acid lime, sweet orange, sour orange, grapefruit, lemon and other citrus species. Stem pitting symptoms were found to be common for CTV

infected trees, while bark scaling was found to be common in some citrus cultivars infected by viroids. CTV, CEVd and HSVd were also detected in 45-79% of citrus seedlings imported from Syria, Lebanon, India, Jordan, Pakistan and Egypt. This provides evidence for circulation of CTV and citrus viroids via seedlings originating in these countries. Cloning and sequencing the CP gene of 6 isolates of CTV showed that the isolates have 96-99% identity with representative isolates from other parts of the world. Phylogenetic analysis of the CTV isolates provided evidence that 4 isolates belong to CTV Group 4. However, 2 isolates formed a clade and were separated from all other CTV groups with 100% bootstrap support, which suggests that the two isolates from Oman constitute a new CTV phylogenetic group. Work is in progress to characterize phylogenetic relationship of citrus viroids from Oman with viroids from other parts of the world.

S14P31

Recent trends in diagnosis, characterization, and management of major virus and virus-like pathogens infecting citrus in India

Ghosh D.K.

Plant Virology Laboratory, NRC for Citrus, Nagpur (44000), Maharashtra, India. ghoshdk@hotmail.com

The field of citrus virology has expanded in the past couple of decades due to the discovery of various virus and virus-like pathogens. The economic importance of these pathogens derives largely from their ability to cause systemic diseases and to persist in vegetative parts of the plants for as long as the trees remain alive. Hence losses are not confined to the season in which infection occurs, but continue as long as the infected plants are in culture. In India about sixteen such pathogens are reported to infect citrus plants among which *Citrus tristeza virus* (CTV), *Indian citrus ringspot virus* (ICRSV), *Citrus yellow mosaic virus* (CiMV), *Citrus exocortis viroid* (CEVd) and the Citrus Greening bacterium (HLB) are of major concern. Nagpur mandarin (*Citrus reticulata*), acid lime (*Citrus aurantifolia*) and mosambi (*Citrus sinensis*) are three important commercial crops in India and are infected by these pathogens resulting in gradual decline of citrus orchards. These pathogens can be diagnosed by conventional bio-diagnosis that has its own limitations. Today a multitude of PCR based techniques, viz. PCR, RT-PCR, IC-PCR, multiplex PCR etc., and protein based diagnosis using pathogen specific polyclonal and monoclonal antibodies are being used routinely for detection of these pathogens in citrus plant samples either as single or as mixed infections. Genomes of these pathogens have been cloned, sequenced and their phylogenetic relationships established. One important component for integrated disease management is to implement a citrus budwood certification program to provide planting material free from the major graft transmissible pathogens. Accordingly by using the standardized biological, serological, and molecular techniques, a citrus budwood certification program has been implemented in India to provide one million virus free citrus plants annually to the citrus growers.

S14P32

The citrus nursery tree certification program in Spain

Pina J.A.[1], Chomé P.[2], Vives M.C.[3], and Navarro L.[3]

[1]Consellería de Agricultura, Pesca, Alimentación y Agua (CAPAA), Servicio de Sanidad Vegetal, Spain; [2]Ministerio de Agricultura, Alimentación y Medio Ambiente (MAGRAMA), Oficina Española de Variedades Vegetales, Spain; and [3]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Moncada, Valencia, Spain. pina_jos@gva.es

This program includes four blocks of trees: 1) The protected foundation block, that is maintained at IVIA and includes healthy plants recovered by shoot-tip grafting *in vitro* (STG) from local or foreign varieties. Plants are grown in containers inside insect-proof screenhouses. 2) Foundation blocks propagated with budwood from the protected foundation block and also grown inside screen- or greenhouses. They belong to individual nurseries or nursery groups. 3) Budwood increase blocks including plants propagated directly from foundation trees to increase the number of buds for propagation of certified trees. They are also maintained inside screen- or greenhouses at each nursery. 4) Certified nursery trees are propagated with budwood from the increase blocks. Certified trees are produced in the open field, in screenhouses or in greenhouses. Plants of each block are periodically indexed by different methodologies according to the regulations. The program is operating with this outline since 1979, when the first healthy plants recovered by STG were released to

the nurseries, which started selling plants from this origin to growers in 1982. Since then, 103 varieties have been propagated with a total of 150 million certified plants, with sweet oranges representing 51% of the certified plants, clementines 27%, other mandarins 13%, lemons 6% and grapefruits 1%. Presently Carrizo citrange is used as rootstock for 59% of the nursery plants and *Citrus macrophylla* for 19%. A total of 40 nurseries are presently operating and they are grouped in 7 foundation blocks. Under this program practically all the Spanish citrus industry has been renewed with healthy plants. Today, traditional graft transmissible pathogens do not pose any problem for our citrus industry.

S14P33

COST FA0806: Plant virus control employing RNA-based vaccines: A novel non-transgenic strategy

Voloudakis A.E.

Agricultural University of Athens (AUA), Dept. of Crop Science, Laboratory of Plant Breeding & Biometry, Greece. avoloud@aua.gr

The current virus control methods are limited in number, efficacy and environmental suitability and current EU decisions restrict crop improvement strategies employing transgenic plants. To protect plants against existing and emerging virus diseases new methods are urgently needed. A very promising approach is the exploitation of RNA silencing, a natural, endogenous mechanism in plants that is a sequence-specific process leading to viral mRNA degradation. COST Action FA0806* (http://www.aua.gr/COSTFA0806) brings together several EU labs in order to develop suitable, efficient and cost-effective methods to induce anti-viral silencing in crops by the transient application of dsRNA, siRNAs and/or artificial small RNAs (collectively designated as "RNA-based vaccines"). These vaccines are produced either *in vitro* or *in vivo* in large quantities and are applied at laboratory or large scale employing specific delivery machinery. FA0806 is structured in three Working Groups (WGs), WG1: Development of novel non-transgenic strategies for plant virus control, WG2: Application of novel non-transgenic strategies for plant virus control, and WG3: Socio-economic evaluation of the impact of the novel application methods. In the frame of FA0806, Training Schools and Short Term Scientific Missions provide instruments for scientific exchange and training for early-stage and senior researchers alike. Currently, 61 members from 28 COST countries and eight non-COST members, from Argentina, Australia, China, Mexico, New Zealand, Peru and South Africa participate in the Action.

Contact information: Andreas Voloudakis (Action Chair): avoloud@aua.gr, Thomas Hohn (Action Vice Chair): thomas.hohn@fmi.ch, Maria Holeva (Action Secretary): m.holeva@bpi.gr.

* COST 257/08 Memorandum of Understanding (2008). COST Action FA0806: Plant virus control employing RNA-based vaccines: a novel non-transgenic strategy.

S14P34

Performance of Maltaise demi-sanguine sweet orange on eight rootstocks inoculated with *Citrus exocortis viroid* (CEVd) and *Cachexia viroid* (CVIIb)

Najar A.[1], Homri N.[2], Bouhlel R.[3], Ben Mimoun M.[2], and Duran-Vila N.[4]

[1]Institut National de la Recherche Agronomique de Tunisie (INRAT), Plant protection, Tunisia; [2]Institut National Agronomique de Tunisie (INAT), Agronomie et Biotechnologie végétales, Tunisia; [3]INRAT, Horticulture, Tunisia; and [4]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal and Biotecnología, Moncada, Valencia, Spain. asmanajara@yahoo.fr

The performance of sweet orange "Maltaise demi-sanguine" infected with two isolates of *Citrus exocortis viroid* and with a cachexia inducing variant of *Hop stunt viroid* (citrus variant CVd-*IIb*) was evaluated on eight different rootstocks (sour orange, Carrizo citrange, *Citrus volkameriana*, Cleopatra mandarin, Swingle citrumelo 4447, Rangpur lime, *C. macrophylla* and *Poncirus trifoliata*) growing in an experimental field at INRAT station in El Gobba, Cap Bon region. The trees were planted in 2005 and yield and fruit quality and size were evaluated every year since 2007. The results obtained showed that HSVd (CVIIb) caused a significant reduction of canopy volume and fruit yield by 20 and 30 %, respectively, of trees grafted on *C. macrophylla* in comparison to non-inoculated trees. In addition, fruit quality was deteriorated. With citrumelo and *C. volkameriana*, canopy volume was decreased, while with sour orange and Cleopatra mandarin, tree volume was similar to non–inoculated trees. Similar yields were recorded for Maltaise demi-sanguine orange trees

grafted on *C. volkameriana*, sour orange and Cleopatra mandarin for the CVIIb inoculated trees and the non-inoculated control. Yield of the Maltaise plants on citrumelo and Carrizo citrange infected with this viroid, was slightly lower without affecting the quality. Concerning Exocortis, data showed that with *Poncirus trifoliata* and Rangpur lime, CEVd infection affected tree height by 25% and 20%, respectively, compared to non-inoculated controls and reduced yield. For Maltaise grafted on Carrizo citrange and citrumelo infected with CEVd, yield was slightly decreased in comparison to control trees, but fruit quality was conserved.

S14P35

Biological and molecular detection of *Citrus psorosis virus* in the Northwest region of Morocco

Achachi A.A., and Ibriz M.

University Ibn Tofail, Faculty of Sciences, Kenitra (FSK), Department of Biology, Genetics and Biometry Laboratory, Morocco.
asmaeachachi@yahoo.fr

Psorosis is a very well known citrus disease causing important damage in many countries, particularly in Mediterranean countries. Based on reports of symptoms such as bark scaling on trunks and branches observed on sweet orange, mandarin and grapefruit trees, this disease is widely present in Morocco. To determine the presence of *Citrus psorosis virus* (CPsV), RT-PCR was performed on 24 samples from trees with psorosis symptoms from the Gharb, the Northwest region in Morocco. To assess CPsV association with psorosis disease in these uncharacterized field sources, samples were examined by biological indexing. Detection of CPsV by RT-PCR did not always correlate with observation of psorosis-like symptoms in field trees or in graft inoculated indicator plants, indicating that psorosis-like symptoms may be also induced by pathogens other than CPsV. These results show for the first time in Morocco that molecular tests reliably reflect Psorosis infection.

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FUNGAL DISEASES

S15O01

The arrival of Citrus Black Spot (*Guignardia citricarpa*) in Florida and current research questions

Dewdney M.M.[1], Peres N.A.[2], Schubert T.S.[3], Mondal S.N.[1], Hu J.[1], and Hincapie Caputo M.[2]

[1]Citrus Research and Education Center University of Florida (CREC UF), Plant Pathology, USA; [2]Gulf Coast Research and Education Center University of Florida (GCREC UF), Plant Pathology, USA; and [3]Department of Plant Industries, Florida Department of Agriculture and Consumer Services (DPI FDACS), Plant Pathology, USA. mmdewdney@ufl.edu

In March 2010, the Florida Citrus Health Response Program discovered Citrus Black Spot (CBS) in juice blocks of 'Valencia' sweet orange in southwest Florida. In the last two years, the area where the disease has been found expanded from 14 km2 to 57 km2. In most of the affected blocks, disease incidence has been low, with one to a few symptomatic trees. In blocks where a few trees were symptomatic, most growers chose to remove trees but wide scale eradication was not a reasonable option. A successful eradication program would require the removal of all infected trees, yet the CBS life cycle, as presently understood, offers no protocol to ensure success. Upon discovery, quarantine measures were immediately applied to reduce further spread including covering fruit loads, safe debris disposal, fruit surface decontamination and fruit movement restrictions. Fungicide programs of copper and strobilurins were promptly initiated in affected groves. To enable better disease management in the humid subtropical climate of Florida, several research questions are being explored: 1) if methods of enhanced leaf litter decomposition reduce the number of *Guignardia citricarpa* pseudothecia in leaf litter; 2) the optimal wetting and temperature regimens to mature pseudothecia; and 3) the baseline sensitivity of *G. citricarpa* isolates to strobilurin fungicides for resistance management. Highlights from these projects will be presented.

S15O02

Predictive model for ascospore release of *Guignardia citricarpa* using climatological data

Dummel D.M.[1], Agostini J.P.[1], and Moschini R.[2]

[1]Instituto Nacional de Tecnología Agropecuaria (INTA), Plant Pathology. EEA Montecarlo, Misiones. Argentina; and [2]Instituto Nacional de Tecnología Agropecuaria (INTA), Instituto de Clima y Suelo, Castelar. Argentina. jpagostini@montecarlo.inta.gov.ar

Citrus Black Spot, caused by *Guignardia citricarpa,* is a cosmetic disease that affects fruit appearance for export. A predictive model for ascospore release was developed using climatological data. Ascospore release was monitored in a 'Valencia' sweet orange grove in Misiones, Argentina on a weekly basis using a Burkard spore trap while climatological data was obtained from a Davis GroWeather station during the 2008/09 season. The daily variables considered in the model were maximum and minimum temperature (°C), rain (mm), and days with more than 10 hours of leaf wetness (DMojt). Cumulative data were calculated for the 7 days prior to the weekly ascospore count for 28 weeks and categorized as high, medium or low. Using stepwise regression of SAS, two models were defined for ascospore release. The first model had only one variable (LogitPrS= – 4,553 1,6015* DMojt) with a correlation coefficient of 67.9% whereas, the second model had one more variable included, viz. the number of days with temperatures between 20 and 29° C (DT) (LogitPrS= –6, 1318 1,8108*DMojt 3,4829*DT) which had a correlation coefficient of 82.1%.

S15O03

Assessment of retention and persistence of copper fungicides on sweet orange fruit and leaves using fluorometry and copper residue analyses

Schutte G.C.[1], Kotze C.[1], Van Zyl J.G.[2,3], and Fourie P.H.[2,3]

[1]Citrus Research International (CRI), Nelspruit, South Africa; [2]Department of Plant Pathology (US), Stellenbosch, South Africa; and [3]Citrus Research International (CRI), Stellenbosch, South Africa. ts@cri.co.za

High volumes of copper hydroxide, cuprous oxide and copper oxychloride were sprayed under natural conditions onto mature sweet orange trees to compare the retention on leaves and fruit over a period of 56 days by means of copper residue analyses and a spray deposition assessment protocol using fluorometry, photomacrography and digital image analyses. Rainfall and increase in fruit size were recorded to determine if it had an influence on weathering of copper residues. Applications with cuprous oxide retained significantly more copper residue and

fluorescent pigment in the first year, while copper hydroxide retained higher copper and pigment levels during the second year. Persistence of copper residues was similar and decreased at the same tempo during both seasons. The loss of copper residues was attributed to weathering, fruit growth and cumulative rainfall as these factors were inversely correlated with copper residue levels (Pearson's r = -0.840, -0.722 and -0.733 respectively). A 76% and 90% correlation was observed between the copper residue analyzed and the quantitative fluorescent pigment measurements on mature leaves and fruit, respectively; showing that fruit is more reliable for fluorometry analyses and that this technique proved to be an effective tool for spray deposition and persistence assessment of copper fungicides. All the copper formulations tested at these registered rates at 35-day spray intervals were effective in controlling Citrus Black Spot caused by *Guignardia citricarpa*.

S15O04

Phyllosticta species associated with citrus diseases in China

Wang X.H., Chen G.Q., Huang F., and Li H.Y.

Wang Xinghong (X.H.), the Institute of Biotechnology, Zhejiang University, China. rm12407@hotmail.com

A total of 496 *Phyllosticta* strains were obtained from mandarins (*Citrus reticulata*), pomeloes (*C. maxima*), sweet oranges (*C. sinensis*) and lemons (*C. limon*) in the main citrus producing regions across China, and 74 strains were selected for phylogenetic analysis. Analyses inferred from the sequences of internal transcribed spacer region (ITS1, 5.8S nrDNA and ITS2), partial translation elongation factor 1-alpha (*TEF1*) and partial actin gene (*ACT*), showed these representative *Phyllosticta* isolates clustered in four distinct clades corresponding to three known, and one undescribed species, which was named as *Phyllosticta citrichinaensis* in this paper. *P. citrichinaensis* was obtained from leaves and fruits of all four citrus species, causing minor damage with irregular spots or freckles. *P. citriasiana*, associated with Tan Spot of pomeloes, was isolated only from this host, but never from lemons, mandarins and sweet oranges. *P. citricarpa*, the causal agent of Citrus Black Spot and presently subjected to phytosanitary regulations in the EU and USA, was isolated from lemons, mandarins and sweet oranges, but never from pomeloes. The isolates of *P. citricarpa* clustered in two subclades, one from mandarins, the other from sweet oranges and lemons. *P. capitalensis* was isolated from all four citrus species as an endophyte, causing false melanose, or occurred together with *P. citricarpa* or *P. citriasiana*. Morphological, cultural and biochemical characters were consistent with the results of phylogenetic analysis. In addition, a specific primer pair Pca8/ITS4 was and selected, and its corresponding PCR procedure was developed for the detection of *P. citriasiana* in this study.

S15O05

Development of an agrotransformation gene-silencing-system for *Phyllosticta citricarpa* and its use in functional analysis of the pathogenic genes

Goulin E.H.[1], Petters D.A.L.[1], Figueiredo J.A.G.[1], Senkiv C.C.[1], Silva JR.G.J.[2], Kava-Cordeiro V.[1], Galli-Terasawa L.V.[1], Peña L.[3], and Glienke C.[1]

[1]Federal University of Paraná (UFPR), Genetics, Brazil; [2]Fundação de Amparo a Citricultura do Estado de São Paulo (Fundecitrus), Brazil; and [3]Instituto Valenciano de Investigaciones Agrarias (IVIA), Spain. eduardo.goulin@gmail.com

The genetics transformation by *Agrobacterium tumefaciens* is a widely tool to genetic modify of a lot of organisms, including phytopathogenic fungi. *Phyllosticta citricarpa* is a plant pathogenic fungus that causes the Citrus Black Spot (CBS) disease. This fungus was successfully agrotransformated by our group, giving its resistance to ammonium glifosinate and green fluorescence emission, due to *bar* and *gfp* genes insertion, respectively. The T-DNA insertion by *A. tumefaciens* can be site directed or not, and in our experiment it was random insertion. Thus, these inserts can produce mutants with diverse modifications, as phytopathogens with alterations in factors that influence the growth up, dispersion and disease development. After the *P. citricarpa* transformation procedure, the transformers were evaluated to their *in vitro* growth up, the *in vitro* pycnidia production and the CBS lesion formation capacity. This test was design using a new methodology of the CBS symptom induction in detached fruits. Different changes were finding when we compared the transformers with the wild type, as low rate of growth, low or any production of picnidia and different size rates of CBS. These alterations have being studying by Chromosome Walking approach, looking for silenced genes involved with pathogenic process of *P. citricarpa*.

S15O06

Epidemiology of Alternaria Brown Spot of mandarins under semi-arid conditions in Spain

Bassimba D.D.M., Mira J.L., and Vicent A.

Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain. dissolu15@hotmail.com

Alternaria Brown Spot (ABS) of mandarins, caused by *Alternaria alternata*, is a serious disease both in humid and semi-arid citrus-growing regions. The pathogen affects leaves and fruit of susceptible cultivars such as 'Fortune', 'Nova', 'Minneola', and 'Murcott'. The epidemiology of ABS was studied mainly in humid areas in Florida but, due to climatic differences, this information cannot be extrapolated to semi-arid regions like Spain. Fields studies were conducted in 2011 and 2012 in the experimental orchards at IVIA, Valencia. Airborne dynamics of *Alternaria* conidia were monitored weekly by a spore trap, and the percentage of pathogenic isolates was determined periodically using the selective medium ARSA and pathogenicity tests. The presence of inoculum on affected leaves, shoots, leaf litter, and weeds was also determined. Infection periods were monitored weekly by exposing trap plants of 'Fortune' and 'Nova', and environmental variables were recorded by an automated meteorological station. *Alternaria* conidia were detected through the period of study, but only 5% of the isolates were pathogenic. Although affected leaves and shoots were the main source of inoculum, the survival of the fungus in the leaf litter was higher than previously reported in Florida. Pathogenic isolates were detected also in weeds, but only at very low levels. Infections in trap plants occurred mainly May-June and September-October. A significant positive correlation was detected between disease incidence and rains. Funding: INIA RTA2010-00105-00-00-FEDER.

S15O07

Spray deposition benchmarks for control of Alternaria Brown Spot and evaluation of adjuvants to improve fungicide spray deposition in citrus orchards

van Zyl J.G.[1], Schutte G.C.[2], and Fourie P.H.[1]

[1]Citrus Research International, Department of Plant Pathology, Stellenbosch University (CRI/USPP), South Africa; and [2]Citrus Research International (CRI), South Africa. gideonvzyl@sun.ac.za

Lack of citrus fruit and foliar disease control is regularly attributed to poor fungicide spray deposition. A deposition assessment protocol using fluorometry, photomacrography and digital image analysis was developed to study improvement of spray application which proved to be very accurate in determining deposition parameters on spray targets. To determine deposition benchmarks, young 'Nova' mandarin leaves were sprayed with copper oxychloride and SARDI Yellow Fluorescent Pigment at different concentrations and spray deposition assessed. Leaves were spray inoculated with *Alternaria alternata* [Alternaria Brown Spot (ABS) of mandarins] and symptoms rated. A strong linear relation was found between treatment concentration, leaf area covered by fluorescent pigment particles (%FPC) and Cu residue analysis. ABS control was modelled on %FPC and benchmarks for 50% and 75% control were calculated. Selected adjuvants were evaluated in laboratory spray- and biological efficacy trials. Treatments varied significantly in deposition quantity and ABS control, but these parameters were poorly correlated, also with control levels predicted from the %FPC benchmark model. Deposition quantity and Cu-residues could only partially explain the level of control achieved following the addition of adjuvants. These anomalous results could be attributed to the effects of adjuvants on deposition quality, on pathogen development and synergistic effects between adjuvant and fungicide.

S15O08

Chemical control of *Colletotrichum acutatum* and *C. gloeosporioides*, causal agents of Citrus Postbloom Fruit Drop in Brazil

Goes A.[1], and Rinaldo D.[2]

[1]Universidade Estadual Paulista (UNESP), Phytopathology, Brazil; and [2]Universidade Estadual Paulista (UNESP), Phytopathology, Brazil. adggoes@yahoo.com.br

Postbloom Fruit Drop (PFD) in Brazil is caused by *Colletotrichum acutatum* and *C. gloeosporioides*. PFD is one of the most important fungal diseases of sweet orange in tropical and subtropical regions of the world.

It produces orange-brown lesions on petals, inducing the abscission of young fruitlets and the retention of the calyces. This study was undertaken to determine the effect of the following treatments (g or mL/hL): T1- nontreated control, T2- quaternary ammonia (QA) 0.5; T3- QA 0.25; T4- famoxadone/mancozeb (FA MA) (0.3125 31.25); T5- (FA MA) QA (0.3125 31.25) 0.5; T6- carbendazim (CA) 2.5; T7- CA QA 2.5 0.25; T8- CA QA 2.5 0.5; T9- fenilpiridinilamina (FE) 0.0125; T10- FE QA 0.0125 0.5, T11- trifloxystrobin/tebuconazole (TR TE) (0.2 0.4); T12- (0.2 0.4) 0.5; T13- conventional farm (CF) difenoconazole 0.3125 at first application plus three additional applications of CA at 2.5; T14- CF QA 0.5. All treatments consisted of four applications of fungicides at the appropriated flowering stages which were begun at pinhead stage. Intervals of applications were 7-13 days. The assessments were made by measuring the amount of flowers with symptoms, effective average number of fruits and number of fruit at harvest. The treatments T-11 and T-12 proved to be the most effective, reducing the abscission of young fruitlets and increasing the retention of the bottom and, consequently improving fruit set (P≤0.05). QA was effective only it was applied plus TR TE.

S15O09

Pathogenicity and genetic relationship of strains of *Elsinoë australis* causing Citrus Scab disease

Hyun J.W.[1], Yi P.H.[1], Yun S.H.[1], Hwang R.Y.[1], and Levy L.[2]

[1]Citrus Research Station, National Institute of Horticultural & Herbal Science, RDA, Jeju, 697-943 (NIHHS, RDA), S. Korea; and [2]National Plant Germplasm and Biotechnology Laboratory, USDA-APHIS-PPQ-CPHST, Beltsville, MD20705, USA. hyunjaewook@korea.kr

Two Scab diseases are recognized currently on citrus: Citrus Scab, caused by *Elsinoë fawcettii*, and Sweet Orange Scab, caused by *E*. *australis*. Six pathotypes of *E*. *fawcettii* and two of *E*. *australis* have been described to date based on host range. The pathogenicity and genetic relationships among 15 isolates of *E*. *australis* from Korea, USA, Argentina and Brazil were investigated in this study. Based on pathogenicity tests on seven differential hosts, all isolates did not induce any symptoms on leaves of the seven differential hosts. In assay on fruit, the isolate Na-1 and KNa-2 induced the symptoms on natsudaidai but not on satsuma mandarin fruits at all. The isolate Ea-2 induced symptoms on sweet orange fruit. By sequence analysis of *Eaut-1*, *2*, *3*, *4*, *EaNat-1* and *2* gene, the isolates were divided into three subgroups, natsudaidai pathotype group, south American isolates group and USA isolates group, and the natsudaidai pathotype isolates and isolates from USA were more closely related. We think that there is possibility that *E. australis* isolates were differentiated to some pathotypes or genotypes in this study.

S15O10

Association and interaction of edaphic factors with root disease related citrus decline

Pretorius M.C.[1], Labuschagne N.[2], Kotze C.[1], and McLeod A.[3]

[1]Citrus Research International (CRI), Nelspruit, South Africa; [2]University of Pretoria (UP), Pretoria, South Africa; and [3]University of Stellenbosch (US), Stellenbosch, South Africa. mc@cri.co.za

Symptoms associated with root disease related citrus decline include sparse foliage, twig die-back and reduced growth, yield and fruit size as well as root rot. The causal factors involved in root disease related decline have not yet been fully elucidated. Invariably tree decline is noticed too late for the implementation of preventative management strategies. The aims of the study are to elucidate edaphic factors associated with citrus decline, so that early-diagnostic tools can be developed. Two declining orchards were selected for the study. Based on visual decline ratings of tree canopies, trees from three to four decline categories were selected. For each tree category several parameters were measured including yield, soil and leaf characteristics, soilborne pathogens and root disease associated symptoms. Multivariate analyses indicated that the two orchards were distinct. Subsequent analyses were therefore conducted separately for the two orchards. Distinct groups were identified for each tree category, indicating that most trees were visually correctly selected. Principal component analyses of the four groups of data revealed that the strongest structure existed for yield data, followed by soil factors, disease associated factors, and leaf parameters describing 81%, 61%, 49% and 39% of the variability respectively. Continued multivariate analyses will allow identification of the progressive changes in soil environmental interactions that lead to tree decline.

S15O11

Searching for citrus rootstocks resistant to Mal Secco disease: a review

Nigro F., Ippolito A., and Salerno M.G.

Department of Soil, Plant and Food Science, University of Bari Aldo Moro, Italy. antonio.ippolito@uniba.it

Mal Secco Disease (MSD) is a severe tracheomycotic disease of citrus caused by the mitosporic fungus *Phoma tracheiphila*. Besides lemon, the most damaged species, MSD also affects other citrus species, such as cedar, lime, bergamot, chinotto, sour orange, rough lemon, Volkamer lemon, etc. When the infection is through a rootlet of a susceptible species, more frequently in the nursery but also in bearing plants in the grove, the pathogen may remain segregated for many years in the inner layers of wood, leading to the particular form of the disease known as 'Mal Nero'; this syndrome is characterized by blackish discoloration of the hard wood and by the absence of almost any external symptom. Eventually, after several years, the pathogen reaches the sapwood, causing a sudden collapse of the canopy, also on resistant scions. Host resistance remains the most desirable goal among the measures to control MSD, and the search for new and resistant rootstocks, to replace the susceptible ones, has long been pursued by researches in Italy. This paper reviews the conclusive data collected over almost three decades of researches, conducted both in the field and in controlled environment, on the behaviour of putative lemon rootstocks towards MSD. The tested rootstocks include: alemow, sour orange (S. Marina and undetermined selections), 'Ichang' lemon, 'Yuzu' orange, 'Nansho Daidai' sour orange, 'Siamelo', Citrange 'Yuma', citrumelos ('Sacaton', 'Swingle 4475', and 'Swingle FF9'), and the hybrids 'Cleopatra' x *Poncirus*, and *Poncirus* 'Christian' x 'Cleopatra'.

S15P01

Endophytic actinomycetes for the biological control of *Phyllosticta citricarpa*

Glienke C.[1], Savi D.C.[1], Goulin E.H.[1], Kava-Cordeiro V.[1], and Silva Jr G.J.[2]

[1]Federal University of Parana (UFPR), Genetics, Brazil; and [2]Fundecitrus Fundo Paulista de Apoio a Citricultura (FUNDECITRUS), Cientifico, Brazil. ch.glienke@gmail.com

The Citrus Black Spot (CBS) disease has caused innumerous losses in citrus producing regions in the world, including Brazil, Australia and South Africa. This disease is caused by the fungus *Phyllosticta citricarpa*. Actinomycetes are widely known due to its enormous capacity to produce secondary metabolites, the pharmaceutical market by providing the most varied treatment options. Looking for biological control of *P. citricarpa*, the present work aimed the isolation of endophytic actinomycetes of the medicinal plant *Vochysia divergens*, and the bioprospecting of its secondary metabolites. The endophytes were tested against the fungus *P. citricarpa in vitro* and *in vivo* using detached orange fruits. The endophytic isolate LGMB259 presented better inhibitory activity *in vitro* and it was confronted with the fungus *P. citricarpa* in fruit tests. The metabolites of other five isolates inhibited totally the emergence of the CBS disease symptoms in tests *in vivo.* To determine the identity of these actinomycetes, the isolates were subjected to DNA sequence analysis of the rDNA 16S gene. Ten isolates were classified as *Microbispora* sp. and two isolates were classified as *Streptomyces sampsonii*. The other two isolates were identified as *Micromonospora* sp. and are apparently undescribed species. This is the first report of the actinomycetes isolation from this medicinal plant, and the first report of actinomycetes with activity against *P. citricarpa.* Financial Support: CNPq/MAPA, Fundecitrus, Fundação Araucária.

S15P02

Population genetics of *Guignardia citricarpa* in South Africa

Carstens E.[1], Linde C.C.[2], Slabber R.[3], Langenhoven S.[4], Schutte G.C.[5], Fourie P.H.[1], and McLeod A.[4]

[1]Stellenbosch University and Citrus Research International, Plant Pathology, South Africa; [2]Australian National University, Canberra ACT, Research School of Biology, Ecology, Evolution and Genetics, Australia; [3]Stellenbosch University, Central Analytical Facilities, South Africa; [4]Stellenbosch University, Plant Pathology, South Africa; and [5]Citrus Research International, South Africa. ec@cri.co.za

Citrus Black Spot (CBS), caused by *Guignardia citricarpa,* is an economically important pathogen of citrus in South Africa (SA). The pathogen produces two types of spores, waterborne conidia on fruit, twigs and leaves on the tree, and windborne ascospores from asci in leaf litter. In SA and Australia, epidemiological studies showed

that only ascospores are responsible for epidemics but in Brazil conidia can also contribute to epidemics. No population genetic approach has been used to study the contribution of ascospores and conidia to epidemics. This study aims to investigate population differentiation of *G. citricarpa* populations in production regions, the relative contribution of sexual and asexual inoculum to epidemics, survival of genotypes between seasons and spatial distribution of genotypes. Since no molecular markers are available for this study, microsatellite markers were developed using next generation sequencing techniques of a SA isolate. A total of 15 polymorphic markers were developed which will be used to genotype populations from four production regions for investigating population differentiation and founder effects. The contribution of sexual and asexual spores and spatial distribution will be studied by genotyping populations from three different orchards during three sampling periods in two consecutive seasons. Altogether this information will indicate whether CBS in SA is a monocyclic disease that can be controlled through destruction of leaf litter containing sexual spores.

S15P03

First report of false melanose symptoms of Citrus Black Spot on sweet orange leaves in Brazil

Silva-Junior G.J., Pereira R.G., Marin D.R., Wulff N.A., Scapin M.S., and Sala I.

Fundo de Defesa da Citricultura (Fundecitrus), Scientific Department, Brazil. geraldo@fundecitrus.com.br

Citrus Black Spot (CBS), caused by *Guignardia citricarpa*, is one of the most serious diseases of citrus around the world. In São Paulo State (SPS), Brazil, CBS was first reported in 1993. Six symptom types have been frequently associated with the CBS on fruits (hard spot, false melanose, lacy spot, freckle spot, cracked spot and virulent spot). On leaves, hard spot have been observed on lemon (*Citrus limon*) and rarely on sweet orange (*C. sinensis*). Recently, we observed false melanose like symptoms on 'Valencia' sweet orange leaves in groves with high CBS incidence in SPS. Initially, the symptoms on leaves, characterized by small and smooth black spot lesions lacking pycnidia, were misidentified as Greasy Spot or Melanose. For the correct diagnosis, lesions were cut from sweet orange symptomatic leaves, disinfested with 1% sodium hypochlorite and transferred to potato–dextrose–agar. After 14 to 21 days, *G. citricarpa* typical colonies were observed on the medium. Positive PCR results for *G. citricarpa* were obtained with both lesions and isolates. This is the first report of CBS false melanose symptoms on citrus sweet orange leaves.

S15P04

Biocontrol of Citrus Black Spot disease: perspectives using fungal endophytes of citrus

Jung L.F., Goulin E.H., Savi D.C., Schuh R., Galli-Terasawa L.V., Glienke C., and Kava-Cordeiro V.

Universidade Federal do Paraná (UFPR), Genética, Brasil. vanessagenetica@gmail.com

Among the diseases affecting citrus production, there is Citrus Black Spot (CBS). This disease is caused by the fungus *Phyllosticta citricarpa* and its control is based on the use of fungicides. Although there is no damage in the internal contents, the pathogen induces lesions on the fruit skin, which lead to refusal to trade *in natura*, and in severe cases promotes premature fruit drop, reducing yield. Phytosanitary restrictions imposed by importing countries lead to the rejection of entire loads of fruit with symptoms, causing great losses to some producing countries. Due to environmental and health concerns, alternatives for the control of this disease must be found. The use of microorganisms for the biological control and biopanning of natural products appears as a promising alternative. Endophytic microorganisms have been highlighted by the growing number of new bioactive compounds. Considering these aspects, hundreds of citrus endophytic fungi were isolated from leaves of sweet orange, in different culture conditions. A total of 67 morphotypes were grouped and investigated for their potential antagonistic effect to the fungus *P. citricarpa*. Among them, six showed to be promising in paired culture test. In tests of volatile metabolites, one isolate showed the best results. This fungus was able to cause inhibition in colony diameter of *P. citricarpa* by 60%, and the volatiles of this fungus inhibited by 100% the formation of pycnidia of the pathogen in citrus leaves assays. In tests with detached sweet orange fruit inoculated with *P. citricarpa*, there was 26% decrease of mean diameter lesions of CBS. This result is of great importance, since the exposure of postharvest fruits to volatile compounds is a process that can be performed, particularly in fruit intended for export. This is the first report of an endophytic fungal volatile compound with antagonist effect against *P. citricarpa*.

S15P05

Modelling of *Guignardia* pseudothecium maturation and ascospore dispersal in citrus orchards

Fourie P.H.[1], Schutte G.C.[1], Serfontein S.[2], and Swart S.H.[2]

[1]Citrus Research International (CRI), Disease Management, South Africa; and [2]QMS-Agriscience (Letsitele), South Africa. phf@cri.co.za

Ascospores are considered the most important inoculum source of Citrus Black Spot (CBS), caused by *Guignardia citricarpa*, but pseudothecium maturation and ascospore dispersal are inadequately studied. *Guignardia* ascospore trapping and concomitant weather data were obtained for three localities for three seasons (July through March from 2006 to 2009) in the Limpopo province of South Africa. Degree-days accumulated until first seasonal ascospore discharge (>10°C with 1 July as biofix; DDtemp), and DDtemp accumulated on rainy (rainfall > 0.1 mm; DDrain) and moist days (vapour pressure deficit < 5 hPa; DDvpd) were used in two Gompertz models to predict onset of ascospore dispersal: a temperature model [Event = exp(-exp(-(-2.725 0.004 × DDtemp)))] and a temperature/moisture model [Event = exp(-exp(-(-3.238 0.008 × DDvpd 0.004 × DDtemp -0.009 × DDrain)))] ($R2$ = 0.608 and 0.658, respectively). Both models predicted a delay in pseudothecium maturation in climates with colder winters and springs, while the temperature/moisture model predicted a further delay in drier seasons or climates. A Gompertz equation was also used to predict the proportion of *Guignardia* ascospores trapped (PAT) per season from DDtemp data accumulated on wet or moist days from the first seasonal ascospore discharge [PAT = exp(-4.096 × exp(-0.005 × DDwet2); $R2$ = 0.908]. These models can be used to predict the onset and dynamics of ascospore dispersal in climatically diverse regions.

S15P06

Infectious period of Citrus Black Spot on sweet orange

Lourenço S.A.[1], Gasparoto M.C.G.[1], Spósito M.B.[1], Amorim L.[1], and Gottwald T.R.[2]

[1]Escola Superior de Agricultura Luiz de Queiroz (ESALQ), Fitopatologia e Nematologia, Brazil; and [2]Agricultural Research Service (ARS), United States Department of Agriculture, USA. salouren@hotmail.com

Citrus Black Spot (CBS) is an important fungal disease, caused by *Guignardia citricarpa.* The risk of introduction and spread of CBS to new unaffected citrus-producing areas is a major concern for those orchards attempting to remain free of the disease. It has never been demonstrated that *G. citricarpa*-infected fruit have established infections in commercial diseased-free areas. The period of production of spores in fruit lesions is an important variable in a model for risk assessment. The objective of this study was to evaluate the infectious period of CBS on sweet oranges. Twenty infected oranges treated with carnauba wax with hard spot symptoms of CBS were kept in a humid chamber until freckle spot symptoms developed. When the first pycnidium formed, 50 µl drop of sterile water was placed on one freckle spot lesion per fruit. After four hours, the water drop was transferred to 250 µl of sterile water with Tween-20 (1%). Every 48 hours over 40 days, the number of pycnidia and the number of conidia formed in each lesion were counted. The experiment was repeated twice. The first pycnidia were observed 2-7 days after the appearance of typical freckle spots. The number of pycnidia increased until 30-40 days, and ranged from 1 to 31 per lesion. Conidia liberation started at 2-12 days and stopped 16-38 days after pycnidia formation. The number of conidia ranged from 0 to 109,875 per lesion. The average infectious period was 15 days and ranged from 0 to 26 days.

S15P07

Characterization of *Guignardia* spp. from citrus fruit in Argentina

Kornowski M.V., and Agostini J.P.

Instituto Nacional Tecnologia Agropecuaria (INTA), Plant Pathology E.E.A. Montecarlo, Argentina. jpagostini@montecarlo.inta.gov.ar

Citrus Black Spot (CBS) is a fruit disease caused by *Guignardia citricarpa* while *G. mangiferae* is a non-pathogenic species that also infects citrus fruit. Four kinds of fruit symptoms are associated with this disease, viz. hard spot, freckle spot, false melanose, and virulent spot. Both species can be characterized by either molecular means or growth on culture media. For molecular characterization with PCR, 25 isolates from

the northwestern and 64 from the northeastern citrus regions of Argentina were analyzed with the primers NP-Br-ITS-Gc and NP-Br-ITS-Gm specific for each *Guignardia* spp. From these orchards, *G. mangiferae* was recovered from 12.5% of the samples as well as from all kinds of symptoms (except virulent spot) as well as 12.5% from hard spot, 37.5% from freckle spot, and 25% from false melanose as well as 25% from a new lesion type, the so-called 'moteado' isolated from lemon fruit. CBS morphology was characterized on oat meal agar for over 257 isolates made from citrus groves of the northeastern parts of Argentina with five kinds of symptoms classified as hard spot (45.5%), freckle spot (35.0%), false melanose (3.5%), virulent spot (7.4%), and 'moteado' (8.6%). Of the isolates collected, 83.6% were *G. citricarpa* that developed characteristic yellow halos on oat meal agar, whereas the rest were of the non-pathogenic species made from hard spot lesions. Both fungal species can therefore be recovered from the same kind of fruit symptoms.

S15P08

Integrated approaches to minimize Alternaria Brown Spot of citrus in Italy

Bella P.[1], Russo M.[2], Tomasello M.[2], Catara A.[2], Catara V.[1], and La Rosa R.[1]

[1]Dipartimento di Scienze delle Produzioni Agrarie e Alimentari (DISPA), Italy; and [2]Parco Scientifico e Tecnologico della Sicilia, Italy. larosar@unict.it

Alternaria Brown Spot of citrus by the fungus *Alternaria alternata* causes considerable damage on hybrids of 'Dancy' mandarin, such as brown to black spots on leaves, fruit and young twigs, resulting in low fruit yield and poor quality. After the first detection in Italy in 2000, the disease has been reported on different mandarin hybrids as 'Nova' and 'Winola' and under specific conditions on sweet orange as 'Valencia'. Epidemiological observations carried out since 2003 in a grove of 'Fortune' mandarin grafted onto Citrange 'Troyer' showed that the disease development is related to rainfall, temperature, and inoculum density. Flower and leaf infections, occurring before fruit set and development, directly affected fruit yield and quality. Infections on leaves and twigs in September/October increased the fungal inoculum, leading to severe infections the following year. Cultural practices such as tree spacing, pruning and intercropping associated to spray programs helped in reducing disease severity on twigs and fruits and concentration of airborne conidia. The number of infected twigs and leaves was reduced up to 30% in wide tree spacing 'Fortune' plots and by 80% in young trees plots. Negative effects on disease pressure was observed on a 'Fortune' plot subjected to early pruning, where infected leaves and twigs increased up to 40% after heavy spring rains. Instead, lower values of disease index were recorded on shoots, leaves and fruits of 'Fortune' plants intercropped with other citrus species or hybrids.

S15P09

Varietal resistance and management of Alternaria Brown Spot in Brazil

Azevedo F.A., Pacheco C.A., Martelli I.B., and Polydoro D.A.

Centro de Citricultura Sylvio Moreira/Instituto Agronômico, Brazil. fernando@centrodecitricultura.br

Alternaria Brown Spot (ABS), caused by *Alternaria alternata*, is the major fungal disease of mandarins in the world. Therefore this study evaluated in Brazil: (i) resistance to ABS in different genotypes; (ii) use of pruning in winter season as way of control to ABS; and (iii) correlation between Citrus Leaf Miner (CLM) and ABS. The severity of the disease (ABS) in fruit of 31 genotypes was evaluated in the field. In parallel, *in vivo* and *in vitro* inoculations with *A. alternata* were performed on leaves. The effects of pruning were evaluated in a 'Murcott' tangor orchard, evaluating percentage of fruit damaged area and disease incidence. Finally, resistant and susceptible varieties of mandarin were evaluated in controlled experiments with CLM and ABS presence. Most of the genotypes were symptomatic in the field and after inoculation with *A. alternata*. The disease was most severe on 'Nova', 'África do Sul', 'Ponkan' and 'Murcott'. Cultivars like 'Cravo', 'Nules' and 'Ortanique' were tolerant, and 'Fremont' was highly resistant to the fungus. Winter pruning reduced the damage caused by ABS. Regarding the correlation between ABS and CLM, the results showed that susceptible varieties had greater damage when the insect was present, while resistant varieties did not lose their resistance characteristic after the CLM attack. Thus, we conclude from this study that there are varieties tolerant and resistant to ABS, and both winter pruning and CLM control are important in ABS management.

S15P10

Outbreak and occurrence of Alternaria Brown Spot in China

Huang F., and Li H.Y.

Institute of Biotechnology, Zhejiang University, China. rm12407@hotmail.com

Symptoms of Alternaria Brown Spot (ABS) caused by *Alternaria alternata* were first reported in Australia in 1903. The disease is currently widespread in America, Africa, and the Mediterranean Basin. ABS is a devastating disease, especially on juvenile tissues of tangerines and tangerine hybrids, and also slightly on grapefruits. Nearly one century after its first description in Australia, ABS is now reported in China. In 2007, the disease was observed in Chongqing, and it was further detected in Guangxi, Hunan, Zhejiang, Yunnan and Guangdong. Currently, ABS affects nearly half of citrus-growing regions in China and it is still spreading. We suggest that ABS might be a long existing disease in China, but it has been ignored because the pathogen affects mainly juvenile tissues, and its isolation is often masked by *Colletotrichum* species, so ABS might be easily confused with anthracnose associated to *C. gloeosporiodes*. Further studies are necessary to determine the prevalence of ABS in all of Chinese citrus-growing regions, and to establish appropriate disease control strategies combining fungicide sprays and cultural practices.

S15P11

Evolution of symptoms caused by *Alternaria alternata* on 'Nova' mandarin fruit and its schematic sequence

Burdyn L.[1], Garran S.M.[1], and Avanza M.M.[2]

[1]Instituto Nacional de Tecnología Agrícola (INTA), Concordia, Entre Ríos, Argentina; and [2]Catedra de Estadística, Facultad de Ciencias Agrarias, Universidad Nacional del Nordeste (FCA-UNNE), Corrientes, Argentina. lburdyn@correo.inta.gov.ar

In the Northeast of Entre Ríos, Argentina, detection of Alternaria Brown Spot (ABS) caused by *Alternaria alternata* is recent. It causes economic damage on 'Murcott' tangerine and 'Nova' mandarin plantations, both of high commercial value for export as well as for the domestic market. ABS causes necrosis and defoliation of shoots. Besides, it causes a variety of symptoms on fruits, affecting their quality and, while in early infections, causing premature fruit drop. In addition, ABS symptoms may also lead to confusion and misdiagnosis of quarantine diseases such as Citrus Canker, Citrus Black Spot and even Scab since some symptom stages of all these diseases are similar in appearance to ABS. In order to clarify the paths followed by ABS symptoms along fruit growth and ripening, they were monitored since their initial stages till complete maturity. An experiment was performed in a 'Nova' mandarin commercial plot with high incidence and severity of ABS and where a chemical control trial was being performed. Fifty fruitlets per experimental unit were labelled, identified, and tracked on monthly bases, counting the type and number of symptoms appearing on each of them. All types of ABS symptoms were classified using a qualitative scale of nine stages previously developed. This monitoring included statistical analysis of the frequency distributions of the different stages and the verification of the proposed sequences. Results helped to clarify ABS symptoms and to facilitate their differentiation from quarantine diseases with similar symptoms.

S15P12

Commercial-scale Alternaria Brown Spot resistance screening as the first step in breeding new mandarins for Australia

Miles A.K.[1], Newman T.K.[2], Gultzow D.L.[2], Parfitt S.C.[2], Drenth A.[1], and Smith M.W.[2]

[1]Ecosciences Precinct, Queensland Department of Agriculture, Fisheries and Forestry, Australia; and [2]Bundaberg Research Station, Queensland Department of Agriculture, Fisheries and Forestry, Australia. malcolmsmith1966@hotmail.com

Rapid-screening-tests and an appreciation of the simple genetic control of Alternaria Brown Spot susceptibility have existed for many years, and yet the application of this knowledge to commercial-scale breeding programs has been limited. Detached leaf assays were first demonstrated more than 40 years ago and reliable data suggesting a single gene determining susceptibility has been emerging for at least 20 years. However it is only recently that the requirement for genetic resistance in new hybrids has become a priority, following increased disease prevalence in Australian mandarin production areas previously considered too dry for the pathogen.

Almost all of the high-fruit-quality parents developed so far by our breeding program are susceptible to this disease necessitating the screening of their progeny. This is done by simply spraying 3-6 month old hybrid seedlings with a spore suspension derived from a toxin-producing field isolate, then incubating these seedlings in a cool room at 25°C and high humidity for five days. Susceptible seedlings show clear disease symptoms and are culled. After using this approach for only two seasons we now have more than 11,000 hybrids growing in field progeny blocks that are known to be genetically resistant to brown spot disease.

S15P13

Difference in susceptibility to Alternaria Brown Spot between young and mature 'Minneola' tangelo leaves

Liarzi O., Sela N., and Ezra D.

A.R.O., The Volcani Center, Bet Dagan, Department of Plant Pathology and weed research, Israel. dezra@volcani.agri.gov.il

Alternaria alternata, the cause of Alternaria Brown Spot, is an important disease of mandarins in Israel. This fungus causes symptoms on fruit, shoots and young leaves of susceptible cultivars. A specific toxin (ACT), produced by the fungus is well characterized but its specific target site in the host is still unknown. The fact that symptoms are visible only on young leaves, suggest that there may be a factor(s) differentially expressed in the young leaves, and absent from mature ones. Using the Suppression Subtractive Hybridization (SSH) method we compared and isolated cDNA molecules expressed only in young (96 sequences) or mature (110 sequences) leaves. All sequences were analyzed using Blast2GO software. Gene ontology of the sequences revealed differences between young and mature leaves. In order to find if these differences play a role in gaining susceptibility or resistance to *A. alternata* of young and mature leaves, respectively, qPCR analysis of selected genes in the presence and absence of the pathogen is in progress.

S15P14

Screening for Alternaria Brown Spot resistance in the triploid mandarin breeding programme in Spain

Bassimba D.D.M., Vicent A., Cuenca J., Aleza P., and Navarro L.

Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain. dissolu15@hotmail.com

About 1,200-2,000 seedless hybrids are obtained each year in the triploid mandarin breeding program in Spain. Some of the parents used, such as 'Fortune', 'Murcott' and 'Minneola', are susceptible to Alternaria Brown Spot (ABS) caused by *Alternaria alternata*. The susceptibility to ABS is partially transmitted to the progeny, so evaluation of genotype susceptibility was a critical target since the beginning of the breeding program. Traditionally, long-term field trials under endemic disease conditions were used to evaluate disease susceptibility. However, these experiments are costly, time-consuming, and difficult to apply with large numbers of genotypes. Therefore, two alternative methodologies were evaluated. Detached young leaves in humid chambers and potted plants with young shoots were inoculated by spraying a spore suspension. Different combinations of genotype, leaf age, spore concentration, temperature, and leaf wetness duration were evaluated and used as explanatory variables. The duration of the incubation period (i.e. from inoculation to symptom expression) and disease severity were used as response variables. The inoculation of detached leaves discriminated only between susceptible and resistant cultivars. The interaction between leaf age and incubation period duration in inoculated potted plants was correlated with the degree of susceptibility observed in the field. The implementation of these two methodologies facilitated the commercial release of new seedless triploid hybrids, such as 'Garbí' and 'Safor', resistant to ABS despite that 'Fortune' is one of the parents. Funding: INIA RTA2010-00105-00-00-FEDER.

S15P15

Enhancing of physiological parameters improves resistance against Alternaria Brown Spot of mandarins

Llorens E., Fernández-Crespo E., Camañes G., Lapeña L., and García-Agustín P.

Universitat Jaume I (UJI), Departamento de Ciencias Agrarias y del Medio Natural. ESTC, Spain. ellorens@uji.es

The appearance of Alternaria Brown Spot (ABS) in Spanish citrus regions forced the abandonment of the 'Fortune' mandarin production due to the difficulty of controlling this disease with fungicides. Environmental

concerns have prompted governments to promote alternatives to the application of synthetic chemicals to reduce risks and impacts of pesticide use. The development of novel resistance-inducing chemicals represents an alternative to protect crops against pathogens. Over the last years we have been working on strategies based on natural compounds and we have demonstrated that soil-drench treatment with hexanoic acid protect citrus against *Alternaria alternata*. In the present work, we have analyzed the effectiveness of hexanoic acid (Hx) in the control of ABS disease in 'Fortune' plants, and the physiological and metabolic changes associated with the Hx treatment. Preliminary results show an improvement of all the physiological parameters analyzed, including transpiration, photosynthesis or chlorophyll levels. Moreover, the metabolomics study of *A. alternata* infection and hexanoic acid treatment show strong changes in the metabolic profile depending on infection and treatment. In conclusion, these studies will shed light into the complexity underlying induced disease resistance. Hx-IR represents an attractive tool for the molecular characterization of the priming phenomenon and for the integrated pest management strategies with the advantage of being a natural compound.

S15P16

Strobilurin resistance of *Alternaria alternata*, the causal agent of Alternaria Brown Spot, in Florida tangerine hybrid groves

Vega B., and Dewdney M.M.

Citrus Research and Education Center, University of Florida (CREC, UF), Plant Pathology, USA. mmdewdney@ufl.edu

In Florida, strobilurin fungicides are commonly used for *Alternaria alternata* control but are becoming less effective. A statewide survey was initiated in 2010 to determine the sensitivity of *A. alternata* populations to azoxystrobin and pyraclostrobin covering 8 counties and 46 blocks. Azoxystrobin and pyraclostrobin sensitivity was determined via a resazurin-based microtiter assay. Resistance was widespread among counties and 69% of isolates were highly resistant with EC50 (effective concentration to inhibit 50% growth) values greater than 5 µg/ml for azoxystrobin and 1 µg/ml for pyraclostrobin, while the mean EC50 values for sensitive isolates were 0.1680 and 0.0216 µg/ml respectively. Cross-resistance was demonstrated by highly significant and strong correlations ($P < 0.0001$). Mixed populations of sensitive and resistant isolates were in 37% blocks and only resistant isolates in 50%. Disease severity was high in the cultivars 'Minneola' and 'Dancy' and low in 'Sunburst' and 'Orlando'. Our observations showed that strobilurin resistance occurred more frequently on susceptible cultivars with intense fungicide use ($P < 0.0001$). Partial sequencing of the cytochrome *b* gene was used to evaluate the resistant genotypes and the typical resistance inducing amino acid substitution of G143A was found. Two introns profiles were found in cytochrome *b* but did not change the isolate phenotype. Profile II was more frequently associated with resistant isolates and sensitive isolates were often profile I.

S15P17

Evaluation of fungicide mixtures as replacement for carbendazim for Citrus Postbloom Fruit Drop control in Brazil

Silva-Junior G.J.[1], Spósito M.B.[2], Marin D.R.[1], and Amorim L.[3]

[1]Fundo de Defesa da Citricultura (Fundecitrus), Scientific Department, Brazil; [2]Escola Superior de Agricultura Luiz de Queiroz (Esalq), Department of Crop Science, Brazil; and [3]Escola Superior de Agricultura Luiz de Queiroz (Esalq), Department of Plant Pathology and Nematology, Brazil. geraldo@fundecitrus.com.br

Control of Citrus Postbloom Fruit Drop (PFD), caused by *Colletotrichum* spp., is based mainly on fungicide sprays. Carbendazim was an important fungicide used for PFD control in Brazil, but recently, it was banned in export citrus production. Thus, the search for other fungicides to control of PFD became necessary. In this study, the effectiveness of carbendazim was compared to the mixtures trifloxystrobin/tebuconazole (Tri/Teb) and cyprodinil/fludioxonil (Cyp/Flu). Two experiments were carried out in 2009⁄2010 season in 18 and 20–yr–old 'Pera' sweet orange groves in Sao Paulo State, Brazil. Four sprays of carbendazim (1000g⁄ha), Tri/Teb (80 and 160g⁄ha) or Cyp/Flu (94 and 63g⁄ha) were performed at 7–day interval, starting at the green bud stage. Untreated control (UTC) trees received no fungicides. The incidence of symptomatic petals, number of persistent calyces and fruit set per branch, and yield per tree were assessed. In both experiments, only Tri/Teb differed significantly from the UTC trees for all variables studied. While trees sprayed with Tri/Teb yielded 88

and 41 kg, UTC trees produced 30 and 6 kg, in experiments 1 and 2, respectively. Fruit yield was intermediary in both experiments for carbendazim (61 and 23 kg) and Cyp/Flu (53 and 13 kg). Therefore, Tri/Teb mixture is a satisfactory, more efficient option for replacing carbendazim for control of PFD. Supported by FAPESP (08⁄54599–2 and 08⁄54176–4).

S15P18

Survival of *Colletotrichum acutatum* in citrus leaves

Pereira W.V.[1], Tanaka F.A.O.[1], Rodrigues M.B.C.[2], and Massola-Junior N.S.[1]

[1]University of São Paulo (USP), Plant Pathology and Nematology, Brazil; and [2]University of São Paulo (USP), Genetics, Brazil. wvpereira@hotmail.com

Citrus Postbloom Fruit Drop (PFD) disease caused by *Colletotrichum acutatum* became recently an important issue for citrus industry in Brazil. The determination of the source of inoculum and its survival period is essential to understand the epidemiology and to develop strategies for the management of this disease. The survival of *C. acutatum* on the surface of orange leaves was investigated. Leaves from 'Pera' sweet orange saplings were inoculated with 105 conidia/mL and kept in a moist chamber for 48 h. For greenhouse and field experiments, the saplings were inoculated with standard isolates. For growth chamber experiments (21°C and 27°C), it was used a *GFP* gene-labeled isolate. Samples of inoculated leaf areas were taken and evaluated monthly. This evaluation was made by the isolation of *C. acutatum* in a semi-selective medium and/or visualization by fluorescence in a confocal microscope. Under controlled conditions, the pathogen survived on the surface of the leaves for seven months at 21°C and for four months at 27°C. In the greenhouse, the survival was three months and two months in the field. Recognition of the isolated cultures was made by PCR. These data indicate that orange leaves can play an important role as source of inoculum for PFD.

S15P19

Pollen exudate stimulates conidial germination of *Colletotrichum acutatum*

Gasparoto M.C.G., Lourenço S.A., Marques J.P.R., Appezzato-da-Glória B., and Amorim L.

Escola Superior de Agricultura Luiz de Queiroz (ESALQ), Fitopatologia e Nematologia, Brazil. mcggaspa@yahoo.com

Postbloom Fruit Drop, caused by *Colletotrichum acutatum* and *C. gloeosporioides*, is an important citrus disease in the southwestern region of São Paulo State in Brazil. The high frequency of rain during the blooming period causes explosive epidemics. Citrus flower extract, resulting from the contact of rainwater with petals, stimulates the germination of appressoria and secondary conidia formation. The objective of this study was to evaluate the conidial germination of *C. acutatum* in the presence of pollen exudate. Anthers were removed from flowers of healthy citrus plants and transferred to 2 mL-sterile water. This mixture was agitated and the suspension was adjusted to 105 pollen/mL. Conidial suspension was adjusted to 105 spores/mL. Three 35 µL-aliquots of conidial suspension plus three 35 µL-aliquots of pollen suspension were placed on polystyrene Petri dishes, totaling 70 µL of a homogeneous suspension in each aliquot. The control was obtained with 70 µL-aliquot of spore suspension. Each dish was placed inside a gerbox containing a filter paper and 30 µL of sterile water. The gerboxes were incubated in growth chambers at 24°C, without light, for 2, 4, 6, 8, 10, 12, 24 hours. Spore germination was calculated observing 100 conidia per aliquot. Twenty-four hours after *in vitro* inoculation, conidial germination was 100% in the presence of pollen exudate and 8% in its absence.

S15P20

Fungal diseases of citrus in Panama

Aguilera-Cogley V.[1], and Vicent A.[2]

[1]Instituto de Investigaciones Agropecuarias de Panamá (IDIAP), Laboratorio de Protección Vegetal, Panamá; and [2]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain. avicent@ivia.es

The citrus-growing area in Panama has increased considerably in recent years. Disease problems are frequently observed in the orchards, but in most cases their etiology is unknown, complicating the design of effective

control strategies. A survey was initiated in 2010, visiting 50 fields in different regions in the country. In each orchard, symptoms were characterized and samples were collected from affected organs. Isolations from symptomatic tissues were made on general and selective media. Fungal isolates were transferred to PDA and V8 for morphological identification. In addition, molecular identification was carried out on representative isolates by amplifying and sequencing the ITS region. Necrotic pustules on the leaves, chlorosis, and premature defoliation were observed in 75% of the surveyed orchards. *Mycosphaerella citri*, the causal agent of Citrus Greasy Spot, was consistently isolated from the affected tissues. Lesions on petals and premature fruit drop were also observed in some sweet orange and lime orchards. *Colletotrichum acutatum* was consistently isolated from these lesions, and pathogenicity tests identified the isolates as the causal agent of Postbloom Fruit Drop. Finally, symptoms of Melanose on sweet orange leaves and fruit were associated with *Phomopsis* sp. In conclusion, the results indicated that Greasy Spot is the prevalent fungal disease of citrus in Panama.

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S15P21

Epidemiology and control of Citrus Greasy Spot in Panama

Aguilera-Cogley V.[1], and Vicent A.[2]

[1]Instituto de Investigaciones Agropecuarias de Panamá (IDIAP), Laboratorio de Protección Vegetal, Panamá; and [2]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain. avicent@ivia.es

Greasy Spot, caused by *Mycosphaerella citri*, is the main fungal disease of citrus in Panama. The disease induces leaf chlorosis, necrotic pustules, and premature defoliation, reducing fruit production and quality. Due to the lack of epidemiological information, strategies for greasy spot control in Panama are not satisfactory. In 2011, a study was set up in a commercial 'Valencia' sweet orange orchard in Cocle Province to characterize the main epidemiological traits of the disease. Airborne ascospore concentration was evaluated weekly using a spore trap. Inoculum dynamics in the leaf litter was assessed by means of a wind tunnel, and infection periods were determined by exposing trap plants. Environmental data were recorded with a meteorological station. In addition, two field trials were conducted to evaluate the efficacy of different spray schedules using the fungicide fenbuconazole. Most ascospores of *M. citri* were detected in the orchard air during March, April and May. Inoculum potential in the leaf litter and disease severity in trap plants was also high in spring. Rainfall was very high through the period of study. A significant negative correlation was detected between rainfall and inoculum potential, due to accelerated leaf litter decomposition under rainy conditions. Fenbuconazole applications significantly reduced disease incidence compared to the non-treated control. Funding: Programa de Formación de los INIA de Iberoamérica.

S15P22

Biological control of Citrus Canker and Melanose using rhizobacteria in Korea

Ko Y.J., Kang S.Y., and Jeun Y.C.

Jeju National University (JNU), Plant Resources & Environment, Republic of Korea. ycjeun@jejunu.ac.kr

Many rhizobacteria are known not only as resistance inducers against various plant diseases, but also as biological control agents in various plant diseases due to their antagonistic effects. In this study bacterial strains were isolated from the rhizosphere of annual plants growing on Jeju Island in Korea. Four bacterial isolates viz. MRL408-3, TRH423-3, TRH415-2, and THJ609-3 were selected from over 100 bacterial isolates using *in vitro* tests against *Xanthomonas citri* subsp. *citri* and *Diaporthe citri,* the cause of Citrus Canker and Melanose, respectively. They are the most important citrus diseases in Korea. Bio-tests on citrus branches showed that the disease severity of both pathogens was reduced by the bacterial isolates after inoculation. Isolates MRL408-3 and TRH423-3 were identified as *Burkholderia gladioli*, TRH 415-2 as *Pseudomonas fluorescens* and THJ609-3 as *P. pudia* by analysis of internal transcript spaces (ITS) in rDNA sequencing. These bacterial isolates may be useful for the control of Citrus Canker and Melanose in Korea on organic farms where the use of fungicides is not permitted.

S15P23

Expression profiles of differentially regulated genes of citrus during infection by *Elsinoë fawcettii*

Hyun J.W.[1], Yi P.H.[1], and Kim Y.J.[2]

[1]Citrus Research Station, National Institute of Horticultural & Herbal Science, R.D.A. Jeju, S. Korea; and [2]College of Life Sciences and Biotechnology, Korea University, S. Korea. hyunjaewook@korea.kr

Citrus Scab is a devastating disease of citrus in JeJu island of Republic of Korea. Citrus Scab is caused by the fungus *Elsinoë fawcettii* and impacts fruit quality and appearance. To identify citrus genes involved in the response to the citrus scab in satsuma mandarin (*Citrus unshiu*), expression profiles were investigated using an citrus oligo array representing 24,100 genes. Expression profiles were captured at four different time-points after inoculation at 12, 24, 48, and 72 h, together with those at 0 h (uninoculated control). A total of about 2,600 genes were found to be significantly modulated in response to at least one of the four time points. The categories included citrus genes associated with sugar metabolism, plant defense, phytohormone, and cell wall metabolism, as well as 10 other gene categories. Over the three time points after inoculation, 635 genes were commonly up-regulated and 374 genes were down-regulated. Both sets of genes were classified based on their functional categories. Those up- or down-regulated genes encode transcription factors, signaling components, defense-related genes, transporter, and metabolism, all of which have been associated with disease response in various plants, suggesting that similar response pathways are involved in Citrus Scab.

S15P24

Biological control of *Phytophthora* sp. and *Fusarium* sp. in citrus nurseries in Egypt

Ahmed Y.[1], Ippolito A.[2], El-Shimy H.[3], D'Onghia A.M.[4], and Yaseen T.[4]

[1]Department of Agri-Food and Environmental Systems Management (DiGeSA), Plant Pathology Section, University of Catania, Italy; [2]Dipartimento di Protezione delle Piante e Microbiologia Applicata, (DPPMA), Università degli Studi di Bari, Italy; [3]Plant Pathology Research Institute (PPATHRI), Agriculture Research Center, Egypt; and [4]Centre International de Hautes Etudes Agronomiques Méditerranéennes (CIHEAM), Mediterranean Agronomic Institute of Bari (MAIB), Italy. yosra242@yahoo.com

Phytophthora sp. and *Fusarium* sp. are the most important soil-borne pathogens of citrus in Egypt. Control methods based on the use of resistant rootstocks and good agricultural practices are not always satisfactory. Application of effective chemical treatments to citrus plants has reduced losses, but development of fungicide resistance is a legitimate concern. Since biological control is an interesting alternative, this study assessed the potential of two commercial biocontrol agents Clontori (*Trichoderma harzianum* and *Clonostachys rosea*) and BioArc (*Bacillus megaterium*) alone or in combination with an organic biofertilizer (Guianto) at 0.5 and 1%. The efficacy of this treatment was evaluated on citrus growth and inoculum density of *Phytophthora* sp. and *Fusarium* sp. Trials were carried out in two Egyptian nurseries on Volkameriana lemon and sour orange seedlings. Results showed that Clonotri and BioArc caused an increase in plant height and root dry weight, and were significantly effective in reducing *Phytophthora* propagules and *Fusarium* populations in the rhizosphere of treated seedlings as compared to the nontreated control. The data revealed that 1% Guanito increased significantly citrus plant vegetative parameters (plant height and root dry weight). The beneficial activities of both Clontori and BioArc were further increased in the presence of 1% Guanito rather than 0.5%.

S15P25

Dry Root Rot, an alliance between *Fusarium solani* and *Phytophthora* or other factors against citrus in California

Adesemoye A.O.[1], and Eskalen A.[2]

[1]Adekunle Ajasin University, Department of Microbiology, Akungba-Akoko, Ondo State, Nigeria; and [2]University of California, Riverside, Dept. of Plant Pathology and Microbiology, U.S.A. akif.eskalen@ucr.edu

Fusarium solani is a weak pathogen of citrus that causes Dry Root Rot (DRR) only when a tree is under stress. Some stressors that results in in DRR are from *Phytophthora* spp., *Citrus tristeza virus* (CTV), wounding by gophers and rodents or girdling practices. This study was conducted to understand the seasonal occurrence, survival, and distribution of *F. solani,* and *Phytophthora* spp. and other factors associated with DRR in California. During a survey in 2009-2010, infected trees were rated for disease severity using a rating scale of 1 to 5. Samples were collected in eight citrus growing counties - Fresno, Kern, Riverside, San Diego, San Luis Obispo,

Santa Barbara, Tulare, and Ventura. Small pieces of tissue from symptomatic root samples were plated onto potato dextrose agar amended with 0.01% tetracycline (PDA-Tet) to isolate fungi and PARPH medium to isolate *Phytophthora* spp. About 450 isolates were recovered from spring 2010 to winter 2011. Isolates were examined for their identification morphologically. Isolates were further identified using molecular methods using Internal Transcribed Spacer, Beta Tubulin, and Translation Elongation Factor. Pathogenecity tests were conducted with healthy citrus trees of the same variety from which initial isolations were made. The results showed that in addition to *Fusarium solani, P. citrophthora,* and *P. nicotianae,* two additional *Fusarium* species - *F. oxysporum* and *F. proliferatum* -are playing important roles in causing DDR disease of citrus. Currently, no correlation has been found between soil nutrient content and the disease. These findings are important for the current management of DRR and will help in designing integrated pest management study on DRR.

S15P26

Efficacy of water soluble silicon in managing Fusarium Dry Root Rot of citrus

Marais L.J.

Leffingwell Ag, Research & Development, USA. lmarais@leffingwellag.com

Dry Root Rot (DRR) of citrus caused by the soil-borne fungus *Fusarium solani* is regarded as a weak pathogen infecting trees under stresses caused by over irrigation and root damage caused by certain fertilizers. Substantial losses in tree mortality and production are caused by this disorder. There are currently no effective fungicidal treatments known for the control of this disease. Control or management is based on cultural practices which include efficient irrigation and fertilizer management. Soluble or aqueous silicon has long been recognized for its prophylactic role in alleviating plant diseases when absorbed by plants. The effect of soil and foliar applications of two aqueous silicon products (SilMatrix, PQ Corp and Carbon Defense, Floratine Biosciences, Inc.) on managing DRR in mature 'Valencia' orange on 'Troyer' Citrange rootstock was investigated. Applications were made to DRR affected trees at 6-8 weekly intervals over a period of three years. Tree condition was rated on a scale of 0 to 4 with 0 = healthy appearing trees and 4 = dead trees. Mortality ratings for untreated controls, SilMatrix and Carbon Defense treatments were 66%, 22% and 11% respectively. SilMatrix enhanced tree condition by 50% in trees rated 1.0 prior to treatment and Carbon Defense enhanced tree condition by 69%. The mean ratings of untreated trees, SilMatrix and Carbon Defense treated trees after three years were 3.5, 0.5 and 0.31 respectively. The preliminary results of this investigation indicate that aqueous silicon is a valuable tool in the integrated disease management of DRR in citrus.

S15P27

Application of artificial intelligence to the visual diagnosis of quarantine citrus diseases

Burdyn L.[1], Garran S.M.[1], Stegmayer G.[2], and Milone D.H.[3]

[1]Instituto Nacional de Tecnología Agrícola (INTA), Concordia, Argentina; [2]Centro de Investigación en Ingeniería en Sistemas de Información (CONICET), Santa Fe, Argentina; and [3]Research Center for Signals, Systems and Computational Intelligence, (FICH-UNL, CONICET), Santa Fe, Argentina. lburdyn@correo.inta.gov.ar

Canker, Scab and Black Spot are endemic diseases in the citrus region of the Uruguay River in Argentina. As being quarantine diseases for the European Union, the main export destination. While biochemical techniques have greatly improved the sensitivity, accuracy and speed of diagnosis, these are not yet readily available in places where diagnosis procedures are necessary. Visual diagnosis currently in use has the main constraint that accuracy is limited to the own experiences of persons in charge of performing it. The aim of this work has been to incorporate to visual diagnosis procedures new artificial intelligence tools based on pattern recognition. These techniques are being applied to the analysis of a database built with all the attributes found while defining the whole set of symptoms of these diseases. A communication platform already available (Frutic), would allow to any person in charge of the diagnostic procedure the query to the intelligent system and its corresponding response be occurring in real time. The database was constructed and continues to be updated by the ongoing contribution of symptom descriptions from different varieties of tangerines and oranges collected along this region by specialists throughout a campaign. Symptoms are visually recognized by using a magnifying glass 20x and described on their different attributes (size, color, embossing, texture, etc.). A first version of this system has shown to be effective in a first step of testing.

S15P28

Optimization of copper application schedules for foliar citrus disease management based on fruit growth, historical rainfall patterns and copper residue decay

Zortea T.[1], Fraisse C.W.[1], and Dewdney M.M.[2]

[1]University of Florida (UF), Agricultural & Biological Engineering, USA; and [2]Citrus Research and Education Center, University of Florida (CREC UF), Plant Pathology, USA. mmdewdney@ufl.edu

Copper applications are essential for foliar citrus disease management in Florida. For effective disease control, copper residues need to be maintained above 0.1 µg/cm2. Simulations show the standard timing of copper applications every 21 days is insufficient in wet years. To minimize copper coverage gaps for grove operations that plan a season's applications in advance, optimized copper application schedules were developed for early, average and late peak bloom. Fifty-five years of historical weather data from the National Weather Service Cooperative Observer Program in five counties (Highlands, Hendry, Indian River, Lake and Polk) were used. The copper residue decay for each year was calculated from equations for fruit growth, copper deposition and residue reduction in the citrus copper application scheduler, a web-based tool to predict residue decay (http://agroclimate.org/tools/cudecay/). To test schedules, intervals (days) between applications were varied ±2 days in a simulation and the best result was rerun until the results converged. Schedules were evaluated based on the sum of unprotected days over the 55 years and a percent reduction of days without coverage was calculated. For all locations combined there was a 51% reduction from 857 to 420 unprotected days for the average bloom and a 19% and 13% reduction for early and late blooms, respectively, with the optimized schedules. The greatest improvement occurred in Highlands County with a 57% reduction of unprotected days.

S15P29

Phytosanitary situation of Chilean citrus industry

Besoain X., Castro M., Camps R., and López E.

Pontificia Universidad Católica de Valparaíso (PUCV), Facultad de Agronomía, Chile. xbesoain@ucv.cl

Chilean citrus industry has experienced a strong growth towards fresh-fruit exports. This situation is partly caused by the Japanese market starting to import lemons and the American market mandarins and sweet oranges. These markets have been accesed due to Chile's competitive advantage since it has no pests and diseases of world impact such as Fruit Fly (*Ceratitis capitata*), Citrus Scab (*Elsinoë fawcettii*), Black Spot (*Guignardia citricarpa*), Citrus Canker (*Xanthomonas citri* subsp. *citri*), and Huanglongbing (*Candidatus* Liberibacter spp.). The main diseases affecting citrus fruit in Chilean farms are: Gummosis caused by *Phytophthora citrophthora* and *P. hibernalis*, Dry Root Rot caused by *Fusarium solani*, leaf and fruit spots caused by *Alternaria* species. In the post-harvest period, the diseases are: Gray Mold caused by *Botrytis cinerea* (also in pre-harvest), Green Mold and Blue Mold by *Penicillium* species, Sour Rot by *Geotrichum candidum* and Black Pit by *Pseudomonas syringae* pv. *syringae*. Regarding nematodes, *Tylenchulus semipenetrans* has caused considerable damages. Some of the virus diseases include Cachexia (*CCaVd*) affecting both lemon trees and mandarins and Tristeza, only stem pitting syndrome caused by severe isolates of *Citrus tristeza virus* (CTV) affecting grapefruit varieties located in the Pica oasis (Tarapaca Region). The main pests are the Chilean False Red Mite (*Brevipalpus chilensis*), Citrus Red Spider Mite (*Panonychus citri*), the Pseudococcidae with predominant species, Olive Black Scale (*Saissetia oleae*), Soft Scale (*Coccus hesperidum*) and Wooly Whitefly (*Aleurothrixus floccosus*).

S15P30

Storage technology for conservation of seeds of citrus rootstocks

Nascimento L.M., Moreira J., Brito M.C.R., Brito P.C.N., and Sanches J.M.

Centro de Citricultura Sylvio Moreira, Brazil. lenice@centrodecitricultura.br

This study evaluated Xtend packaging and several fungicide treatments to improve the conservation of seeds of 'Swingle' citrumelo and lemon 'Cravo'. Seeds of 'Swingle' citrumelo were harvested, washed, and divided

into two groups: one dried in the shade for four hours (77%) and the other dried with forced air for 72 hours (54 %). Seeds of lemon 'Cravo' were dried in the shade at room temperature for four hours (71%). Three fungicide pretreatments were evaluated: 1) fungicide dip (imazalil- 2mL/L) for two minutes; 2) fungicide spray (captan- 0.5g/L); 3) fungicide dip (imazalil- 1mL/L) for two minutes followed by fungicide spray (captan- 0.5g/L) after drying. Treated seeds were divided in lots of 200g, packed in common plastic bags or micro perforated Xtend polyethylene bags, and further stored in cold chamber at 5°C and 70% HR. Seed viability in each treatment was evaluated by periodic sowing for 10 months and the percentage of germinated seeds was calculated. Seed weight loss during storage was also evaluated. The packaging Xtend showed promising results for the storage of seeds of 'Swingle' citrumelo and lemon 'Cravo', compared with the package currently used in citrus nurseries. Seed longevity was improved, without reducing seed germination, and the appearance of mold fungi was prevented.

S15P31

Morphological and molecular diversity of *Phytophthora nicotianae* strains isolated from citrus plantations in Cuba

Llauger R., Coto O., Peña M., Zamora V., and Collazo C.
Instituto de Investigaciones en Fruticultura Tropical, Cuba. direccion@iift.cu

A collection of *Phytophthora* spp. isolates was built with strains collected from citrus plants showing symptoms of Gummosis. Sampled areas belong to two of the most important Cuban citrus enterprises (Jagüey Grande and Ceiba del Agua). Eight *P. nicotianae* strains isolated from citrus plants were identified considering sporangia length and width and its ratio. A *P. nicotianae* strain isolated from mango and a well characterized strain isolated from citrus plantation in Brazil were used for comparative purpose. Only two strains were identical in the cluster analysis done based on the sporangia traits measured and a morphological diversity was observed between Cuban *P. nicotianae* isolates obtained from citrus. Sporangia were variable in shape, mostly ovoid-ellipsoid, obpyriform, spherical and distorted. Pathogenicity assays showed the capacity of the strains to affect Mexican lime fruits and rootstocks plants under *in vitro* conditions and re-isolation of the pathogens were done from infected fruits. Molecular identification using primers pairs Pn5B/Pn6 specific to *P. nicotianae* confirmed the previous morphological identification. The sequences of amplified products obtained using ITS5/ITS4 primers pairs with DNA template of four of the strains were compared in the NCBI Genbank- using BLAST and confirmed the identification as *P. nicotianae* isolates and the diversity of the Cuban strains. Those sequences were included in the NCBI Genbank with the accession numbers: GUO73388, GUO73387, GUO73392 and GUO73389.

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ENTOMOLOGY AND PEST CONTROL

S16O01

The status of citrus IPM in California

Grafton-Cardwell E. E.

University of California Riverside (UCR), Entomology, USA. eegraftoncardwell@ucanr.edu

Citrus is grown in 4 distinct regions of California and climatic differences influence the citrus varieties grown and the pests and natural enemies that develop. The heaviest insecticide treatments occur in the San Joaquin Valley where extremes of heat and cold reduce the efficacy of natural enemies. In the San Joaquin Valley, citrus thrips *Scirtothrips citri* and California red scale *Aonidiella aurantii* have been primary pests for many decades. Forktailed bush katydid *Scudderia furcata* and citricola scale *Coccus pseudomagnoliarum* became primary pests when selective insecticides replaced organophosphate and carbamate insecticides. San Joaquin Valley growers maintain their integrated pest management (IPM) program by utilizing low rates of pyrethroids or organophosphate insecticides for katydids and applying organophosphates for citricola scale in alternate years – resulting in an average of 3-4 treatments for all pests per year. In 2008, the Asian citrus psyllid, *Diaphorina citri,* was discovered and in 2012 huanglongbing disease was found in California. While the majority of find sites of the psyllid have been in residential areas of the state, it is expected that the pest and disease will become established in commercial citrus in the near future. Because the disease is difficult to detect when trees first become infected and there is no cure for the disease, psyllid population reduction in combination with infected tree removal is currently the most effective strategy for preventing disease spread. Because the most effective treatments for Asian citrus psyllid are broad spectrum in nature, California citrus IPM will experience major disruptions. Regional differences in the risk of Asian citrus psyllid and huanglongbing establishment and the impact of treatments for the psyllid on the IPM program are discussed.

S16O02

The status of citrus IPM in South Africa

Grout T.G.

Citrus Research International (CRI), Nelspruit, South Africa. tg@cri.co.za

Four years ago citrus IPM in South Africa was largely defined by the use of biorational control strategies such as microbial control, sterile insect release, mating disruption, attract-and-kill and soil- and stem-applied systemics. This is still the case but pressure from export markets on quarantine pests and diseases has increased, together with further residue restrictions. Due to concerns about citrus black spot, *Guignardia citricarpa*, mancozeb applications have increased in the Eastern Cape province and are likely responsible for a decline in numbers of the phytoseiid mite *Euseius addoensis.* This has resulted in greater populations of citrus thrips, *Scirtothrips aurantii*, early in the season and led to the use of longer-residual thripicides such as chlorfenapyr. This product is harmful to both phytoseiids and hymenopterous parasitoids of quarantine pests such as certain mealybug species and false codling moth (FCM), *Thaumatotibia leucotreta*. Attempts to redress these imbalances are sometimes made by releasing the FCM parasitoid *Trichogrammatoidea cryptophlebiae* and *Coccidoxenoides perminutus* for mealybug, but the latter is not very effective against indigenous mealybug species. Although California red scale (CRS), *Aonidiella aurantii*, is not a quarantine pest for most export markets, it must be controlled preventively where biological control is compromised by disruptive thripicides. This requirement, coupled with the increased price of spray oil, has resulted in widespread use of generic imidacloprid SC formulations as soil drenches. Although spirotetramat was recently registered in South Africa for the control of CRS and it appears to be IPM-compatible, its cost is high relative to other scalicides. The only new citrus pest that has become established in the last four years is the woolly whitefly, *Aleurothrixus floccosus.* The fruit fly *Bactrocera invadens* has spread throughout the rest of Africa south of the Sahara but is not yet established in South Africa.

S16O03

Integrated pest management in Spanish citrus: current status of biological control.

Urbaneja A.[1], Tena A.[1], and Jacas J.A.[2]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Protección Vegetal y Biotecnología, Spain; and [2]Universitat Jaume I (UJI), Ciències Agràries i del Medi Natural, Spain. aurbaneja@ivia.es

Biological control has been and will definitively continue to be an increasingly important part of citrus crop protection practices in Spain and elsewhere. Classical Biological Control (BC) has been actively practiced in

Spanish citrus orchards for many decades. Most of the citrus pests occurring in Spain at present are under satisfactory natural BC, either by indigenous or introduced natural enemies However, a few key pests are not controlled by natural enemies. The management of these species has been traditionally based on chemical control. Nevertheless, recent restrictions on pesticide usage within the EU have led to a renewed interest on alternative BC strategies. Present trends indicate that augmentative and conservation BC will probably play an increasing role in the Spanish citrus industry. Additionally, strategies aimed at the conservation of native natural enemies of all these pests are in progress.

S16O04

Status of citrus IPM in the Southern Mediterranean basin (North Africa): case of Morocco

Mazih A.

Institut Agronomique & Vétérinaire Hassan II (IAVHII), Plant Protection, Morocco. ahmedmazih@gmail.com

More than 30 phytophagous arthropods and snail species have been reported on citrus in Morocco. Hemiptera represents the largest number of pest species. These include armored scales, soft scales, whiteflies, aphids, mealybugs and leafhoppers. The remaining, are represented by the Med fruitfly, Lepidoptera and mites species. The key pests around which control strategies pivot are Mediterranean fruit fly,-*Ceratitis-capitata*-Wiedemann (Diptera: Tephritidae.), California red scale,-*Aonidiella-aurantii*-Maskell (Hemiptera: Diaspididae), mites mainly citrus red mite, *Panonychus-citri*-McGregor (Acarina: Tetranychidae), and the citrus leafminer,-*Phyllocnistis-citrella*-Stainton (Lepidoptera: Gracilariidae). The outbreak of some secondary pests during last years (e.g.-*Icerya-purchase* Mask (Hemiptera: Margarodidae) could be interpreted as the secondary effects of some pesticides, used against key pests, to predatory Vedalia beetle-*Rodolia-cardinalis* Mulsant (Coleoptera: Coccinellidae). In addition, the recent invasion of a new mite species-*Eutetranychus-orientalis*-Klein (Acari: Tetranychidae), recorded for the first time in 2008, became a big concern, because of its rapid spreading and huge number of generations that often necessitate frequent sprays. The most effective products used are mineral oils or sulphur. Up to now, pest management in Moroccan orchards still heavily relies on chemical control. However, the implementation of ecological methods is slowly taking place. Some have already been developed by research, and could be made ready for use in practice, in order to meet the new requirements of the market regarding fruit quality, environment, health, and the good agricultural practices. Thus, biological control is in progress and alternative methods to chemical control such SIT are underway.

S16O05

Integrated Pest and Disease Management in New Zealand- progress,changes and challenges since 2004.

Pyle K.R.[1], and Jamieson L.E.[2]

[1]Pyle Orchards and Consulting Pty Ltd, New Zealand; and [2]The New Zealand Institute for Plant and Food Research Limited, New Zealand. kpyle@xtra.co.nz

A three year integrated pest and disease management research programme financed by the New Zealand government and citrus growers ended in 2005. We give an overview of the key results and the changes and challenges since then. The Australian citrus whitefly *Orchamoplatus citri* (Hemiptera:Aleyrodidae) was first detected in New Zealand in 2000 and it spread to all growing areas by 2006.It has resulted in significant changes to programmes including additional insecticide sprays. Its phenology, economic importance and chemical control strategies are outlined. The prospect for biological control using imported parasitoids from field surveys in Australia is described. In lemons, the focus has been on the citrus flower moth *Prays nephelomima* (Lepidoptera: Praydidae) which causes rind spotting and yield reduction, mainly on the YenBen lemon. The pest's phenology, susceptibility to insecticides, and the response to mating disruption trials using 500 to 1000 dispensers per hectare containing the female sex pheromone are described. Further work is planned using lure and kill techniques. Citrus disease research has focused on understanding the disease cycle and control of anthracnose (*Colletotrichum gloeosporioides*) a pre-harvest disease of Satsuma mandarins.

S16O06

Progress toward integrated management of Asian Citrus Psyllid in Florida

Stansly P.A.

University of Florida (IFAS), SW Florida Research and Education Center, USA. pstansly@ufl.edu

Asian citrus psyllid (ACP) *Diaphorina citri* was first detected in Florida in 1998 and quickly spread throughout the state, followed in 2005 by huanglongbing (HLB) or citrus greening disease vectored by ACP. High incidence of HLB was soon found in many citrus orchards, rendering the recommended practice of removing symptomatic trees impractical and leaving vector control as the principal means of HLB management. Two important early advances were the "stem tap" technique for rapid monitoring of ACP populations and the "dormant spray" that provided effective suppression of overwintering ACP adults. Extensive field testing furnished a suite of effective broad-spectrum and selective contact insecticides for foliar application and systemic insecticides used as soil drenches to protect young trees. Foliar nutrient programs have proved effective in mitigating impact of the disease on mature tree health and productivity when coupled with ACP control, even where HLB incidence is high. Area wide cooperative spray programs were organized and executed, and their effectiveness documented by statewide monitoring of over 5,000 citrus blocks at 3-week intervals using the tap sample. These measures have enabled the Florida citrus industry to maintain viability in the face of HLB, thanks in part to a favorable market which has so far covered additional costs. Present efforts are directed at refining and improving the program by definition of economic thresholds, better timing and selectivity of spray programs, and integration with biological and cultural strategies such as mass release of the parasitoid *Tamarixia radiata* and UV reflective mulches to better protect young plantings.

S16O07

Perspective of the Indonesian Citriculture in the Presence of Huanglongbing Disease

Supriyanto A.S, and Nurhadi N.

Balai Penelitian Tanaman Jeruk dan Buah Subtropika (Balitjestro), Agriculture, Indonesia. arry_supriyanto@yahoo.com

Citrus is one of the most important horticultural crops in Indonesia, with a production area of around 57,083 hectares produced 2,028,904 tons. Mandarins and tangerines are the main citrus grown (95.5%), and others such as; pummelo, oranges, limes and lemons represent less than 4,5%. Citrus import during the last decade showed a significant increase of about 96 tons in 2000 to 193,462 tons in 2011 with a value of US $53,000 and of US $171,000,000 respectively, placing Indonesia as the eigth citrus imported country of the world and the biggest one in Asia. Citrus plantations in Indonesia can not be avoided from the threat of pests and diseases complex, particularly Huanglongbing disease (HLB) that proven causes degeneration of tree growth, declining productivity and quality, death; and even threaten the sustainability of the citrus agribusiness in citrus plantations of Indonesia. The results of HLB disease epidemic research in the last 10 years provide a more comprehensive understanding of: 1) the interactive relationship between the citrus pathogen HLB vector *Diaphorina citri* Kuw. (Hemiptera: Psyllidae) the rate of disease progression of citrus HLB, 3) the spread pattern of disease by *D. citri* in the field, and 4) factors which influence the rate of disease progression and spread of HLB in the field. This information is expected to provide more comprehensive understanding of how to control HLB properly in the citrus grower situation in Indonesia. This paper provide information and discussion dealt with the implications of HLB epidemic studies results and its contribution to the components of HLB disease epidemics through the concept of 'toward precision farming for sustainable citrus health'.

S16O08

Nontarget effects of cultural practices to manage the bacterial disease huanglongbing on soil food webs that affect the insect pest *Diaprepes abbreviatus*

Campos-Herrera R.[1], El-Borai F.E.[2], Schumann A.[2], and Duncan L. W.[2]

[1]Instituto de Ciencias Agrarias (ICA-CSIC), Contaminación Ambiental, Spain; and [2]University of Florida, Citrus Research and Education Center (UF-CREC), Entomology and Nematology, United States (US). r.camposherrera@ufl.edu

The 'Advanced Citriculture Production System' (ACPS) can mitigate the impact of the devastating bacterial disease huanglongbing (HLB) by bringing citrus trees into production more quickly than conventional citriculture methods.

Daily fertigation required by ACPS changes soil physico-chemical properties compared to those in conventionally managed orchards. Real-time PCR was used in a field trial to investigate the effects of ACPS compared to conventional grower practices (G) on more than a dozen microorganisms in soil food webs that affect larvae of a major arthropod pest of citrus, *Diaprepes abbreviatus* (*Diaprepes* root weevil, DRW). A second treatment involves the use of polypropylene mulch as a barrier to DRW entering or exiting soil. To date, greater numbers of the EPN *Steinernema scapterisci* and more nematophagous fungi (*Catenaria* sp. and *Paecilomyces lilacinus*) were recovered from bare compared to mulched soil ($P < 0.05$). Steinernematid entomopathogenic nematodes (EPNs) were more prevalent in G, whereas more *Heterorhabditis indica* were detected in ACPS ($P < 0.05$). ACPS increased citrus fibrous roots and the numbers of plant parasitic nematodes, free living nematodes and DRW emerging from soil ($P < 0.05$). These results demonstrate the need to understand how the management tactics used to mitigate HLB might be modified to avoid exacerbating other serious biotic perturbations to tree health.

S16O09

Protection of young trees from HLB through disruption of *Diaphorina citri* (Kuwayama) feeding behavior.

Rogers M.E., Ebert T.A., Kim K.D., and Weaver C.E.

University of Florida, Citrus Research & Education Center (UFCREC), Entomology and Nematology, United States. mrgrs@ufl.edu

The long-term economic viability of a citrus grove is dependent on the ability to replant new trees and bring those trees into fruit production. In areas where HLB is endemic, young trees that become infected with the HLB pathogen are unlikely to become productive fruit-bearing trees. Thus, it is of utmost importance to determine ways to prevent young trees from becoming infected with the HLB pathogen. Using an electrical penetration graph (EPG) monitor, we determined the ability (and duration) of foliar and soil-applied insecticides to disrupt the feeding behaviors of *D. citri* postulated to be responsible for successful pathogen transmission. In the field, caging studies with adult *D. citri* were conducted every 3 months to determine the residual activity of selected insecticides (in terms of direct insect mortality) under field conditions during the different seasons of the year. Results of this work were then used to design a multi-year field trial to validate whether certain season-long insecticide programs can prevent young trees from becoming infected with the HLB pathogen. Immediately following the planting of a 4-ha block of 'Valencia' orange, replicated plots were established and assigned to one of six insecticide programs. Biweekly psyllid counts were made for each plot and trees were tested every 3 months for the presence of the HLB pathogen. Results will be discussed in terms of developing pest management programs to protect young tree plantings from HLB.

S16O10

Effect of UV-blocking plastic films on plant location and spread of the Asian Citrus Psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) on citrus.

Miranda M.P.[1], Marques R.N.[1], Santos F.L.[1], Felippe M.R.[1], Moreno A.[2], and Fereres A[2].

[1]Fundecitrus (Fundecitrus), Scientific Department, Brazil; and [2]Institute of Agricultural Sciences (I.C.A), Crop Protection, Spain. mpmiranda@fundecitrus.com.br

Diaphorina citri (ACP) is a major pest of citrus worldwide as it transmits Huanglongbing (HLB). Vision, behavior and performance of insect pests can be manipulated by using UV-blocking materials. Thus, the aim of our study was to evaluate how UV-blocking films affect the spread and host plant finding ability of ACP. Screen houses (5m x 2.5m x 2m) either covered with a UV-blocking film (98.9% UV-block) or with a non UV-blocking film (control) were evaluated. In a first trial, 100 ACP adults were released at the center of each screen house to assess their ability to locate citrus plants arranged around the release point. In a second trial, 100 ACP adults were released at one end of the screen house to assess their ability to spread along citrus plants placed in 3 rows of 4 plants spaced 1 m apart. In both trials, the number of psyllids/plant at different time intervals was counted. A low percentage of psyllids were able to locate citrus plants under the UV-blocking film: 9% (UV-blocking) and 60% (non UV-blocking) in the first trial, and 2.5% (UV-blocking) and 46% (non UV-blocking), in the second trial. This represents a 90% reduction in the ability of psyllids to find their host plants. In the second trial, psyllids concentrated mainly in the first rows under the UV-blocking films whereas distribution

was uniform under the non-UV-blocking films. Our results show that UV-blocking films can effectively limit the spread of ACP in citrus grown in enclosed environments.

S16O11

Ontogenic variation in citrus flush shoots and its relation with host plant finding and acceptance by Asian Citrus Psyllid (Hemiptera: Psyllidae)

Setamou M.[1], and Patt J. M.[2]

[1]Texas A & M University-Kingsville Citrus Center (TAMUK-CC), Agriculture, Agribusiness and Environmental Sciences, USA; and [2]U.S. Horticultural Research Laboratory (USDA-USHRL), Horticulture and Breeding Research, USA. mamoudou.setamou@tamuk.edu

The Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Psyllidae) is a destructive insect mainly because it vectors the bacterial pathogens that cause the deadly and incurable citrus greening disease. *Diaphorina citri* adult females lay eggs and immature development occurs exclusively on new flush shoots of their Rutaceae host plants. Although citrus trees are evergreen vegetation that holds green foliage all year round, *D. citri* is particularly abundant in citrus groves when new flush shoots are present. Thus, population dynamics of this pest are strongly determined by flush cycles of citrus trees. New flush shoots have distinctive physical and chemical characteristics that may facilitate host finding by adult psyllids and their suitability for psyllid oviposition and immature development. Consistent with this hypothesis, *D. citri* adults preferentially selected younger flush shoots over mature ones for feeding and oviposition. Appearance of young flush shoots as measured by their spectral reflectance, their softness and volatile composition provided evidence of their roles in nutritional ecology of *D. citri*.

S16O12

Targeting juvenile hormone metabolic genes in the Asian citrus psyllid (*Diaphorina citri*) as a strategy to reduce the spread of citrus greening disease.

Van Ekert E.[1], Borovsky D.[2], Powell C. A.[3], Cave R. D. [1], Alessandro R. T. [4], and Shatters R. G. [4]

[1]Indian River Research and Education Center, University of Florida, Entomology and Nematology, USA; [2]Borovsky consulting, Vero Beach, FL, USA; [3]Indian River Research and Education Center, University of Florida, Plant Pathology, USA; and [4]US Horticultural Research Laboratory, USDA - ARS, USA. belgica@ufl.edu

Diaphorina citri Kuwayama, the Asian Citrus Psyllid (ACP), is a devastating citrus pest due to its transmission of a phloem-limited bacterial pathogen, *Candidatus Liberibacter asiaticus* Jagoueix that causes citrus greening. Psyllid control is a major part of effective greening disease management, and our research targets perturbation of insect juvenile hormone metabolism as a new psyllid control strategy. Previous studies have shown that application of a juvenile hormone (JH) analogue, pyriproxyfen, produces ovicidal/nymphicidal effects, morphological abnormalities, and reduced fecundity in ACP adults. These observations prompted us to identify JH biosynthetic and degradative pathways as targets for biologically-based control strategies, including RNA interference, as alternatives to heavy reliance on broad-spectrum pesticides. First, candidate genes/cDNAs encoding the JH metabolic enzymes, juvenile hormone acid methyl transferase (JHAMT) and juvenile hormone esterase, were identified through computational analysis of the ACP genome. Second, JHAMT cDNA was cloned, expressed in *E. coli* and a functional protein was purified. This JHAMT had a high affinity for substrates leading to JHI and JHIII synthesis, making it plausible that both juvenoids are present in the ACP. Results are discussed with respect to mechanism(s) of JH biosynthesis/catabolism in the ACP and targeting this process as an interdiction point for a biorational ACP control strategy.

S16O13

Biological control of red scale on citrus on the central coast of New South Wales.

Dao H.T.[1], Beattie G.A.C.[2], Holford P.[2], Spooner-Hart R.[2], Meats A.[3], and Burgess L.[3]

[1]Plant Protection Research Institute (PPRI), Vietnam; [2]University of Western Sydney (UWS), Australia; and [3]University of Sydney, Australia. daothihang@hotmail.com

Most studies on the ecology and biological control of California Red Scale (*Aonidiella aurantii*) in citrus orchards have been undertaken in warm dry regions where species of *Aphytis*, particularly *A. melinus*, are viewed as the

most important of a narrow-range of natural enemies. Our research was undertaken in unsprayed orchards on the relatively humid central coast of New South Wales where three annual generations of the scale and a wide range of key natural enemies, comprising five parasitoids, four native coccinellids, and six entomopathogenic fungi, occur. Scale populations were maintained below economic thresholds despite marked variation in levels of parasitism and incidence of predators and entomopathogens among study orchards. Winter temperatures and natural enemy activity in summer-autumn were the key factors limiting abundance of the scale. The most important natural enemy was the steelblue ladybird, *Halmus chalybeus*. Incidence of the five parasitoids, *A. chrysomphali*, *A. melinus*, *Encarsia citrina*, *E. perniciosi* and *Comperiella bifasciata*, varied among orchards. Two strains of *A. melinus*, one of uncertain origin, were recorded. *E. citrina* and *E. perniciosi*, both of unknown origin, played important roles. Parasitism was not density-dependent. Competitive displacement was not evident. Intraguild predation by *Aphytis* on *Encarsia* was rare. Three fungi had not been previously recorded in Australia. Current diversity and abundance of the fungi may stem from reduced use of fungicides.

S16O14

Sugar provisions improve fitness and efficacy of the parasitoid *Aphytis melinus* in the field.

Tena A.[1], Cano D.[2], Pekas A.[3], Wäckers F.[4], and Urbaneja A.[1]

[1]instituto Valenciano De Investigaciones Agrarias (IVIA), Protección Vegetal y Biotecnología. Entomología, Spain; [2]Bioinsumos Agrícolas (SAC), PERÚ; [3]Universidad Politécnica de Valencia (UPV), Instituto Agroforestal Mediterráneo, Spain; and [4]Lancaster Environment Centre, Lancaster University (ULAN), Centre for Sustainable Agriculture, UK. atena@ivia.es

Many adult parasitoids depend on sugar-rich foods such as nectar and honeydew to meet their energy requirements. We used HPLC (high performance liquid chromatograph) analyses to assess the sugar reserves (total sugar per parasitoid) and honeydew consumption of individual field collected *Aphytis melinus* parasitoids, the most successful biological control agent of California Red Scale *Aonidiella aurantii*. The number of honeydew producers was also measured when *A. melinus* were collected. Our data show that A. melinus, whose host does not produce honeydew, fed commonly on honeydew from other abundant hemipterans in spring and summer. However, when the number of honeydew producers decreased, as occurred in fall, less than 20% of *A. melinus* were found to have fed on honeydew, and their average total sugar content was reduced three-fold. In a second assay, we determined whether the addition of sugar provisions might improve the fitness and performance of *A. melinus* when honeydew producers were scarce. We compared sugar reserves of *A. melinus* using HPLC analysis, and evaluated population density, parasitism rates on *A. aurantii* and migration in trees with and without sugar subsidies. Our results showed that the number of adult *A. melinus* per tree, migration, parasitism rates and the number of parasitoids classified as sugar fed (those that had previously fed on sugars) were all greater in trees where sugars were added.

S16O15

Ground cover management in citrus affects the biological control of aphids.

Gómez-Marco F.[1], Tena A.[1], Jacas J.A.[2], and Urbaneja A.[1]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Unidad Asociada de Entomología UJI-IVIA; Departamento de Entomología, Spain; and [2]Universitat Jaume I (UJI), Unitat Associada d'Entomologia IVIA-UJI, Spain. fmgomez@ivia.es

The citrus aphids, *Aphis spiraecola* Patch and *A. gossypii* Glover (Hemiptera: Aphididae), are key pests of citrus clementine in Spain. A rich complex of natural enemies (NE) exploits these two species. However, NE usually arrive too late, when aphid population have exceeded economical thresholds. Hence, successful biological control should be based on anticipating the arrival of NE. This could be achieved by use of reservoir plants. *Festuca arundinacea* Schreb has proved as a suitable ground cover enhancing biological control of different citrus pests, as *Tetranychus urticae* Koch and *Ceratitis capitata* (Wied.). We have compared the dynamics of aphid colonies for two consecutive seasons in orchards with a *F. arundinacea* ground cover versus bare soil, which is the traditional citrus ground management. Aphid colonies were smaller and lasted shorter in trees associated with *F. arundinacea* than in those grown on bare soil. These differences have been attributed to an earlier appearance of aphid NE in *F. arundinacea*, which were much more abundant than in bare soil. The final cause of these differences is probably related to the presence of alternative prey/host in the grass (cereal specific aphid species).

S16O16

Field evaluation of some pesticides and biological control against citrus mealybug *Planococcus citri* Risso (Hemiptera:Pseudococcidae)

Kararacoglu M.[1], Kutuk H.[1], Tufekli M.[1], Satar G.[2], and Yarpuzlu F.[1]

[1]Biological Control Research Station, Turkey; and [2]Department of Plant Protection, Faculty of Agriculture, University of Cukurova, Turkey. h_kutuk@hotmail.com

This study was conducted to compare efficiency of some insecticide and biological control against citrus mealybug, *Planococcus citri* Risso (Hemiptera:Pseudococcidae), caused important economic losses in recent years in the Mediterranian region of Turkey. It was investigated at 23 different citrus orchards ranged 0.6-17 ha in Finike districtive of Antalya province in 2011. Summer oil, Chloropyrifos-ethyl, Spirotetramat and biological control agents, (*Cryptolaemus monrozieri* Mulsant and *Leptomastix dactylopii* How.) were applied under farmer conditions. Biological control agent was sampeled by stenier funnel and made visual observation of 100 fruit for infestation rate 30-45 days after application. There weren't found any big differences between insecticide application and biological control. While the lowest infestation value was Summer oil (% 5.91); Spirotetramat (% 6.88), Chloropyriphos-ethyl (%7.69) and biological control (% 8.66) fallowed it. The highest average number of predator (biological control agent) was determined as 20.57 individulas per steiner funnel for biological control applied citrus orchard, followed by summer oil as 4.25 individuals, spirotetramat as 2.67 individuals. But no biological control agent observed at Chloropyriphos-ethyl applied citrus orchards. Because of possibility of fitotoxity effect of summer oil at hot weather condition, spirotetramat looks an alternative to biological control or has a change in IPM application for managing citrus mealybug.

S16O17

Can imidacloprid cause lepidopteran pest repercussions?

Moore S.D.[1], Van der Walt R.[2], Kirkman W.[1], and Du Preez D.[3]

[1]Citrus Research International (CRI), South Africa; [2]Nelson Mandela Metropolitan University (NMMU), Biochemistry and Microbiology, South Africa; and [3]Nelson Mandela Metropolitan University (NMMU), Botany, South Africa. seanmoore@cri.co.za

Imidacloprid has been registered on citrus for the control of California red scale and aphids for many years. Higher levels of false codling moth (FCM), *Thaumatotibia leucotreta*, on citrus have anecdotally been associated with the use of systemically applied imidacloprid for a number of years. However, these observations have not been scientifically supported until now. Two analytical methods were used to determine the physiological effect of imidacloprid on adult female FCM: mass spectrometry for ovarian protein and HPLC for quantifying Juvenile Hormone (JHIII) levels. Elevated ovarian protein and JHIII levels were recorded in moths which had developed from imidacloprid-treated fruit or diet. Additionally, fecundity of moths which developed from imidacloprid-treated fruit was significantly higher than that of moths which had developed on untreated fruit. In a field trial, FCM infestation in the imidacloprid-treated half of an orchard was almost double that in the untreated half of the orchard. This comparison was reliable, as there were no other differences in the pesticide programme and FCM levels in the two halves of the orchard had been almost identical during the previous season. This trend was, however, not repeated in a second orchard. Although final repetitions of ovarian protein, JHIII, laboratory fecundity studies and studies on field levels of FCM will be conducted, it can already be concluded that imidacloprid treatment of citrus trees can lead to elevated levels of FCM in orchards. It is possible that the same effect could also occur with other Lepidoptera.

S16O18

Ecology and management of Kelly's Citrus Thrips in Eastern Spain

Navarro-Campos C.[1], Pekas A.[2], Aguilar A.[1], and Garcia-Marí F.[1]

[1]Universidad Politécnica de Valencia (UPV), Instituto Agroforestal del Mediterráneo, Spain; and [2]Biobest Belgium N.V., R&D Department, Belgium. crinacam@posgrado.upv.es

Kelly's Citrus Thrips (KCT), *Pezothrips kellyanus*, is a recently reported citrus pest worldwide. In eastern Spain citrus KCT was first identified in 2005 and nowadays is the most abundant thrips species found in

citrus flowers causing important economic losses due to fruit scarring. We studied different aspects of KCT´s biology and ecology in order to improve its management. KCT populations were sampled throughout the year in 14 citrus orchards in 2008 and in eight citrus orchards in 2009 and 2010 by sampling citrus flowers, fruitlets and mature fruits, as well as employing ground and aerial sticky traps. Additionally, soil samples were extracted using Berlese funnels in order to determine the presence and abundance of soil predatory mites. KCT individuals were captured in the citrus groves all year round: for the three years of the study the highest KCT population density was observed during the petal fall and the beginning of the fruit growing periods. Differences in the population density and damage by KCT were observed among years, apparently related with air temperature. With the data obtained, the aggregation pattern, sampling plans, and intervention threshold for KCT were determined. With respect to soil predatory mites, 15 species from eight families were identified being *Parasitus americanus* and *Gaeolaelaps* (*Hypoaspis*) *aculeifer* the most abundant. Higher populations of some predatory mite species were associated with lower fruit damage caused by KCT suggesting potential for biological control.

S16O19

Monitoring and management of *Brevipalpus chilensis* Baker (Acarina: Tenuipalpidae) in citrus

Olivares N.[1], Vargas R.[1], and Ripa R.[2]

[1]Instituto de Investigaciones Agropecuarias (INIA), CRI, La Cruz, Chile; and [2]Centro de Entomología Aplicada (CEA Ltda.), Chile. nolivare@inia.cl

The mite *Brevipalpus chilensis* Baker is an endemic phytophagous pest in Chile. It has a wide host range including citrus, grapes, custard apple and kiwi. The objective of this study was to evaluate *B. chilensis* population fluctuation in *Citrus sinensis*, *Citrus limon* and *Citrus reticulata*. The studies were conducted in three commercial orchards located in Valparaíso Region, Chile. Samples were taken every 15 days from lignified and non-lignified branches, fruits, and leaves. The sampled structures that displayed the largest abundance of mites were branches and fruits; the mites were not found on citrus leaves. In periods of fruit absence, branch monitoring is the most suitable tool for determining the density of *B. chilensis*. The results of this study show that *B. chilensis* is present all the season on lignified and non-lignified branches. This would allow a more precise monitoring and control preventing the migration of the mites towards the fruits. With these results we conclude that the optimal integrated management of *B. chilensis* in citrus orchards must include practices such as opening tree canopy by selective pruning, removal of non-harvested fruit, and chemical spray applications, one at the beginning of summer plus a second spraying post-harvest.

S16O20

Host adaptation of *Tetranychus urticae* populations in clementine orchards with a *Festuca arundinacea* cover may contribute to its natural control.

Aguilar-Fenollosa E., Pina T., Gómez-Martínez M.A., Hurtado M.A., and Jacas J.A.

Universitat Jaume I (UJI), Ciències Agràries i del Medi Natural, Spain. aguilare@uji.es

Tetranychus urticae Koch (Acari: Tetranychidae) is a key pest of clementine mandarins, *Citrus clementina* Tanaka (Rutaceae), in Spain. This mite is highly polyphagous and can be easily found in clementine orchards, both in the trees and in the associated flora. In a previous study we found that the use of a cover of *Festuca arundinacea* Schreber (Poaceae) offered a better regulation of *T. urticae* populations than either bare soil or the traditional wild cover, which included a mix of weed species. We hypothesized that the selection of two host races of *T. urticae*, specialized in *F. arundinacea* and *C. clementina*, could partly explain the results obtained in field studies (bottom-up regulation). Reciprocal transplant experiments show that sympatric deme × host combinations had higher mean fitness values than the allopatric combinations in clementine, but not in *F. arundinacea*, for most of the fitness parameters evaluated in the present study. Because local adaptation implies mean deme fitness to be systematically higher for the sympatric deme × habitat combinations than for the allopatric ones, these results can be taken as indicative of occurrence of local adaptation in *T. urticae*. Molecular genetics analyses with microsatellite markers support this conclusion and indicate that host adaptation of *T. urticae* found in our system may indeed contribute to a better natural regulation of this mite.

S16P01

Analysis of population trends of Citrus pests from an area-wide field survey and monitoring network established in eastern Spain

Garcia-Mari F.

Universitat Politècnica de València (UPV), Instituto Agroforestal Mediterráneo, Spain. fgarciam@eaf.upv.es

The establishment of scientifically sound warning, forecasting and early diagnosis systems is one of the priorities established by EU directives for pest management. Programs of field survey and monitoring generate also a large amount of information which can be useful to identify and study factors which influence pest populations. We present results of an area-wide survey and monitoring network established by the autonomic Government of the Comunitat Valenciana in eastern Spain citrus crops between 2004 and 2009 for quarantine purposes and to assist farmer in pest management decisions. Four hundred orchards were monitored biweekly all along the year, determining population levels of the most important pests. Population trends and patterns of change in pest abundance were determined along the season each year, from year to year, among citrus species and geographically, between different regions of the area surveyed. Depending on the species or group of arthropod, factors as macro and microclimate, biological control, plant species preference, cultural practices or interactions between these factors, were considered responsible for the demographic patterns observed.

S16P02

Arthropod pest composition and farmers' perceptions of pest and disease problems on citrus in Kenya

Ekesi S.

International Centre of Insect Physiology & Ecology (ICIPE), Plant Health, Nairobi, Kenya. sekesi@icipe.org

A survey was conducted in citrus orchards in lowland and highland areas of Kenya to assess arthropod pest composition and damage on three citrus species as well as farmers' perceptions of pest and disease problems in their farms. Various arthropod pests were identified attacking citrus. In the lowlands, fruit flies infested (38.4%) of the fruits, false codling moth (FCM) (22.6%), and citrus rust mite (CRM) (7.6%). In the highlands, fruit flies infested 36.4% of fruits, FCM (20.2%) and CRM (9.4%). The major leaf feeders in the lowlands were scales that infested a mean of 18.1% of trees, aphids (16.2%), whiteflies (12.6%), swallowtail butterfly larvae (SB) (11.8%), thrips (11.4%) and mealybugs (4.8%). In the highlands, African citrus psyllid (ACP) infested a mean of 33.2% of the trees, followed by scales (27.1%), whiteflies (15.4%), thrips (12.1%), aphids (10.1%), SB (9.8%) and leafminers (4.2%). Among the arthropod pests, farmers ranked fruit flies at 84.2%, and FCM, scales and ACP at a mean of 66.3% as the major pest constraint in citrus production. Greening ranked highest (85.4%) among diseases followed by fruit and leaf spot (72.4%) and citrus tristeza virus (44.5%). Only 24.6% of farmers used some form of chemical insecticide (modern or traditional) in citrus production, largely due to lack of capital, efficacy or availability of the products.

S16P03

Thresholds for HLB vector control in infected commercial citrus and compatibility with biological control.

Monzó C.[1], Stansly P.A.[1], and Urbaneja A.[2]

[1]University of Florida (IFAS), SWFREC, USA; and [2]Instituto Valenciano de Investigaciones Agrarias (IVIA), Entomology, Spain. aurbaneja@ivia.es

Control of the HLB vector, *Diaphorina citri* Kuwayama, is considered a basic component for management this disease, even in a high HLB incidence scenario. Such control is mostly chemically oriented. However, overuse of insecticides would increase costs and be incompatible with biological control. Establishment of economic thresholds for psyllid control under different price scenarios could optimize returns on investment. Two 3-year experiments are being conducted in commercial orange blocks with high HLB incidence. Experimental

design is RCB with 4 treatments and 4 replicates: no insecticide, calendar applications, insecticide applications according to a threshold of 0.2 psyllids/stem tap sample, and applications according to a 0.7 threshold. Vector populations are monitored biweekly by tap sampling. Differences in vector abundance among treatments are being correlated to HLB infection levels estimated by Q-PCR and most importantly, to fruit yields. Consequences of each vector control strategy on beneficial arthropod fauna are also being evaluated, as well as potential negative impacts on biological control processes in the crop. After two years, a yield increase was observed with the calendar treatment, but so far additional costs would require high juice prices scenarios to be sustainable. Negative impacts of calendar sprays on biological control of mites and leafminers have also been observed

S16P04

Development of the Huaglongbing (HLB) vector, *Diaphorina citri* Kuwayama, 1908 (Hemiptera: Psyllidae), in different host plants.

Alves G.R., Diniz A.J.F., Lima A.A., Vieira J.M., and Parra J.R.P.

Escola Superior de Agricultura Luiz de Queiroz - Universidade de São Paulo (Esalq/USP), Entomologia e Acarologia, Brazil. gustavo_ralves@yahoo.com.br

Huanglongbing (HLB) or greening is considered the most important Citrus disease in the world. In Brazil, it is transmitted by the Asian citrus psyllid *Diaphorina citri*. Knowledge on the life cycle of *D. citri* in natural and non-citrus hosts may help Integrated Pest Management in *citrus*. The objective of this work was to evaluate the effect of the most common cultivars grown in the state of São Paulo (Hamlin, Pêra, Natal, Valencia and Ponkan) and that of the orange Jessamine, *Murraya paniculata*, used in Brazil as hedgerow and considered a very important non-citrus host for this psyllid. *D. citri* couples were individually confined in cages for egg laying during 24 h, under laboratory conditions (temperature: 25±2°C; RH: 60±10%; photophase: 14h). Egg hatching and nymph mortality were daily observed until adult emergence. The egg viability ranged from 83.3 % to 56.8% for Valencia and Hamlin cultivars, respectively. The nymphal viability was also the lowest in the Hamlin cultivar (57.4%), with no differences among the other treatments. The highest viability was observed in the Valencia cultivar (65.9%) with the lowest in the Hamlin cultivar (32.6%).The life cycle lasted from 18.4 (Natal) to 17.2 (orange Jessamine) days.

S16P05

Agreggation and sampling plans for *Diaphorina citri* (Hemiptera: Psyllidae) immature stages in citrus.

Asplanato G.[1], Amuedo S.[1], and Franco J.[2]

[1]Facutad de Agronomía - Universidad de la República (FAGRO-UDELAR), Protección Vegetal, Uruguay; and [2]Facultad de Agronomía - Universidad de la República (FAGRO-UDELAR), Biometría y Estadística, Uruguay. gasplana@fagro.edu.uy

The Asian Citrus Psyllid *Diaphorina citri* is an efficient vector of the associated bacteria to Huanglongbing, considered the most destructive citrus disease in the world. In Uruguay, the psyllid was reported in 1991 but the disease has not been discovered yet. Studies were conducted to determine the distribution pattern of *D. citri* eggs and nymphs on flush shoots and to develop reliable sampling plans. The samplings were carried out from 2006 to 2009 in five citrus orchards located in the main citrus growing region of Uruguay. Taylor´s power law was appropriate and adequate to describe the variance-mean relationship of all *D. citri* development stages on the five orchards. The dispersion index *b* from Taylor's power law did not vary among orchards and development stages. Data from all orchards were pooled and common regression slope for eggs and nymphs were estimated. The distribution pattern of immature stages was aggregated among flush shoots as indicated by the dispersion index b=1.42, that was significantly > 1. The minimum sample size to estimate densities above five immature stages (eggs nymphs) per flush shoot was 175 flushes for a precision level of 0.25, appropriate for management decisions. To provide a quick estimation of *D. citri* in citrus orchards, a presence – absence sampling plan was evaluated.

S16P06

Use of oils to control the Asian Citrus Psyllid (*Diaphorina citri*) in Mexican lime under dry tropic conditions in Mexico

Orozco-Santos M., Velázquez-Monreal J.J., García-Mariscal K., Manzanilla-Ramírez M.A., Carrillo-Medrano S.H., and Robles-González M.M.

Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Campo Experimental Tecomán, Mexico. orozco.mario@inifap.gob.mx

Mexico is the main world producer of Mexican lime (*Citrus aurantifolia*). The Asian Citrus Psyllid (ACP; *Diaphorina citri*) is the most important pest affecting this citrus species which is the vector of Huanglongbing disease. ACP control is based on the use of synthetic insecticides. Although, oils have a long history as insecticides to control citrus pests, only a few cases have been reported against *D. citri*. In this study, the effects of 9 oils against ACP were evaluated in Colima, Mexico. Three assays were carried out in trees heavily infested with the insect. One foliar application of each treatment was done on tagged shoots of 4-6 days old. Temperature during application was 32 °C±1. Live nymphs were evaluated after the application. In the first test, three doses of the paraffinic oil (PureSpray Foliar 22E®: 1, 2 and 3%) reduced ACP population from 9.3-13.2 nymphs/shoot (before application) to 0.2-2.7 nymphs (at 7 days). The control registered 11.5 nymphs. In the second and third test, all paraffinic oils tested (PureSpray Foliar 22E®, Saf-T-Side®, Apoyador®, Stylet-oil®, and Banasole®), cooking oil (Cristal®), petroleum oil (Citrolina) and citrus oil (Oroboost®) showed the lowest infestations of ACP relative to the control. At 7 days, oils registered 0.7-3.3 nymphs/shoot, 0.0-1.8 nymphs/shoot were found in the second and third tests, respectively. Contrarily, control had 6.1 and 11.6 nymphs/shoot. No oil caused phytotoxicity was observed. These results indicate that the oils can be included in programs for ACP control in Mexican lime.

S16P07

Seasonal patterns in the proportion of Asian citrus psyllid (*Diaphorina citri*) carrying *Candidatus* Liberibacter asiaticus.

Ebert T.A.[1], Brlansky R.H.[2], and Rogers M.E.[1]

[1]University of Florida - Citrus Research & Education Center (UFCREC), Entomology and Nematology, United States; and [2]University of Florida - Citrus Research & Education Center (UFCREC), Plant Pathology, United States. mrgrs@ufl.edu

Candidatus Liberibacter asiaticus (Las) is the putative causal agent of Huanglongbing disease in Florida citrus where pathogen spread occurs by the Asian Citrus Psyllid (*Diaphorina citri* Kuwayama). Insecticide use to control *D. citri* in attempts to manage the spread of Las occur year-round, resulting in the application of up to 12 or more insecticides applications per year. However, it is unknown if there are periods of the year when psyllids may be less likely to be carrying Las and thus insecticide applications may not be warranted. To determine if there are times of the year when pathogen spread may be less likely to occur, *D. citri* populations at six locations in central Florida and one location in southern Florida were sampled monthly over a 48 month period. The proportion of adult *D. citri* carrying Las was measured using QPCR of pooled samples. Prior to DNA extraction, psyllids were sorted by gender and color. Florida Automated Weather Network was used to estimate environmental conditions at these locations. Las was present at all times of year, but prevalence was highest October through December. Seasonal patterns in sex ratio and color were correlated with weather patterns and Las prevalence in *D. citri*.

S16P08

Effectivness of Imidacloprid (Winner) by trunk application for psyllid (Diaphorina citri – Hemiptera: Psyllidae) control on citrus.

Lozano Leonel Junior F.[1], Soares R.D.J.[1], and Moraes J.P.[2]

[1]BayerCropScience (BCS), Agronomic Development, Brazil; and [2]Escola Superior de Agronomia Luiz de Queiroz (ESALQ), Brazil. francisco.lozano@bayer.com

The citrus psyllid (*Diaphorina citri*) injures citrus by: (1) distortion and curling of leaves, (2) withdrawal of sap from the foliage, and (3) transmission of the bacterium that causes the greening disease (Huanglongbing).

Suppression of citrus psyllid populations is recommended for slowing disease spread. The efficacy of the Winner® (imidacloprid) by trunk application was tested against the citrus psyllid in plants with different ages. This experiment was conducted in a citrus grove of different tree sizes in Bayer CropScience's Experimental Agricultural Station, Paulínia, SP, Brazil. The study block was divided into four replicates within which 7 treatments were randomly assigned: (1 – 5) Winner 200 CS (Imidacloprid 200 g/L) at 0.2 g ai/cm trunk diameter in plants of 2, 5, 10, 15 and 20 cm diameter, (6) Winner 200 SC at 0.3 g ai/cm trunk diameter on 20 cm diameter plants, and (7) a blank application as untreated control. Treatments were applied by a spray applicator with the full strength product without water, around the whole diameter of the trunk 20 cm below the lowest branches. Psyllid adults were confined by sleeve cages to plant shoots and the number of alive adults and nymphs inside the cages were later counted to assess efficacy. The interval of the infestations was according the presence of new shoots. Efficacy was calculated by the formula of Abbott and the data were transformed using the arcsine square root functions and subjected to ANOVA and means compared using Tukey's test ($P < 0.05$). -Control was 100% in plants of 5 cm diameter but variable results against adults were seen on larger trees.- Nymphs controlled better than adults regardless of tree size, with suppression observed up to 214 days.

S16P09

Movement of Asian citrus psyllids in HLB-affected and healthy citrus trees

Cen Y.[1], Wu F.[1], Liang G.[1], Chen J.[2], Deng X.[3], and Xia Y.[4]

[1]Laboratory of Insect Ecology, South China Agricultural University, China; [2]Crop Diseases, Pests and Genetics Research, San Joaquin Valley Agricultural Sciences Center, USDA, USA; [3]Citrus Huanglongbing Research Laboratory, South China Agricultural University, China; and [4]Center for Integrated Pest Management, North Carolina State University, USA. cenyj@scau.edu.cn

Asian Citrus Psyllid (ACP) *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) is the vector of Huanglongbing (HLB). *Diaphorina citri* showed different tropism to HLB-infected and healthy citrus trees. The presence or absence of new flushes had a significant influence on the movement of ACP in HLB-affected citrus hosts. With new flushes, HLB-affected citrus trees were more attractive to adults of ACP. The psyllids fed on the host for a longer time period. In the absence of new flushes, psyllids preferred leaves with yellowing symptoms. After feeding for about 36 hours, psyllids moved to health-looking leaves. This behavior appeared to facilitate pathogen spread. In a color board experiment (yellow, green or white), it was observed that the yellow color board attracted more adult psyllids, indicating that psyllid adults preferred yellow color. Late stage psyllid nymphs (3rd to 5th instar) could make significant horizontal and vertical movements. In HLB-affected trees, nymphs were more attracted towards yellowing young shoots. Yet, movement was slower in HLB-affected trees than that in healthy trees. In healthy trees, number of nymphs moving downwards was significantly higher than that in HLB-affected trees.

S16P10

Effectivity of Huanglongbing vector (*Diaphorina citri* kuw.) control citrus grower group based in regency of Sambas, West Kalimantan, Indonesia

Supriyanto A.S.[1], Nurhadi N.[1], Zuhran Z.[2], and Purbiati P.[3]

[1]Balai Penelitian Tanaman Jeruk dan Buah Subtropika (Balitjestro), Agriculture, Indonesia; [2]Balai Pengkajian Teknologi Pertanian Kalimantan Barat (BPTP Kalbar), Agriculture, Indonesia; and [3]Balai Pengkajian Teknologi Pertanian Jawa Timur (BPTP Jatim), Agriculture, Indonesia. arry_supriyanto@yahoo.com

The purpose of this study was to determine the effectiveness of Huanglongbing vector control recommendation citrus grower group based. Studies have been carried out in 2010 in Tebas Sungai village, Sambas district, with 11 tangerine groves owned by growers in the citrus grower association of Sambas district. The tangerine grove that been used are, one grower's orchard as a demonstration plot in a particular citrus grower group (orchard I); five other citrus orchards with different ownership at the same citrus grower group (orchard II), as well as five other citrus orchard with different ownership which each of them spreads over five different citrus grower groups outside the farm demonstration plots (orchard III). The recommendation technology for controlling Huanglongbing vector which applied in this experiment, included bark painting by systemic insecticide of imidacloprid for two each 1.5-month and spray using contact insecticide with dimethoate to the plant crown which application time been alternated after bark painting application. The effectiveness of technology implementation is measured by a decrease psyllid populations found in citrus samples in adult

stage, nymphs and eggs that were observed at regular intervals every two weeks during the flushing to the 14th week after the first treatment. The results showed if the recommended treatment technology were absolutely proven to reduce Huanglongbing vector population in significant, namely in the orchard I, II, and III respectively at 95.3%, 84.7%, and 72% for stage imago; 97.3 %, 80%, and 100% for stage nymphs; and 98.5%, 100% and 100% for the egg stage.

S16P11

Behavioural responses of *Diaphorina citri* to host plant volatiles.

Fancelli M.[1], Birkett M.A.[2], Pickett J.A.[2], Moraes M.C.B.[3], Laumann R.A.[3], and Borges M.[3]

[1]Embrapa Cassava & Fruits (EMBRAPA/CNPMF), Laboratory of Entomology, Brazil; [2]Rothamsted Research (RRES), Biological Chemistry and Crop Protection, UK; and [3]Embrapa Genetic Resources & Biotechnology (EMBRAPA/CENARGEN), Laboratory of Semiochemicals, Brazil. fancelli@cnpmf.embrapa.br

The Asian Citrus Psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) is a vector of the greatest threat to the citrus production and industry in the world known as HLB (*Huanglongbing* = ex-greening). Differences in host plant suitability for insect development and disease incidence have been reported. Thus, *D. citri* responses to plant volatiles were investigated in order to identify attractants from host plants. Volatiles were collected from host plants, *Citrus sinensis* cv. Pera D6, *C. reshni* - Cleopatra, *Citrus limettioides* and *Poncirus trifoliata.* A four-arm (Pettersson) olfactometer was used to determine behavioural responses of *D. citri* females to the volatiles. One treated arm (plant volatiles) was compared against control arms (hexane). Air was drawn from the olfactometer at the rate of 200 ml/min. One adult female starved for 1 h was exposed to the volatiles for 16 min, and every 2 min the position of the olfactometer was rotated by 90°. The time spent by *D. citri* in the different arms of the olfactometer was recorded using OLFA software. The data were analysed using a two-sample unequal variance *t*-test. *D. citri* spent more time ($P < 0.01$) in the arm treated with *Citrus sinensis* cv. Pera volatiles when compared to control arms, but no differences between treatments and controls were observed for the other plants. These data so far provide evidence for differences in susceptibility of potential host plants.

S16P12

Efficacy of selected insecticides for the control of the California Red Scale in Southern Italy.

Campolo O., Grande S.B., Chiera E., and Palmeri V.

University of Reggio Calabria (UNIRC), Department of Agricultural and Forest Systems Management, Italy. orlando.campolo@unirc.it

The California Red Scale (CRS), *Aonidiella aurantii* (Maskell), is considered one of the most important pests of citrus in the Mediterranean basin as well as in other citrus growing areas worldwide. In Southern Italy, citrus is the most widely cultivated crop and the control of the CRS relies mainly on the application of synthetic insecticides. During 2009 and 2010, selected insecticides (spirotetramat, chlorpyriphos and pyriproxyfen) were evaluated against this pest in two Calabrian citrus orchards under integrated pest management. Treatments were performed according to the dosage reported on the label of the commercial products. Two treatments, at 15-day interval, were performed. Control plots were sprayed with water only. Efficacy was assessed 7, 14, 21, and 28 days after the first treatment and at the harvest. In both trials, spirotetramat showed the highest levels of efficacy against CRS and other citrus pests (citrus leafminer, aphids and mites) that were adequately controlled until the harvest. At harvest, spirotetramat also had the best visual acceptance by a panel of consumers.

S16P13

Altea (Eastern Spain) area-wide project to control California Red Scale *Aonidiella aurantii* (Hemiptera: Diaspididae) based on conservation and release biological control agents.

Laborda R.[1], Garcia-Mari F.[2], Sanchez A.[1], Xamani P.[1], Garcia A.[3], Punset C.[4], Bernabeu P.[5], Aznar M.[5], and Bertomeu S.[1]

[1]Universitat Politécnica de Valencia (UPV), Ecosistema Agroforestales, Spàin; [2]Universitat Politécnica de València (UPV), Instituto Agroforestal Mediterráneo, Spain; [3]Universitat Politècnica de València (UPV), Hidráulica, Spain; [4]Ayuntamiento de Altea, Spain; and [5]Cooperativa Agrícola de Altea, Spain. rlaborda@eaf.upv.es

The municipality of Altea includes a large rural area in which citrus orchards and country cottages coexist. A municipal initiative instituted to reduce the risk of pesticides used to control the principal pest of citrus crops

in the area, the California Red Scale (CRS) *Aonidiella aurantii* (Maskell). A surface area of 66 ha surrounding the main urban area was selected for study in 2010. The alternative to the use of synthetic organic pesticides was the mass release of biological control agents (*Aphytis melinus DeBach (Aphelinidae)*, *Rhyzobius lophanthae* (Blaisdell)(Coccinellidae) and *Comperiella bifasciata* (Howard*)* (Encyrtidae) and the application of essential oil (Orizone®) when population of CRS reached a threshold of 2% infested fruits. Additionally, a cover crop was mechanically managed in a 30 ha surface area and -beneficial fauna identified. The results showed that the intervention threshold was not reached in 93% of the surface area due to the biological control exerted by the parasitoid releases as well as by the activity of naturally occurring populations of beneficial insects.

In 2011 we analyzed the real effect of mass releases of *A. melinus*, considering the high levels of native fauna observed in the previous year. Different release rates, ranging from 0 to 100,000 *A. melinus*/ha, were compared. No differences were observed between orchards with or without releases, nor among different levels of release. These results confirm the importance of conservation biological control strategies in CRS management.

S16P14

Hyperparasitism may prevent efficient regulation of *Aphis spiraecola* Patch. (Homoptera: Aphididae) in citrus orchards by primary parasitoids.

Gómez-Marco F.[1], Tena A.[1], Jacas J.A.[2], and Urbaneja A.[1]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Unidad Asociada de Entomología UJI-IVIA; Departamento de Entomología, Spain; and [2]Universitat Jaume I (UJI), Unitat Associada d'Entomologia IVIA-UJI, Spain. fmgomez@ivia.es

Aphis spiraecola Patch., is a key pest of clementine mandarins in Spain. The biological control of this species is far from satisfactory and we have tried to unravel the reasons for this failure. We sampled mummies of *A. spiraecola* during the spring population that this species exhibits in Valencia and kept them until emergence of the developing parasitic wasp. At least two plausible explanations for deficient biological control have been found. On the one hand, very few parasitoid species (n=440) could complete their development in this aphid. Parasitic wasps belonging to the genus *Trioxys* were the only specimens successfully reared out of these mummies. Percent parasitism by these wasps accounted for 14 % of total mummies caught. On the other, hyperparasitism was extremely high (67%) and *Syrphophagus aphidivorus*, a primary hyperparasitoid, alone represented more than 55% of all hyperparasitoids found. Molecular tools could probably help to shed light into the complicated trophic relationships established among *A. spiraecola* parasitic guild. Disentangling these relationships will probably pave the way for improving the biological control of this species.

S16P15

Citrus mealybug biological control strategies and large scale implementation on citrus in Turkey.

Erkilic L.B., Demirbas H., and Guven B.

Biological Agriculture Consulting and Engineering Co (BIYOTAR), Biological Control, Turkey. lerzane@superonline.com

Citrus Mealybug, *Planococcus citri* (Risso) (Hem.: Pseudococcidae) is one of the main pests on citrus in Turkey. Since 1970 biological control implementation in citrus orchards continue on Citrus mealybug with *Cryptolaemus montrouzieri* Mulsant (Col.:Coccinellidae) and *Leptomastix dactylopii* Howard (Hym.:Encyrtidae). The interests in biological control tend to be increase due to human and environmental health, food safety and pesticide residue issues. The application dosages of *C. montrouzieri* and *L. dactylopii* range between 10 to 20 individuals per tree according to the mealybug population level and also the citrus variety. The main strategy is to use both beneficials *C. montrouzieri* and *L. dactylopii* at the early stage of mealybug population. *L. dactylopii* can be released at the beginning of mealybug population build up, before mealybug egg production, and *C. montrouzieri* should be released when the mealybug population is producing eggs. If the mealybug population has both, adults and egg masses, then both beneficials should be released together. In large scale application the early usage sometimes is not possible, because other main pests such as the California Red Scale and Rust mite are present and chemical applications may take place at the beginning of season. In this case a compatible pesticide use is preferred to carry out the biological control releases.

In Turkey due to food safety reasons and also Min. of Agriculture subvention given for the usage of biological control agents would be increase the application area in coming future.

S16P16

Repellency and acceptability of several substances by ant species in citrus groves in Spain.

Campos Rivela J.M., Martínez-Ferrer M.T., Fibla J.M., and Pla M.

IRTA - Amposta, Sustainable Plant Protection - Entomology, Spain. jmiguel.campos@irta.cat

Ants are associated with outbreaks of insects principally because of their interference with the biological control of these pests. The control of ant populations should be considered as a key component in the development of strategies for pest management. Ants communicate between individuals by using semiochemicals for several purposes. Then again, the success of chemical control of ants is based, among others, on the acceptability of the ant worker to bring the toxic to the colony, and especially to the queen. Field bioassays were performed on *Lasius grandis*, *Pheidole palidulla* and *Linepithema humile*. Bioassay arenas were located under citrus trees, where the selected species was actively foraging before. Only one species was allowed to forage in each experiment. One of the bioassay was carried out to quantitatively determine the repellent activity of semiochemicals farnesol and methyl eugenol against these species. Circular barriers of beeswax containing different concentration of the semiochemicals (0.03%, 0.06%, 0.12%, 0.24%, 0.48%, 0.96%, 2%, 3% and 5%) were used. The number of ants crossing these barriers during 2 minutes in four samples each was recorded. A non-choice test field bioassay was conducted to qualitatively determine the acceptability of some toxicants formulated in 25% sucrose. Toxicants used were: pyriproxyfen, boric acid, spinosad, imidacloprid, and abamectin. The obtained results are presented and discussed.

S16P17

Taxonomic studies for classical biological control of *Delottococcus aberiae* (Hemiptera: Pseudococcidae).

Beltrà A.[1], Navarro-Campos C.[1], Garcia-Marí F.[1], Malausa T.[2], Giliomee J.H.[3], Addison P.[4], and Soto A.[1]

[1]Universitat Politècnica de València (UPV), Instituto Agroforestal Mediterráneo, Spain; [2]Institut National de la Recherche Agronomique (INRA), Biologie des Populations Introduites, France; [3]University of Stellenbosch (US), Department of Botany and Zoology, South Africa; and [4]University of Stellenbosch (US), Department of Conservation Ecology and Entomology, South Africa. albeliv@etsia.upv.es

Delottococcus aberiae Brain (Hemiptera: Pseudococcidae) is a mealybug of Afrotropical origin that has been recently introduced in eastern Spain. The absence of efficient natural enemies has led to population outbreaks in some citrus orchards reducing fruit quality and production. This species was described as a minor pest of citrus in South Africa and shows small morphological differences with the Spanish strain. Mealybugs are typical invasive pests and classical biological control programs have been traditionally applied against them. However, the misidentification of the mealybug species and their natural enemies has led to the failure of some parasitoid introductions. In this work we carried out a survey of *Delottococcus* mealybugs and their parasitoids in different orchards and natural ecosystems in South Africa as a first step towards a classical biological control program. We provide a multi-criterion characterization of mealybugs and parasitoids combining morphological and genetic techniques. DNA was amplified from five different loci in mealybugs and four loci in parasitoids. Genetic analysis revealed significant differences among Spanish and South African mealybug populations. Moreover, new parasitoid species were recorded and their DNA sequences are provided. The potential use of these parasitoids in biological control of *D. aberiae* in Eastern Spain is discussed.

S16P18

Canibalism and intraguild predation in citrus mealybug natural enemies.

Gkounti V.T.[1], Savvopoulou-Soultani M.[1], Kontodimas D.C.[2], and Milonas P.G.[2]

[1]Aristotle University Thessaloniki (AUTH), Phytopathology, Greece; and [2]Benaki Phytopathological Institute (BPI), Entomology and Agricultural Zoology, Greece. vgkounti@agro.auth.gr

Cannibalism and interspecific interactions among insect natural enemies are common, yet these behaviors are frequently omitted from predatory-prey theory and empirical studies. These interactions may disrupt, facilitate or augment biological pest control.- In the present study intra- and interspecific interactions

among three natural enemies of the citrus mealybug *Planococcus citri* (Hemiptera: Pseudococcidae) were empirically investigated. The natural enemies' guild consisted of the predatory coccinellids *Nephus includens* and *Cryptolaemus montrouzieri* and the endoparasitoid *Anagyrus pseudococci*. Cannibalism and intraguild predation between the two predators have been studied in presence and absence of prey. It has been showed that *N. includens* adults readily consume conspecific eggs despite the availability of prey. However, as prey availability increases their tendency to cannibalism decreases. They also consume *C. montrouzieri* eggs in the absence of prey, while avoid them when alternative prey is offered. *C.montrouzieri* preys on eggs of *N. includens*, regardless of prey presence. We also investigated predation risks for immature stages of *A. pseudococci* by *N. includens* adults. Our results showed that larvae of *A. pseudococci* face increased intraguild predation at early developmental stages, before mummification of the host. After mummification *N. includens* does not prey upon it. The strength of these interactions is discussed in relation to sustainable biological control.

S16P19

Biological control of the citrus mealybug: dispersion pattern of the parasitoid *Anagyrus* sp. Near *pseudococci,* during augmentative releases.

Franco J.C.[1], Zina V.[1], Silva E.B.[1], Steinberg S.[2], Belda J.E.[3], Branco M.[1], Suma P.[4], and Mendel Z.[5]

[1]Instituto Superior de Agronomia, Universidade Técnica de Lisboa (ISA/UTL), Centro de Estudos Florestais, Portugal; [2]BioBee Sde Eliyahu Ltd. (BioBee), Research & Development, Israel; [3]Koppert Espana SL (Koppert), R&D Dept, Spain; [4]Università degli Studi di Catania (DGSAA/UniCT), Dipartimento di Gestione dei Sistemi Agroalimentari e Ambientali - Sez. Entomologia Agraria, Italy; and [5]Agriculture Research Organization, Volcani Center (ARO), Dep. Entomology, Israel. verazina@isa.utl.pt

Anagyrus sp. near *pseudococci* (Hym.: Encyrtidae) is the major parasitoid of the Citrus Mealybug (CM), *Planococcus citri* and the Vine Mealybug (VM), *Planococcus ficus* (Hemip.: Pseudococcidae). The wasp is commercially available to be used in augmentative releases to control both mealybug species. The effectiveness of this biological control tactic is dependent, among other factors, on the release rate and distribution pattern of the wasps within the target area. Therefore, knowledge on the dispersion of the parasitoid is of importance to optimize the protocols of the augmentative release. Recently, we showed that the sex pheromone of the VM attracts females of *A.* sp. near *pseudococci* and increases parasitization levels of CM by the wasp. In the present study, we employed VM pheromone baits to study the spatial and temporal dispersion pattern of *A.* sp. near *pseudococci* in augmentative releases. The experiment was set up in a 5 ha maritime pine stand, ca. 20 Km south of Lisbon. The absence of a local population of the parasitoid was verified by activation of traps baited with VM pheromone before the release. Sticky plate traps baited with 50 µg of VM pheromone were suspended up to 100 m away from the wasp release point along six different transects and six distances (10, 20, 40, 60, 80 and 100 m), in a circular layout. At the release point, we installed 18 commercial containers of *A.* sp. near *pseudococci* (CITRIPAR), each with 500 mummies. Daily monitoring of wasp captures showed that the parasitoid was able to disperse up to 100 m within first two days after release. The mean number of wasps captured within six days in pheromone traps decreased exponentially in function of distance from the release point. Mean captures at 100 m-correspond to-ca. 11% of-those at 10 m. Our results suggest that 3-4 release points per hectare are sufficient for a good cover of a target area by the parasitoid in augmentative release schemes.

S16P20

Study on biological control and trap techniques of *Thrips flavidulus* (Thysanoptera: Thripidae) in citrus orchards

Yao H., Yu F., and Zhang H.

Huazhong Agricultural University (HZAU), College of Plant Science and Technology, China. hongyu.zhang@mail.hzau.edu.cn

Thrips flavidulus has caused great damages to citrus orchards in China. In this study, the functional and numerical responses for the adults of the predatory mites *Neoseiulus cucumeris*, *Neoseiulus barkeri* and *Euseius nicholsi* on *T. flavidulus* first-instar larvae were studied to evaluate their predation ability on *T. flavidulus*. The results showed that the functional response of adult *N. cucumeris*, *N. barkeri* and *E. nicholsi*

could be described by the Holling II functional response type. The results suggested that predation ability of *N. cucumeris* was strongest to *T. flavidulus* among three predatory mites and prey density significantly influence reproductive ability of *N. cucumeris* and *E. nicholsi*, but not *N. barkeri*. Among 10 types of colors sticky cards i.e., yellow, blue, deep-blue, white, red, green, purple, gray and pink in citrus orchards, *T. flavidulus* had the strongest preference to the blue and deep blue sticky cards. The thrip numbers on those cards were significant different from those on the other eight colors sticky cards. The trapping techniques of blue sticky cards were discussed. Effect of 16 different plant volatile compounds on the behavior of adult female *T. flavidulus* thrips was tested with a Y-tube olfactometer. The results showed that *T. flavidulus* were attracted by the compound of benzenoids and monoterpenes isoprenoids. The compound A and E have the significant attraction to thrips at concentration 10% and 1%.

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S16P21

Thysanoptera occurring in clementine mandarin under three ground cover management strategies: species composition, abundance and distribution.

Aguilar-Fenollosa E., and Jacas J.A.

Universitat Jaume I (UJI), Ciències Agràries i del Medi Natural, Spain. aguilare@uji.es

There is a complex of Thysanoptera species inhabiting citrus and some of them are considered pests. This study was aimed at studying the effect of ground cover management on thrips species composition, abundance and distribution in commercial clementine mandarin orchards. Three ground cover management systems were compared: resident vegetation cover, *Festuca arundinacea* Schreber (Poales: Poaceae) cover, and bare soil. The ground cover influenced the abundance of some thrips species in the cover. Among the main species causing fruit damage in citrus, we found *Frankliniella occidentalis* (Pergande) and *Thrips tabaci* Lindeman. The *F. arundinacea* cover strongly depressed the abundance of these thrips species in ground cover vegetation. The resident vegetation cover could provide a range of suitable hosts supporting populations of potential thrips pest species out of the main citrus peak blooming period. Apart from that, the *F. arundinacea* cover, which hosted Poaceae-specific thrips species, could provide alternative food enhancing populations of predatory phytoseiid mites which could reduce populations of potential citrus pests below economic injury level. Our results confirm the suitability of *F. arundinacea* as a cover crop for clementine mandarins.

S16P22

Effect of biotic and abiotic factors on the insurgence of fruit scars in Italian orange orchards.

Siscaro G.[1], Zappalà L.[1], Biondi A.[1], Conti F.[2], and Fisicaro R.[2]

[1]University of Catania (DiGeSA), Department of Agri-food and Environmental Systems Management, Italy; and [2]Sicilian Region - Assessorship of Agricultural and Food Resources (SFR - OMP), Regional Plant Health Service, Italy. fconti@regione.sicilia.it

Citrus fruit rind scars are corky tissue formations, the structure, shape and distribution of which may be very variable on the fruit surface. Such phenomena have been increasingly recorded during the last few years on lemon and orange fruits, as confirmed by surveys carried out in several Italian packing houses. They are referred to fruit scars induced by various biotic factors such as thrips feeding and physical damage, mainly caused by brunch rubbing due to wind or spraying equipments. In 2011, a field survey was conducted in a Sicilian organic orange orchard, to evaluate possible correlation between thrips infestation, brunch rubbing and fruit scars. Two plots were evaluated; one planted with the variety Navelina and the other with Tarocco Scirè. No insecticides were applied during the observation period. Thrips infestation was monitored weekly from the flowering to fruit-set, and type, incidence and severity of fruit scars were assessed by field direct observations at fruit-set and harvest. In addition, a survey was carried out in 7 packing houses located in the same production area to assess the rate of downgrading due to fruit scars. Although very few flowers and young fruits were found infested by thrips (mainly *Pezothrips kellyanus* and *Frankliniella occidentalis*),

a high incidence of damaged fruits (up to 40%) was observed in both varieties, with scars apparently due to mechanical damage. The same trend, both in terms of type and incidence, was observed during the packing houses survey with an average of 35% of scared fruits (min 20-max 50%). The results showed that few scars are attributable to thrips infestation, while physical factors, notably wind and spraying equipment, play the key role. This finding also stresses the urgent need to carefully discriminate the origin of fruit scars in order to avoid unnecessary insecticide applications in citrus.

S16P23

Thrips population dynamics, composition and first survey of ground-dwelling predatory mites in lemon orchards in Italy.

Biondi A.[1], Zappalà L.[1], Tropea Garzia G.[1], Perrotta G.[2], and Siscaro G.[1]

[1]University of Catania (UNICT), Department of Agri-food and Environmental Systems Management, Italy; and [2]Assessorato regionale delle Risorse agricole e alimentari, Regione Siciliana (U.P.A.), Dipartimento Regionale Azienda Foreste Demaniali, Italy.
antonio.biondi@unict.it

Several thrips species are known to infest citrus orchards worldwide. Flowers are usually preferential feeding sites, but developing fruits are also attacked. *Pezothrips kellyanus* has emerged as a key thrips pest on citrus in Italy, and knowledge on its biology and natural enemies are still largely insufficient. We conducted a survey in two Sicilian lemon orchards, aimed at assessing the thrips species composition and population dynamics on lemon flowers, young fruits and weed flowers. Thrips ecology was also studied placing double-sided sticky traps under the tree canopy. Ground-dwelling predatory mites were collected in the soil samples from below the canopy using a Berlese apparatus. Kelly's thrips was the most abundant species (88.6-91.2%) infesting lemon and its presence was well correlated to blooming. On the 23 weed species sampled, the commonest thrips were *Frankliniella occidentalis* and *Megalurothrip* ssp., while *P. kellyanus* was observed only on 6 species, mainly Asteracee. The data obtained confirmed a significant soil phase of the pest, since in the up and bottom trap sides there were two peaks of nymphs and adults, respectively. Of sampled mites, 20% were predaceous Laelapidae, Macrochelidae and Phytoseiidae. This study is the first step towards establishing a scientific basis for an effective soil and weed management system aimed at reducing thrips infestation in Italian lemon orchards.

S16P24

Pollen availability in the cover crop may affect the biological control of *Tetranychus urticae* in citrus.

Pina T.[1], Argolo P.S.[2], Urbaneja A.[2], and Jacas J.A.[1]

[1]Universitat Jaume I (UJI); Unitat Associada d'Entomologia UJI-IVIA, Departament de Ciències Agràries i del Medi Natural, Spain; and [2]Instituto Valenciano de Investigaciones Agrarias (IVIA); Unidad Asociada de Entomología UJI-IVIA, Departamento de Entomología, Spain. pina@uji.es

Tetranychus urticae Koch (Acari: Tetranychidae) populations in citrus can be regulated through the management of the cover crop. A mono-specific cover of the grass *Festuca arundinacea* Schreber (Poaceae) enhanced the diversity and abundance of Phytoseiidae mites compared with a multifloral cover. This increase resulted in a better control of *T. urticae*. Pollen availability and quality could be behind this result. Whereas multifloral cover offers a long-term source of a wide variety of pollen, *F. arundinacea* offers one single type of pollen in spring only. As a consequence, the generalist pollen feeder *Euseius stipulatus* (Athias-Henriot), which exerts direct and indirect negative effects on specific predators as *Neoseiulus californicus* (McGregor), is highly favored in multifloral covers. To determine the role of pollen quality on the biological control of *T. urticae* by phytoseiid mites, the effect of two types of pollen on the efficacy of *E. stipulatus* and *N. californicus* to regulate *T. urticae* populations was studied under semi-field conditions. Results suggest that pollen provision does not enhance the ability of these phytoseiids to control *T. urticae*. However, pollen quality directly affected the numbers of *E. stipulatus*. Therefore, poor quality pollen may prevent pollen feeders from reaching high numbers and indirectly benefit *T. urticae* control by specialist phytoseiids.

S16P25

Assessment of trophic interactions among citrus mites by DNA-based gut content analysis.

Pérez-Sayas C., Pina T., Gómez-Martínez M.A., Jacas J.A., and Hurtado M.A.

Universitat Jaume I de Castelló (UJI), Ciencias Agrarias y del Medio Natural, Spain. mhurtado@uji.es

Tetranychus urticae Koch and *Panonychus citri* (McGregor) (Acari: Tetranychidae) are the most important tetranychid mites affecting citrus orchards in the Region of Valencia (Eastern Spain). Their main natural enemies are different phytoseiid mites. These predators can prey on both tetranychid mite species but their actual effect in the field remains unknown. The analysis of the phytoseiid's gut content using molecular techniques can provide a new tool to quantify predation. These techniques can reveal the real prey spectrum of a polyphagous predator, and the range of predators feeding on a particular species. Squash-capture and tissue-print of field-collected phytoseiid mites followed by Multiplex PCR has revealed to be a valid alternative to classic taxonomy for mite identification. Furthermore, it has allowed establishing prey preferences and mite trophic interactions under real field conditions. In the citrus agroecosystem, the specialist *Phytoseiulus persimilis* Athias-Henriot clearly prefers *T. urticae* even when this prey is scarce, whereas the generalist pollen-feeder *Euseius stipulatus* (Athias-Henriot) behaves as an omnivorous species that does not seem to prey preferentially on *T. urticae* even when this species is highly abundant.

S16P26

Effectiveness and economics of a novel proactive approach for managing the Citrus Rust Mite in Texas.

Setamou M., and Sekula D.

Texas A&M University-Kingsville Citrus Center (TAMUK-CC), Agriculture, Agribusiness and Environmental Sciences, USA. mamoudou.setamou@tamuk.edu

The Citrus Rust Mite (CRM), *Phyllocoptruta oleivora* (Ashmead) (Acari: Eriophyidae), is considered the most economically important pest affecting citrus production in Texas citrus because the majority of citrus produced is destined for fresh fruit market. Feeding damage of the pest causes conspicuous injury to fruit leading to blemish and discoloration of the rind. In light of the absence of any effective alternative CRM control method, the use of chemical miticides is the only management strategy used by growers. Traditionally, growers scout for mite population on fruit and once a certain population level is reached starting from April-May, they decide to begin spraying. This traditional approach provides temporary control of CRM in citrus orchards. Since CRM also reproduces on leaves and citrus trees have evergreen vegetation, effective control will require targeting CRM when its populations are at their lowest during the dormant period. Thus, pre-bloom proactive spray initiation dates were compared to the traditional post-bloom date. Significant and long term reduction of CRM densities were observed with the proactive spray programs. The gross returns were 1.1 to 1.3-fold higher with the proactive spray. This novel approach also preserves predatory mite populations which would provide additional control to various mites. Although this novel approach did not reduce chemical input and associated costs of control, the better CRM control achieved made its adoption an effective alternative both ecologically and economically.

S16P27

Spider mite response in citrus rootstocks is mediated by the oxylipin pathway.

Agut B.[1], Hurtado M.A.[1], Jacas J.A.[1], and Flors V.[2]

[1]Universitat Jaume I (UJI), Unitat Associada d'Entomologia IVIA-UJI, Spain; and [2]Universitat Jaume I (UJI), Metabolic Integration and Cell Signaling Group, Spain. bagut@uji.es

Citrus rootstock selection has been traditionally based on their resistance to pathogens and tolerance to abiotic stresses. However, the resistance to arthropod pests has been consistently neglected. Recently, it has been shown that citrus rootstocks can influence the life history of the phytophagous spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae). To compare the usefulness of pathogen resistance-based rootstock selection with arthropod tolerance/resistance, we have performed comparative experiments of

spider mite performance on citrange Carrizo (Citrus tristeza virus, CTV, tolerant), Cleopatra mandarin (CTV and salt tolerant) and Sour orange (CTV sensitive and limestone tolerant). Sour orange showed reduced leaf damage symptoms and supported lower mite populations. Taking together the genetic analysis of defense pathways and the phenotype, results suggest a relevant role of the oxylipin pathway in the plant defense against *T. urticae*. Nevertheless the SA-related responses cannot be neglected, since the most resistant rootstock, Sour orange, still displayed enhanced levels of this hormone and *PR5* marker gene expression upon spider mite infestation. Because the LOX inhibitor Phenidone totally abolished the enhanced response of Sour orange, oxylipins are probably essential signals controlling citrus defense responses against arthropod infestations.

S16P28

Impact of three ant species on pest populations in Mediterranean citrus orchards.

Calabuig A.[1], Pekas A.[2], and García Marí F.[1]

[1]Instituto Agroforestal Mediterráneo (IAM), Entomología, Spain; and [2]Biobest Belgium NV (Biobest), R&D, Belgium. alteakova@hotmail.com

We studied the influence of three ant species, the native to the Mediterranean *Pheidole pallidula* (Nylander) and *Lasius grandis* (Forel) and the invasive *Linepithema humile* (Mayr), on the population densities of three citrus pests: the honeydew producer woolly whitefly *Aleurothrixus floccosus* (Maskell) and the non-honeydew producers, California Red Scale (CRS) *Aonidiella aurantii* (Maskell) and Citrus leafminer *Phyllocnistis citrella* (Stainton). From April to November 2011 we monitored ant activity, CRS densities on fruits and twigs as well as whitefly and citrus leafminer population densities in ant-allowed and ant-excluded trees in three citrus orchards each one dominated by one ant species. The most active ant species was *L. humile* (average for the observation period 64.5 ants/minute climbing up or down the tree trunk) followed by *P. pallidula* (4.5 ants/minute) and *L. grandis* (2.2 ants/minute). On fruits, *A. aurantii* densities in the ant-excluded trees were on average 31% lower for the three ant species studied than in the ant-allowed trees.- On twigs no differences in *A. aurantii* densities were detected between treatments. In the case of *A. floccosus* results varied depending on the ant species; in the presence of *L. humile,* whitefly population densities increased by 66%. Likewise, for *P. citrella* we found significant differences in densities between treatments only for *L. humile* (increase of 15% when ants were present). These results show that ant activity may have an effect on the abundance of honeydew and non-honeydew producing pests, yet this effect depends on the ant species involved.

S16P29

Ant nests distribution in citrus groves under different ground cover management strategies in Spain.

Martinez-Ferrer M.T., Campos Rivela J.M., Fibla J.M., and Pla M.

Institut de Recerca i Tecnologia Agroalimetàries (IRTA), Sustainable Plant Protection Entomology, Spain. teresa.martinez@irta.cat

Ants represent a large proportion of the arthropods present in citrus ecosystem. Their activity may have a striking impact on the abundance of the rest of the arthropods, pests and natural enemies. The structure of ant communities may change as a result of changes in habitat, differences in growth or in the physical structure. Ground cover management can modify the inter-specific competition between ant species. The ant nests spatial distribution, diversity and abundance were studied in eight citrus groves in northeastern Spain (Tarragona) under different ground cover management: a) resident wild cover, b) sown cover of *Festuca arundinacea* and *Poa annua*, and c) bare soil. The area covered by 60 trees -ten trees per six contiguous rows- and the corresponding transects among the rows was monitored in spring, summer and autumn. The number of active nests of each species and the position of the nests were recorded. Ant nests distribution was obtained by the distance to the nearest neighbor method. The intra-annual nest permanence and the nest renovation indexes were determined for the most abundant ant species. The results obtained are presented and discussed.

S16P30

Composition and flight activity of Staphylinidae (Coleoptera) in citrus orchards

Adorno A.[1], Biondi A.[2], Zappalà L.[2], Siscaro G.[2], and Sabella G.[1]

[1]University of Catania (UNICT), Department of Biological, Geological and Environmental Sciences, section of Animal Biology "M. La Greca", Italy; and [2]University of Catania (UNICT), Department of Agri-food and Environmental Systems Management, Italy.
antonio.biondi@unict.it

Staphylinid beetles are among the most important epigeic polyphagous arthropods living in various agro-ecosystems and represent an important component of functional biodiversity. Their flight activity in citrus orchards is still poorly investigated and, since most of them are insect predators, many species may play important roles in pest control. The aim of this study was to gain knowledge of the flying staphylinid fauna and its temporal distribution in citrus orchards. A survey was carried out, from December 2009 to August 2011, in a 70ha citrus farm located in the typical orange production area of Sicily. Two types of flight interception trap techniques were used: (i) 12 window traps maintained over one year and the trap content was serviced fortnightly, and (ii) 21 samplings carried using a net-car trap. The sampled Staphylinidae belong to 150 species, with 30 representing more than 90% of the whole sampled material. The most abundant, presented in decreasing order of collected number of specimens, were: *Megarthrus bellevoyei, Anotylus nitidulus, Gabronthus maritimus, Paraphloeostiba gayndahensis, Scopaeus debilis, A. speculifrons, Cordalia obscura, Amarochara umbrosa, Proteinus atomarius, Carpelimus corticinus, Philonthus concinnus, Heterothops minutus, S. mitratus, Tachyporus nitidulus, Aloconota gregaria, Atheta palustris*. Peak of flight activity for most species was typically in spring or spring-autumn, while species with strictly summer and winter peaks were a minority. Only some of the sampled species have been reported as potential biocontrol agents of citrus pests and further investigation is needed to assess the ecological services that they can provide in an integrated pest management framework. The results proved that several predatory staphylinid species do fly actively within a citrus orchard, suggesting the importance of increased knowledge on the dynamics of movement from neighboring ecological infrastructures to citrus cultivations.

S16P31

Entomo-pathogenic nematodes to combat -infestations at ticofrut

Camacho H.[1], Yglesias G.[2], and Faerron P.[2]

[1]Tico Frut Company. University of Costa Rica - Fabio Baudrit Agricultural Research Station, Costa Rica; and [2]TicoFrut, Agricultural Department, Costa Rica. hcamachov@hotmail.com

Tico Frut´s 15,000 hectares of orange groves in Costa Rica undergo frequent infestations of *Phyllophaga* spp. beetle larvae (Coleoptera: Scarabedidae) and *Nasutitermes* spp. termites (Ispotera: Termitidae) both of which cause major losses. In order to eliminate these pests by means of environmentally low-impact strategies, a program was implemented to apply the nematode *Heterorhabdites bacteriophora* (Nematoda: Heterorhabditidae). The nematodes are raised en masse in the larvae of *Galleria mellonella*. Each moth produces an average of 517 eggs during its life cycle, and lays them on wax paper in jars. 0.5 grams of eggs (approximately 1,100 ova) are placed on 100 grams of artificial food, where eclosion then occurs. Once they hatch, the larvae develop on a simple diet, with a density of 11 larvae per gram of food, during 15 days. 50% of the larvae survive. To complete their growth, the larvae are put into larger containers holding with 14,000 larvae apiece for 15 days. The *Galleria* larvae are then parasitized by the nematodes in containers: in each one 200 *Galleria* larvae are placed along with 100 infectious nematode. After thirteen days, the infectious juveniles are collected in white traps. For this year´s planting of 500,000 orange tree seedlings, 1000 nematodes were put into five liters of water per tree; this approach was needed due to the fact that the replanting had to be carried out during the period prior to the start of the rainy season, when the soil was very dry. In order to apply the nematodes to the termite mounds, an opening was made in the middle section of each one, and it was then sprayed with the nematode-water solution with a backpack pump unit. Both the beetle larvae and the termites were infected with the bacteria transmitted by the nematodes, and they died within a 24 to 28 hour period. This control method does not pollute the environment; it works quickly without damaging water sources or harming humans, and is highly effective.

S16P32

Monitoring and Management of *Naupactus (= Asynonychus) cervinus* in citrus orchards in Chile.

Luppichini P., and Olivares N.

Instituto de Investigaciones Agropecuarias (INIA), CRI, La Cruz, Chile. pluppich@inia.cl

Naupactus cervinus (Boheman) (Coleoptera: Curculionidae) is a pest rarely treated in Chilean citrus orchards. However, the detection of several egg masses under orange and lemon fruit calix has been reported as a refusal cause of the exported Chilean fruit. In order to improve-*N.-cervinus* control and to reduce its incidence in Citrus orchards, this study raises the hypothesis that an optimal pest management requires the design of an integrated monitoring system. Integrated pest management (IPM) assays were conducted in citrus orchards (orange and lemon) within the Valparaíso Region. Commercial *N. cervinus* pesticides and a toxic barrier were used as treatments. Prior to the application of the treatments, *N. cervinus* adult stage presence was monitored in tree canopies by shaking the branches and collecting the fallen insects in a plastic sheet placed underneath the trees. The pesticides (azinphosmetil, indoxacarb and methidathion) were applied to the tree's canopy at manufacturer's recommended dose. The toxic barrier (a.i. azinphosmetil) was physically placed around the trunk of the trees. Adults were collected from tree leaves every 15 days, 90 days after the application of the treatments, for evaluation. Foliar applications of azinphosmetil and indoxacarb display an effective control in orange and lemon orchards. However, the toxic barrier showed superior control performance compared to the sprayed pesticide treatments. Therefore, we conclude that the toxic barrier is an alternative for *N. cervinus* control in IPM.

S16P33

Population dynamics of citrus blackfly in Tahiti lime in the state of São Paulo, Brazil.

Felippe N.[1], and Raga A.[2]

[1]Pontificia Universidade Católica de Campinas, Brazil; and [2]Instituto Biológico, Brazil. adalton@biologico.sp.gov.br

Field investigations on the seasonal population dynamics of the Citrus Blackfly (CBF) *Aleurocanthus woglumi* Ashby (Hemiptera: Aleyrodidae) in Tahiti lime (*Citrus latifolia*) orchard were conducted at Artur Nogueira municipality, São Paulo State, Brazil, from August 2011 to June 2012. Twenty leaves per mature tree were collected at fortnightly intervals from ten previously marked trees. Under stereoscopic microscope we evaluated the spiralling oviposition and nymphal population. CBF showed the highest peak of oviposition during August 2011, reaching 52.9 eggs/leaf. From overall collections we obtained 23.0 eggs/oviposition, ranging from a minimum of 7 eggs and a maximum of 53 eggs/oviposition. Approximately 92% of the ovipositional masses had between 12 and 39 eggs. CBF egg-laying is confined to the lower leaf surface (abaxial). The highest nymphal population occurred during early April 2012, with average of 13.0 nymphs/leaf. CBF immature population declined during the wetter season (October to March). The entomopathogenic fungus *Aschersonia aleyrodis* and *Aegerita webberi* occurred all year long and during the rainy season.

S16P34

Integrated strategies to monitor and control Citrus Flower Moth, *Prays citri* Mill. (Lepidoptera: Yponomeutidae) on nursery trees of the Mediterranean area

Conti F., and Fisicaro R.

Sicilian Region. Assessorship of Agricultural and Food Resources (SFR - OMP), Regional Plant Health Service, Italy. fconti@regione.sicilia.it

Prays citri Mill. (Lepidoptera: Yponomeutidae), the Citrus Flower Moth (CFM), is regarded as a key pest of nursery trees in Sicily. Larvae damage flowers and new shoots. Furthermore, young larvae can penetrate small fruits. Growers may spray several times per year mainly with organophosphate insecticides. During the last 5 years, a new IPM approach has been evaluated. Different pesticides were assayed and the possibility to predict infestation by means of monitoring male flights with pheromone traps was assessed. In 2007,

2010 and 2011, the efficacy of spray applications of fosmet, flufenoxuron and *Bacillus thuringiensis* was evaluated on 2-yr old lemon trees. Spray applications were carried out weekly from May to June, for a total of 6 treatments per year. Results suggest that OPs could be substituted, or at least alternated, with IGRs and BT. Starting from 2006, pheromone traps (Z-7 tetradecenale) were placed in lemon plots and the infestation level on flower, young fruits and new shoots was recorded. All set of data produced a robust correlation (r^2= 0.82) between male captures and infestation percentage. Depending on the economic target chosen, a maximum level of 120 captures/trap per week could be set as the action threshold.

S16P35

Biology of Citrus Fruit Borer, *Citripestis sagittiferella* Moore and its control.

Srijuntra S., Manusmunkong B., Sahaya S., Jumroenma K., and Srikachar S.

Plant Protection Research and Development Office), Department of Agriculture, Thailand. nutaa2000@yahoo.com

Studies on the biology of the citrus fruit borer, *Citripestis sagittiferella* Moore were carried out at the laboratory of Entomology and Zoology Research Group, Plant Protection Research and Development Office, Bangkok, and pummelo orchards at Trat and Chumphon province during October 2005 to April 2007. Results showed that females lay eggs in clusters (2-29 eggs) on the fruit at night. The egg is round in shape and white in color. The incubation period is 5.30±0.87 days. The larvae of *C. sagittiferella* molt four times and total larval stages last 14.60±0.52 days. The growth increment of larvae based on the width of head capsules assumed a geometric progression with a ratio of 1.61. The final instar larva pupates in the soil and requires 6.77 ± 1.88 and 5.83±1.54 days for male and female, respectively, to reach adulthood. The longevity of adult male and female are 5.72±1.18 and 5.88±1.24 days, respectively. The survey of *C. sagittiferella* natural enemies yielded one species, *Trichogrammatoidea* sp., an egg parasitoid. Damaged and fallen fruit should be removed from orchards to control this moth. Infested areas should be sprayed with insecticides four times on a weekly basis after fruit setting. Then fruit should be wrapped to prevent infestation by *C. sagittiferella*.

S16P36

Spirotetramat (Movento®): a new tool for *Aonidiella aurantii* Maskell management in Spain.

Izquierdo J., and Fullana J.

Bayer CropScience, Spain. josep.izquierdo@bayer.com

Spirotetramat (Movento®) is a new insecticide (full translocation in xylem and phloem) which has been developed for worldwide use in numerous crops. Spirotetramat is listed in Group 23 of the IRAC (Insecticide Resistance Action Committee) mode-of-action classification scheme as an inhibitor of lipid biosynthesis in targeted pest species. It displays a completely new mode of action for control of several sucking insect pests which are known to seriously damage agricultural crops. California red scale (CRS), *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae), is a key pest of citrus in the Mediterranean basin where it is widely distributed. Despite the few available insecticides, chemical control is still the commonest method used against this pest. Herein, we show the efficacy of spirotetramat against CRS in citrus and enhance some of its advantages for the practice. Spirotetramat showed high levels of efficacy against CRS in the numerous field trials carried out in Spain since 2002. Several characteristics of spirotetramat explain these results. The foliar penetration is of utmost importance to allow the transport of the product in the plant vascular system and to exploit its full biological potential. The phloem-mobile molecule like spirotetramat, acting mainly via ingestion, favours the control of hidden targets as CRS in comparison to contact products. Spirotetramat affected all immature stages of CRS offering an interesting flexibility in the application periods according to the specific targets and needs of the growers.

Finally, the low impact of spirotetramat on key natural enemies in citrus allows the inclusion of this product in IPM programs where biological control is promoted and improves the results by the integration of methods.

S16P37

Carbendazim, mancozeb and pyraclostrobin residues resulting from the chemical control of black spot (*Guignardia citricarpa*) in Valencia oranges in NE

Kulczycki Waskowicz C.[1], Sosa A.[1], Becerra V.[2], Navarro R.[2], Burdyn L.[1], Garran S.M.[1], and Mousqués J.[1]

[1]National Institute of Agricultural Technology, Concordia Agricultural Experiment Station (INTA, EEA Concordia), Pesticides Residues Plant Protection, Argentina; and [2]National Institute of Agricultural Technology, Mendoza Agricultural Experiment Station INTA (INTA, EEA Mendoza), Phytopharmacy Study Center, Argentina. ckulczycki@correo.inta.gov.ar

Black Spot, a severe disease caused by the fungus *Guignardia citricarpa* Kiely affects citrus orchards decreasing both yield and fruit quality. Chemical control using different fungicides is the most effective control method. However, legal and food safety requirements make imperative to know actual level of pesticide residues at harvest. The objective of this study was to determine fungicide residue levels on ripe orange fruit resulting from different chemical control programs. Trials were conducted on a commercial orchard of Valencia oranges, using a randomized four blocks design where four different fungicide treatments besides the control were tested. The fungicides mancozeb, pyraclostrobin, and carbendazim, were applied at different times, from October 2009 to April 2010. Once fruit reached commercial maturity as required, in October 2010, fruit samples were collected and immediately processed. Pyraclostrobin and carbendazim residues were analyzed using liquid chromatography (detection limits of 0.03 mg/kg and 0.02 mg/kg, respectively). Mancozeb was analyzed by a colorimetric method based on a process of spectrometric quantification of CS2 (detection limit 0.1 mg/kg). In all cases, residues were below detection limits.

S16P38

Evaluation of a novel insecticide, DPX-HGW86 10% OD (Benevia™) and DPX-HGW86 20% SC (Verimark™), for *Diaphorina citri* (Hemiptera: Psyllidae) control.

Miranda M.P., Felippe M.R., Garcia R.B., Caldeira R.E., and Noronha Jr N.C.

Fundecitrus (Fundecitrus), Scientific Department, Brazil. mpmiranda@fundecitrus.com.br

Diaphorina citri is the vector of huanglongbing in Brazil and elsewhere. Insecticidal control is the primary tactic against this insect. Rotation of insecticidal modes of action is important to avoid psyllid resistance and therefore new chemistry is for management of this pest. Thus, this research was carried out to evaluate the efficacy of DPX-HGW86 formulated either as a 10%OD (Benevia™) or a 20%SC (Verimark™). These products represent a novel diamide chemical class of insecticide for *D. citri* control either as a foliar spray or drench application respectively. For the foliar spray trial, citrus nursery trees with fully expanded leaves were treated with the following products and doses (ml commercial product/100L water): Benevia at 50, 75, 100 and 125ml; imidacloprid 20%SC at 20ml and Lambda-cyhalothrin 5%CS at15ml. For the drench application trial, nursery trees with new shoots were treated with Verimark at 1.75, 3.5, 7ml commercial product/plant and imidacloprid 20%SC 1.75ml c.p./plant. Both experiments were conducted in a greenhouse. Mortality was assessed every week up to 50 days after application (DAA) for spray treatments, and for drench treatments up to 104 DAA. Both applications resulted in good efficacy (mortality≥80%) against *D. citri* adults, similar to the standard treatments. The systemici activity observed with Verimark™ is important because presently, only the neonicotinoid class is available with this feature for adult psyllid control. Therefore, both Benevia™ and Verimark™ will be useful for insecticide resistance management.

S16P39

Effects of foliar applications of pyrethroid and neonicotinoid insecticides on the populations of *Panonychus citri* (McGregor, 1916) (Acari: Tetranychidae) in laboratory.

Zanardi O.Z., Rugno G.R., Bordini G.P., Beloti V.H., and Yamamoto P.T.

Escola Superior de Agricultura Luiz de Queiroz (ESALQ), Entomologia e Acarologia, Brasil. gabrielrugno@hotmail.com

Overuse of pyrethroids and neonicotinoid insecticides could be one a cause of outbreaks of *Panonychus citri* (McGregor, 1916) in citrus orchards. This work investigated the effect of foliar application of these insecticides on intrinsic rate of increase (r_i) of *P. citri* in the laboratory. Treatments and doses (g a.i./100 L) evaluated

were: esfenvalerate (0.25); deltamethrin (0.75); lambda-cyhalothrin (1.0); imidacloprid (4.0); thiamethoxam (2.5) and control (distilled water). Citrus seedlings 30 cm in height with eight fully-developed leaves where sprayed. After residues dried, two newly emerged *P. citri* females were placed on each leaf. The plants were placed in a room with controlled temperature at 25±2°C, RH 70±15% and photophase of 14h. The design was completely randomized with five treatments and three replications. Results were evaluated 28 days after application by counting all living individuals. Intrinsic rate of increase was determined by the equation r_i = [ln (N_f/ N_0)/Δt], where: N_f = final number of individuals, N_0= initial number of individuals and Δt = duration of experiment. The r_i ranged from 0.014 to 0.054, not significantly different among treatments. These results suggest that the insecticides evaluated did not affect the mite population, being, therefore, one of the causes for the outbreak population of *P. citri*.

S16P40

Side effects of some pesticides under field conditions on important parasitoids and predators in citrus ecosystem

Simsek V.M.[1], Uygun N.[2], and Satar S.[2]

[1]Bayer CropsScience (BCS), Bayer CropsScience, Adana-Turkey; and [2]Çukurova University (ÇU), Department of Plant Protection, Adana - Turkey. hserhat@cu.edu.tr

Side effects of pyriproxyfen, buprofezin, chlorpyrifos-ethyl, spirotetramat, mineral oil and paraffinic mineral oil on some predators and parasitoid were investigated in a 12 years old orange orchard infested with *Panonychus citri* (McGregor) (Acari: Tetranychidae) and a 16 years old lemon orchard infested with *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae). Trials were carried out under field conditions in 2008 and 2009 for *P. citri* and 2007 and 2008 for *A. aurantii*. All treatments were replicated four times in a randomized block design. After insecticide treatment, predators were collected with Steiner funnel and parasitoids for *A. aurantii* were obtained by collecting plant materials at -1st, 7th, 14th and 21st days for both years. Effects of pesticides were classified according to IOBC standards. *Chilocorus bipustulatus* (L.) (Coleoptera: Coccinellidae) (74.31%) and *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) (>90%) for *A. aurantii* infested orchard, and *Stethorus gilvifrons* (Mulsant) (Coleoptera: Coccinellidae) (85.06%) for *P. citri* infested orchard were recorded as main predators and parasitoid in 2007 and 2008. The side effects of the pesticides on predators of *P. citri* were found statistically significant for both years, but they were found moderately harmful (51-75%) for IOBC field evaluation. Similar results were also obtained from *A. aurantii* infested orchards, as predators were affected from pesticide at statistically different level. The lowest populations reduction was calculated for spirotetramat (45.8% for 2007, 55.1% for 2008) and paraffinic mineral oil (45.8% for 2007, 54.3% for 2008), while the highest level of reduction in predators populations were observed in chlorpyrifos-ethyl (91.7% for 2007, 87.9% for 2008) and pyriproxyfen (91.7% for 2007, 85.6% for 2008). Although effect of pesticides on *A. melinus* were statistically different from each other, all pesticides were grouped as moderately harmful (51-75%) or harmful (>75).

S16P41

Side effects of some pesticides under laboratory conditions on important parasitoids and predators in citrus ecosystem.

Simsek V.M.[1], Uygun N.[2], and Satar S.[2]

[1]Bayer CropsScience (BCS), Bayer CropsScience, Adana-Turkey; and [2]Çukurova University (ÇU), Department of Plant Protection, Adana - Turkey. mehmet.simsek@bayer.com

The laboratory tests were conducted to evaluate side effect of insecticides pyriproxyfen, buprofezin, chlorpyrifos-ethyl, spirotetramat, mineral oil, paraffinic mineral oil and spinosad on *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae), *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae), *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) and *Sympherobius pygmaeus* (Rambur) (Neuroptera: Hemerobiidae). Contact toxicities of insecticides on beneficials and their progenies were monitored under guidelines of IOBC. According to IOBC classification, pyriproxyfen was harmless on *A. melinus*, *L. dactylopii* and *C. montrouzieri* adults and harmful on *C. montrouzieri* and *S. pygmaeus* larvae; buprofezin showed similar

result except *S. pygmaeus* larvae; chlorpyrifos-ethyl was harmful on all parasitoids and predators except *C. montrouzieri* larvae and adult; spirotetramat was harmless on parasitoids and predators except *A. melinus*, it slight harmful on it; mineral oil was harmless on *L. dactylopii* and *C. montrouzieri* adults, slightly harmful on *A. melinus* adult, *C. montrouzieri* and *S. pygmaeus* larvae; paraffinic mineral oil was harmless on *A. melinus*, *L. dactylopii* and *C. montrouzieri* larvae-adults, and slightly harmful on *S. pygmaeus* larvae; Spinosad was harmless on *C. montrouzieri* larvae-adults and *S. pygmaeus* larvae, moderately harmful on *L. dactylopii* and harmful on *A. melinus*. The progeny production of each surviving beneficial treated with spirotetramat at adult and larval stages were not affected by treatment and similar results were also observed pyriproxyfen and buprofezin except larval stage of predators, meanwhile spinosad has slightly effect on fertility of predators. The adult or larval stages of *C. montrouzieri* have slightly reduced number of progeny after chlorpyrifos-ethyl treatment.

S16P42

New book on citrus pests: "Citrus pests. Integrated pest management in countries of Mediterranean climate" (Plagas de los cítricos. Gestión integrada en países de clima mediterráneo).

Garcia-Mari F.

Universitat Politècnica de València (UPV), Instituto Agroforestal Mediterráneo, Spain. fgarciam@eaf.upv.es

This book, in Spanish and appearing in October 2012, includes published information on citrus pests throughout the world in general, and particularly from Mediterranean countries. It gathers and synthesizes in a descriptive way the up to date knowledge on pest identity, morphology, biology, seasonal cycle and management methods, including sampling methods, treatment thresholds and the main natural enemies, as well as chemical and biological control. The book includes 180 color plates, with more than 1,200 pictures, to help recognize the most important phytophagous arthropods which affect the crop, their symptoms and damage, and their most relevant natural enemies. On the other hand, the book includes an analytical study, from a demographic point of view, of the pest situation in citrus crops in the east of the Iberian Peninsula, discussing the main factors which influence the structure and development of pest populations and determine its abundance and evolution. For this purpose, many experimental data from samplings and trials are presented in more than 60 tables and near 200 figures. The observations carried out have allowed the development of this book which reflects accurately the current situation of arthropod pests in citrus crops.

S16P43

Impact of pesticides used in citrus orchards on the beneficial insects *Cryptolaemus montrouzieri* Mulsant

Rahmouni R., and Chermiti B.

High Agronomic Intitute of Chott-Mariem (ISACM), Laboratory of Entomology and Biological Control, Tunisia.
chermiti54@yahoo.fr

Adults and larvae of *Cryptolaemus montrouzieri* Mulsant, a predator of the mealybug *Planococcus citri* Risso, were exposed to direct sprays and to leaf discs treated with Malathion, Acetamiprid, Cyhexatin, Tetradifon, Methidathion, and Spinosad. Active substances were very toxic when directly sprayed on larvae with mortality rates around 80.0% except for Spinosad, which was less toxic (39.0% mortality). Direct spray of adults caused mortality rates of 100% for Acetamiprid and 18.8% for Spinosad. On treated leaf discs, larval mortality rates varied between 73.0 and 84.0% except for Spinosad that showed 48.0% mortality only. In relation to adults, only Acetamipride and Methidathion were very toxic with mortality rates higher than 70.0%, whereas Malathion, Cyhexatin Tetradifon and Spinosad caused mortalities of 47.5, 31.0 and 18.8%, respectively.

S16P44

Preliminary survey of the Green Lacewings (Neuroptera, Chrysopidae) in citrus orchards in Northern east of Tunisia (Cap Bon).

Limem Sellami E., and Chermiti B.

Higher Agronomic Institute of Chott-Mariem (ISACM), Laboratory of Enomology and Biological Control, Tunisia. chermiti54@yahoo.fr

Aphids are one of the key pests affecting citrus in Tunisia. Nowadays, their control is mostly chemical. However, because of the need for a more sustainable citrus industry, other biologically-based control methods are under study. One of them involves the biological control by means of natural enemies. As a first step, increased knowledge on the actual diversity of-natural enemies in this citrus system is needed. Because lacewings (Neuroptera: Chysopidae) are one of the most important group natural enemies of aphids, a survey of lacewings occurring in citrus orchards in NE Tunisia (Cap Bon) was conducted. 3 organic-and 4 conventional citrus orchards were compared during two consecutive years (2010, 2011). Adults were collected with avacuum aspirator; whereas pre-imaginal instars were collected from aphid infested shoots. Six green lacewing species were identified: *Chrysoperla lucasina*, *C. carnea*, *C. affinis*, *Dichochrysa flavifrons*, *Dichochrysa prasina* and *Chrysopaformosa*. *C. lucasina*-was the dominant species (53%) in all orchards. Adults of the complex *C. carnea* were recorded-from February to December and showed two peaks-in May-June and in September. The occurrence of pre-imaginal instars is related to aphid activity. Diversity of green lacewing is generally low (H'= 1.27). However, Shannon index was highest in organic orchards. Dominance and equitability index (respectively 0.8 and 0.2) showed a dominance of the *C. carnea* complex in all orchards. This species could be managed for the biological control of aphids in citrus orchards.

S16P45

Mating disruption technique as a control method for California red scale: a review of doses, efficacy and date of application

Navarro-Llopis, V., Alfaro C., Vacas S., and Primo Millo J.

Instituto Agroforestal del Mediterráneo (CEQA). Universitat Politècncia de València. vinallo@ceqa.upv.es

California red scale, Aonidiella aurantii Maskell, is a key pest for citrus fresh market around the world. Chemical control with organophosphates, insect growth regulators or oils has been widely used, although the increasing resistance to organophosphates, the effect of IGR over non-target organisms and the difficulties in applying oils suggest the development of other IPM control methods. In the present work, the use of mating disruption is proposed as a new control method which can be applied alone or combined with other methods. The pheromone dose, the number of release points per surface and the date of application have been studied for an efficient application of the mating disruption technique. The importance of emitting the pheromone with zero order kinetics is well known in mating disruption. For this purpose, we designed a new mesoporous dispenser that releases the pheromone in an efficient way during more than 150 days. After 6 years of studies, results suggest a minimum dose of 35 g of pheromone ha-1, a minimum density of 450 dispenser ha-1 and the application of dispensers just before the second male's flight. In these conditions, an efficacy of at least 90% was obtained in field trials, taking special importance to the reduction in the percentage of fruit with more than 5 scales. The effect of this method over A. aurantii parasitoids was also studied suggesting a total compatibility of augmentative releases and mating disruption.

S16P46

Physiological selectivity of insecticides used for control of *Diaphorina citri* (Kuwayama, 1908) on larvae of *Ceraeochrysa cubana* (Hagen, 1861).

Rugno G.R., Zanardi O.Z., Beloti V.H., Cunha R.A., Barbosa V.G., Parra J.R.P., and Yamamoto P.T.

Escola Superior de Agricultura Luiz de Queiroz (ESALQ), Entomologia e Acarologia, Brasil. gabrielrugno@hotmail.com

This study investigated the effect of 11 insecticides used for control of *Diaphorina citri* (Kuwayama) on the larval survival of *Ceraeochrysa cubana* (Hagen). First instar lacewing larvae 48 h old were sprayed using

a Potter spray tower (spray deposit 1.9 mg /cm^2). After application, larvae were placed in flat-bottomed glass tubes and held at 25±2°C, 70±15% RH and 14 h photophase and fed eggs of *Anagasta kuehniella* (Zeller). Experimental design was completely randomized with four replicates and 10 larvae/replicate. Larval survival was assessed 24 h after application and categorized according to the IOBC/WPRS classification. The insecticides azadirachtin (AzaMax®), imidacloprid (Evidence 700 WG®) and phosmet (Imidan 500 WP®) were classified as innocuous to *C. cubana* (Class I, <30% mortality). Thiamethoxam (Actara 250 WG®), thiamethoxam (Engeo Pleno®), imidacloprid (Provado 200 SC®), esfenvarelate (Sumidam 150 SC®) and pyriproxyfen (Tiger 100 EC®) were slightly harmful (Class II, 30-79%) and chlorantraniliprole (Ampligo®), chlorpyriphos (Lorsban 480 BR®) and malathion (Malathion 1000 EC®) were moderately harmful (Class III, 80-99%). The insecticides AzaMax, Evidence and Imidan are therefore considered compatible to larvae of *C. cubana*. None of the tested insecticides were considered harmful (Class IV, >99%).

S16P47

Distribution of the Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Psyllidae) in Iran

Parsi F.[1], Manzari S.[2], Askari M.[3], Amin G.[4], Ranjbar S.[5], and Naseri M.[6]

[1]Iranian Research Institute of Plant Protection (IRIPP), Agricultural Entomology, Iran; [2]Iranian Research Institute of Plant Protection (IRIPP), Insect Taxonomy, Iran; [3]Agricultural and Natural Resources Research Center of Hormozgan, Agricultural Entomology, Iran; [4]Agricultural and Natural Resources Research Center of Fars, Agricultural Entomology, Iran; [5]Agricultural and Natural Resources Research Center of Jiroft and Kahnouj, Agricultural Entomology, Iran; and [6]Agricultural and Natural Resources Research Center of Kerman, Agricultural Entomology, Iran. farparsi@yahoo.com

The Asian Citrus Psyllid *Diaphorina citri* Kuwayama, is one of the most important pests of citrus orchards in Iran. The insect transmits the pathogen that causes citrus greening disease. It was firstly discovered in Balouchestan province by Bove in 1999. In order to manage and conduct a programme to control Greening disease, it is necessary to have distribution information on *D. citri*. Hence, its different populations were monitored in 8 provinces in the north and south Iran from 2010 t0 2011. Over 2,300 records have been established by GPS and data mapped in ArcGIS9.3.1. A distribution map based on two samplings per year is presented. The ACP was found in Kerman, Balouchestan, Fars and Hormozgan provinces but not in Gilan, Mazandaran, Khozestan and Boushehr. In 2010, *D. citri* was discovered in Fars province and one year later the population was found to be expanded significantly.

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VARIETIES

S17O01

New citrus variety evaluation in Australia 2005-2012

Sanderson G.[1], Creek A.[2], Lacey K.[3], and Wallace M.[4]

[1]Dareton Primary Industries Institute (DPII), NSW Department of Primary Industries, Australia; [2]Centre for Irrigated Agriculture, Griffith (CIA), NSW Department of Primary Industries, Australia; [3]Intensive Plant Research Branch (IPR), West Australian Department of Food and Agriculture, Australia; and [4]Orchard Monitoring Services (OMS), Citricare, Australia. graeme.sanderson@dpi.nsw.gov.au

Evaluating new citrus varieties is a national project in Australia led by the New South Wales Department of Primary Industries (NSW DPI) and is a continuance of the Australian Citrus Improvement Program. This long term program is an industry driven initiative aimed at maintaining productivity and profitability. A cooperative agreement was developed between NSW DPI, Horticulture Australia Ltd (HAL), Auscitrus and local variety managers of Plant Breeders Rights (PBR) varieties to undertake independent evaluation from 2005 to the present. Thirty one new varieties were included in the evaluation and their performance assessed in the major citrus growing regions of Australia. The varieties are predominantly mandarin and mandarin hybrids and 20 of the 31 have PBR protection. Varieties have been progressively released from Australian Post Entry Plant Quarantine with the final variety being field established in November 2011. Evaluation activities include standard fruit quality and yield determination as well as providing larger volumes of fruit from topworked trees to approved agents for test marketing. Evaluation site visits to view PBR varieties are organised in conjunction with variety managers and fruit displays provided in the major citrus regions of Australia. New variety managers are encouraged to become part of the program and seven new varieties were included in 2011. The Australian Citrus Industry's 'Strategic Research and Development Plan 2012-2017' recognises the need for continued evaluation of new citrus introductions and on-going funding will be sort from Horticulture Australia Ltd to continue the program.

S17O02

'Sweet Sicily' and 'Early Sicily', two new triploids from the program of CRA-ACM, Centro di- Ricerca per l'Agrumicoltura e le Colture Mediterranee, Acireale, Italy

Russo G., Reforgiato Recupero G., Recupero S., and Pietro Paolo D.

Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee (CRA-ACM), Italy. giuseppe.russo@entecra.it

The development of triploid hybrids by crossing a monoembryonic 2x female parent with a 4x male parent has been the successful breeding strategy carried out by the CRA-ACM since 1978. Through this strategy hybrids issued in the past allowed the obtainment of seedless fruits, in some cases pigmented, of good size, mature in different periods. In 2011 other two promising hybrids, 'Sweet Sicily' and 'Early Sicily', due to their positive characteristics, were released. The former was obtained by the cross 'Comune´ clementine X 4x 'Tarocco' sweet orange, the latter by the cross 'Oroval' clementine X 4x 'Tarocco' sweet orange. The fruits of these hybrids, very early in ripening time (mid-November) showed a fresh market suitable fruit size, excellent organoleptic properties and a typical taste intermediate between the clementine and 'Tarocco' orange.

S17O03

Comparison of performance of different late-maturing navel selections around the world.

Rabe E.[1], Turner P.[2], and Chavarria J.[3]

[1]BiogoldUSA and Paramount Citrus, California, USA; [2]Biogold International, South Africa; and [3]BiogoldEM, Spain. erabe@paramountcitrus.com

During the late 1980's and early 1990's, a number of late navel selections of Australian origin were commercialized around the world. In addition to the earlier-discovered and distributed 'Lane Late' selection, the newer Australian selections included: 'Barnfield', 'Powell', 'Autumn Gold', 'Chislett' and a host of other's, most originating from the 'Lane Late'. Data including acreage levels, yield, internal quality, pick dates and general industry perspectives from various production regions will be presented. Regions

included represent California, South Africa, Spain, Australia and Chile. In general, yields have been sub-optimal in certain regions due to late picking, especially in California, fruit sizes have been too large, granulation prevalent and general profitability not desirable. Lately, a number of new late-maturing selections have been found in South Africa. These include 'Cambria' (commercialization have commenced already), 'Witkrans', 'Glen Ora', and 'Karninka'. Initial internal quality and maturity data will be presented. Although, in South Africa, these selections have not been picked as late relative to what the practice is in California, the characteristics claimed include better return crops, smoother rinds and even later hanging ability, as compared to the Australian selections under similar conditions. In California, late navels are expected to hang into May and June, past the next season bloom period, ie up to 14 months after flowering and set. There is thus a distinct commercial gap for late selections with better cropping behavior, a smaller size manifest, retaining juice levels, less granulation and smoother rinds. The new South African late navel selections are seen as possibly meeting some of these requirements.

S17O04

Three new varieties of clementine: 'Clemenverd', 'Nero' and 'Iviaman1957'

López-García A., Terol J., Tadeo F.R., Herrero A., Ibáñez V., and Talón M.

Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Genómica, Spain. talon_man@gva.es

The Genomics Center (Instituto Valenciano de Investigaciones Agrarias) has generated in recent years various collections of citrus varieties. One objective of these collections is the selection of genotypes of commercial interests. In the present communication we described 3 lines derived from clementine mandarin (*Citrus clementina*) through physical mutagenesis, called 'Clemenverd', 'Nero' and 'Iviaman1957'. These lines that show enhanced agronomic traits as related to the parental genotype have already been registered. Thus, 'Clemenverd' shows a delayed striking external maturation. 'Nero' is an early variety of seedless clementine that maintains the excellence proper to clementine. On the other hand, 'Iviaman19571' shows well into February, commercial organoleptic qualities similar to the parental 'Clemenules'. 'Iviaman19571' also has excellent perdurability in the tree, making it an ideal option to extend the supply of clementines during winter. These 3 varieties have good production and post-harvest conditions and do not develop spines.

S17O05

Relationship between sensory and physico-chemical quality parameters of 'Lane Late' with Protected Geographical Indication

Duarte A.[1], Mendes S.[2], Nunes S.[2], Sustelo V.[2], and Gomes C.[3]

[1]Institute of Mediterranean Agricultural and Environmental Sciences, University of Algarve (ICAAM/UAlg), Faculty of Sciences and Technology, Portugal; [2]University of Algarve (UAlg), Faculty of Sciences and Technology, Portugal; and [3]Direção Regional de Agricultura e Pescas do Algarve (DRAPALG), Portugal. aduarte@ualg.pt

The determination of the optimal harvest time is extremely important in order to obtain a product of high quality, highly valued by the market. This is particularly important when the production is covered by a certification system. This paper establishes a relationship between physico-chemical quality parameters (soluble solids content, titratable acidity, maturity index, and others) and the acceptance of the fruits by a panel of consumers. The study was conducted over three months in two orchards of 'Lane Late' sweet orange with different climatic conditions. The assessment by consumers is related to the evolution of the maturity index. However, consumer preferences may be very different. Fruits with the same maturity index can be regarded as too acid for some consumers, while others consider them too sweet. On the other hand it was concluded that some consumers have a high requirement level for fruit tagged with Protected Geographical Indication "Citrus of Algarve." In some cases we found that although the fruits presented a high quality does not correspond to what consumers consider should be required for a "Citrus of Algarve".

S17O06

New promising citrus hybrids for the ornamental use

Recupero S., Russo G., and Reforgiato Recupero G.

CRA- Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee (CRA-ACM), Italy. giuseppe.reforgiato@entecra.it

In Italy for the production of ornamental citrus lemon (*Citrus limon*), 'Oval' kumquat (*Fortunella margarita*), 'Calamondin' (*C. madurensis*) and 'Small leaf' chinotto (*C. myrtifolia*) represent the most utilized species. Considering that the market offer is increasing also in others competitor's countries, there is the risk of a surplus of production. So is very important for revitalizing the market the availability of new genotypes. In 2002 the CRA-ACM started a breeding program with the intent to produce new genotypes with favourable and variable ornamental traits. Two new genotypes showing interesting ornamental characteristics recently released are reported. The former is a hybrid obtained by cross of 'Meyer' lemon and a blood orange, showing original striped fruits as an apparent form of orange-over-red periclinal chimera. In 2012 this genotype was proposed for the Italian patent. The latter is a chance seedling of 'Oval' kumquat, showing small, rounded, yellow and very fragrant fruits. Due to its late blooming fruits persist on the trees until the late summer, when other genotypes are not able to maintain fruits on the tree.

S17O07

'Meirav' seedless, a new promising Israeli mandarin: effects of rootstocks and growth regulators on yield and fruit quality

Kanonich Y.K., Holsman S.H., Oren Y.O., Kaplan I., Tagari E., Fainzack M., and Giladi B.

Ministry of Agriculture Extension Service, Israel. shukan@shaham.moag.gov.il

'Meirav' seedless mandarins naturally ripen from December till late January. The fruit are entirely seedless, have an attractive orange/red color, and are very tasty and easy to peel. The variety was tested during 4 consecutive growth seasons for yield, fruit size and quality on 4 different rootstocks: '812', sour orange, 'C-35' and 'Volkameriana' in two different climates in the desert and coastal areas. The rootstocks '812' and sour orange were most preferred regarding their effects on fruit quality. In the desert area, TSS and acid levels on '812' and sour orange rootstocks were ~12.0% and ~0.85%, respectively, and the annual average yield was 39 ton/ha. 'Volkameriana' rootstock provided a higher average yield of 58 ton/ha, but fruit internal quality was poor, and TSS and acid levels were just 10.9% and 0.7%, respectively. In the coastal area, '812', sour orange and 'C-35' rootstocks had more or less similar effects on yield (3.8-4.1 ton/ha), TSS (13.6-13.7%) and acidity levels (0.94-1.08%). However, the 'C-35' rootstock caused severe scion-rootstock incompatibility observed already after 7 years. Double sprays with Naphthalene acetic acid (NAA, 300 mg/L) combined with 'Bonus-NPK' fertilizer (4%) on medium size fruitlets (16 and 20.5 mm in diameter), did not affect fruit thinning, but significantly increased fruit size and consequently total yield. Overall, our data suggest that '812' and sour orange are the preferable rootstocks for 'Meirav' seedless mandarin due to their positive effects on yield and fruit quality. NAA treatments further increased fruit size and yield.

S17O08

How many seeds are acceptable in commercial citrus varieties derived from irradiation?

Williams T.E.

University of California Riverside (UCR), Botany and Plant Sciences, United States. timwill@ucr.edu

Mutation breeding, specifically through the use of irradiation directed at reducing seed numbers in normally high-seeded varieties, has resulted in the production and release of a number of new varieties from various citrus breeding programs. The goal of these breeding efforts, quite obviously, has been to achieve zero seeds under all conditions of cross pollination in the irradiated selection. But is this necessary for commercial success in new varieties developed from irradiation, or can low seed counts, in the 1-3 mean seed/fruit range, fulfill commercial expectations for specific varieties?. This presentation will explore current experiences with commercially released varieties developed using irradiation, explore the importance of seed count in relation to other specific fruit quality characteristics and suggest some useful breeder guidelines for future selections.

S17P01

Plant growth, initial fruit production and yield efficiency of twelve early maturing sweet orange cultivars in São Paulo State, Brazil

Caputo M.M.[1], Mourão Filho F.A.A.[1], Silva S.R.[1], Stuchi E.S.[2], and Bremer Neto H.[1]

[1]Universidade de São Paulo (USP), Produção Vegetal, Brazil; and [2]Embrapa Mandioca e Fruticultura (EMBRAPA), Estação Experimental de Citricultura de Bebedouro, Brasil. marinamaittocaputo@hotmail.com

One alternative to minimize the effects of highly destructive diseases on sweet oranges used for industrial processing is the establishment of new plantings in regions of less incidence, such as the Southwestern São Paulo State, Brazil. However, the different sweet orange cultivars have distinct vigor which can influence tree growth and development and, consequently, affect production. The objective of this study was to evaluate the plant growth, initial fruit production and yield efficiency of twelve early maturing sweet orange cultivars (*Citrus sinensis*) grafted on 'Sunki' mandarin (*C. sunki*) and identify those superior to 'Hamlin', the main early maturing cultivar planted in São Paulo State. The sweet orange cultivars evaluated were 'Hamlin', 'Westin', 'Pineapple', 'Ruby', 'Seleta Vermelha', 'Mayorca', 'Valencia 2', 'Olivelands', 'Kawatta', 'IAPAR 73', 'Salustiana' and 'Valencia Americana'. The experimental design was completely randomized with four replications, consisting of three plants of each cultivar per plot. Data of plant growth (plant height, canopy diameter and volume), fruit production and yield efficiency collected on 2010 and 2011 harvest seasons were obtained and analyzed by Dunnett test (P<0.05). 'Olivelands' was the cultivar with highest vigor, with highest plant height and canopy volume. Moreover, this cultivar also had higher initial fruit production and yield efficiency compared to 'Hamlin'.

S17P02

Initial production of 17 mid-season sweet orange cultivars in Southwestern São Paulo State, Brazil

Ramos Y. C.[1], Mourão Filho F. A. A.[1], Stuchi E. S.[2], Silva S. R.[1], Caputo M. M.[1], Bremer Neto H.[1], and Fadel A. L.[1]

[1] (ESALQ/USP), Produção Vegetal, Brazil; and [2](EMBRAPA), Estação Experimental de Citricultura de Bebedouro, Brasil. cairesramos@usp.br

The high incidence of diseases and their destructive impact on sweet orange in Brazil has led to changes in the growing regions. Thus, it is critical to conduct studies to evaluate the performance of different materials as potentially productive cultivars in such new regions. This study evaluated the initial production of 17 mid-season sweet orange cultivars (*Citrus sinensis*) ['Seleta Rio', 'Seleta Amarela', 'Homossasa', 'Finike', 'Biondo', 'Bidewells Bar', 'Sanguinea', 'Vaccaro Blood', 'Torregrossa', 'Jaffa' oranges and seven selections of 'Pera' ('Pera Rio', 'Pera 2000', 'Pera 2', 'Pera 3', 'Pera 4', 'Pera AM', 'Pera MT')] grafted on 'Sunki' mandarin (*Citrus sunki*) in the Southwestern region of São Paulo State, Brazil. The experiment was planted in 2007, at 6.0 x 2.5 m spacing. The data regarding accumulated fruit production in 2010 and 2011 were submitted to variance analysis and the means were compared by Scott-Knott test (P<0.05). 'Pera AM', 'Biondo', 'Vaccaro Blood' and 'Jaffa' sweet orange cultivars had significantly higher accumulated fruit production (2010 and 2011).

S17P03

Determination of yield and fruit quality characteristics of several local and foreign originated nucellar orange clones in Adana province of Turkey

Polatöz S.[1], and Tuzcu Ö.[2]

[1]Alata Horticultural Research Station (ALATA), Breeding, TURKEY; and [2]University of Çukurova, Faculty of Agriculture, Horticulture, TURKEY. sefapolatoz@gmail.com

Due to its various climatic and soil characteristics, many fruits can be grown economically in Turkey. Citrus are grown for many centuries in the Mediterranean region of Turkey, especially in the southern part of Toros Mountains. In recent years, citrus cultivation increased year by year. In this study, second level selection lines were obtained from controlled cross pollination of trifoliate orange (*Poncirus trifoliata*) with local and other 20 orange varieties. Nucellar progenies were planted in the research plot during 1975

to 1980. These genotypes were examined for 22 pomological and four plant characteristics. Fruits were harvested at optimal maturation stage and yield/tree rates were calculated individually. Selections of 20 nucellar oranges, which were named as 'Hamlin (14-17)', 'Hamlin (14-15)', 'Magnum Bonum', 'Trovita (A-35)', 'Parson Brown', 'Portakal (P-24)', 'Portakal (P-28)', 'Shamouti WN', 'Yafa (14-4)', 'Yafa (A-21)', 'Balady', 'Madame Vinous', 'Dörtyol Yerli', 'Pineapple', 'Barile', 'Kozan Yerli', 'Biondo', 'Biondo Ricco', 'Paperrind' and 'Valencia' were evaluated in terms of their yield and fruit quality characteristics. Nucellar plants are very important because they have the same genetic characteristics of their mother plants and are free of most viruses. Among the results generated, it was found that 'Hamlin (14-15)', 'Balady', 'Madame Vinous', 'Trovita (A-35)' and 'Valencia' were very promising.

S17P04

Yield and fruit quality of two early maturing orange cultivars, 'Navelina' and 'Fukumoto', in Andalusia (Spain)

Merino C., Hervalejo A., Salguero A., and Arenas Arenas F.J.

Instituto de Investigación y Formación Agraria y Pesquera (IFAPA), Cítricos, Spain. carlos.merino.luna.ext@juntadeandalucia.es

Spain has positioned itself as the sixth largest producer of citrus fruit and the number one exporter worldwide. However the profitability of Spanish citrus orchards is threatened by the globalization of markets which has led to Spanish citrus growers to be interested in new citrus cultivars in order to obtain greater profits and to be more competitive by improving some aspects of the crop and marketing such as productivity and fruit quality, and avoiding the concentration of the supply. This study was carried out to evaluate the yield and fruit quality of 'Navelina' and 'Fukumoto', two early ripening sweet orange cultivars, in Andalusia (Spain). During the 2010/2011 season, yield and fruit quality: size, weight, shape and external fruit colour, juice content, total soluble solids concentration, total acids concentration and soluble solids/acid ratio were evaluated in a experimental field, located in western Andalusia (Huelva), planted in June, 2006, in a radomized block design, with 'Carrizo' citrange as rootstock and 'Valencia Late Frost' as interstock, thus being a double-budded plantation. The results showed significant difference between 'Navelina' and 'Fukumoto' in fruit quality. Thus 'Fukumoto' induced more attractive fruit colour, while 'Navelina' had more rounded fruits, as a result of a bigger height. Finally 'Navelina' and 'Fukumoto' had similar yields.

S17P05

Phenological growth stages of oranges and mandarins in Entre Ríos province (Argentina)

Rivadeneira M. F.

Instituto Nacional de Tecnología Agropecuaria. Estación Experimental Agropecuaria Concordia (INTA EEA Concordia), Entre Ríos, Argentina. frivadeneira@correo.inta.gov.ar

The objective of this research was to evaluate flowering growth stages and ripening time of commercial oranges and mandarins varieties in Entre Ríos, Argentina. The study was carried out in Concordia in a plot located at the Estación Experimental Agropecuaria Concordia Instituto Nacional de Tecnología Agropecuaria. The observations were made in four trees from 2005 to 2011 growing seasons and flower phenology was recorded every two weeks in oranges ('Newhall', 'Lane Late', 'Valencia Late' and 'Salustiana'), mandarins ('Okitsu' satsuma and 'Clemenules' clementine), and hybrids ('Ellendale' and 'Nova') grafted on trifoliate orange (*Poncirus trifoliata*) rootstock. Fruit quality was determined at commercial harvest. Dates were standardized for ANOVA analysis and means compared with LSD Fisher. Date of full bloom was year dependent (p<0.0001), the earliest were in 2006 and 2008 (11st September and 4rd September respectively) and variety dependent (p=0.03) with 'Clemenules', 'Ellendale' and 'Valencia Late' being the first blooming varieties, 'Nova' and 'Salustiana' intermediate and 'Okitsu' having the latest bloom. Mean harvest date depend on the variety (p<0.05) for the earliest mandarins 'Clemenules' and 'Okitsu' was the 10 and 8 April, respectively, and the maturing date was the longest for 'Ellendale' (21 July) and 'Valencia Late' (9 September). This data shows the significance of phenological stage recognition for rational management practices.

S17P06

Fruit characteristics of some late maturing commercial mandarin cultivars in Spain

Simón-Grao S.[1], Simón I.[2], Lidón V.[3], Nieves M.[4], Carbonel A.A.[5], Manera J.[2], Hernández F.[3], and García-Sánchez F.[1]

[1]Centro de Edafología y Biología Aplicada del Segura (CEBAS), Plant Nutrition, Spain; [2]Escuela Politécnica Superior de Orihuela (EPSO), Física y ATC, Spain; [3]Escuela Politécnica Superior de Orihuela (EPSO), Producción Vegetal, Spain; [4]Escuela Politécnica Superior de Orihuela (EPSO), Agroquímica y Medio Ambiente, Spain; and [5]Escuela Politécnica Superior de Orihuela (EPSO), Tecnología Agroalimentaria, Spain. fgs@cebas.csic.es

Spanish's annual mandarin production is about 2 million tons, of which over the last twenty years the major group has been clementines and 'Clemenules' in particular. Currently a largely new clementine and other varieties are flooding the fruit markets and nurseries. For a citrus grower to choose the right mandarin cultivar for a given region or market, it is very important to know the characteristics of that cultivar particularly in terms of the development of its internal as well as external quality attributes. In this study we characterized the chemical and physical aspect of some late maturing commercial mandarins in the Spanish southeast. We analyzed the fruits of the clementine cultivars: 'Fina', 'Clemenules', 'Hernandina'; the tangor cultivars: 'Ortanique', 'Nadorcott', 'Mor' and 'Ellendale'; and the hybrids cultivars: 'Fortune', 'Kara', 'Nova' and 'Yosemite'. These mandarin cultivars were analyzed for their physical and chemical characteristics. 'Ellendale' and 'Kara' provided the biggest fruit size; on the other hand, 'Hernandina' produced the smallest fruits. 'Ellendale' also showed the highest amount of juice expressed as relative percentage to fruit weight. However, this cultivar had the highest number of seed with 9 per fruit, while 'Nova' was the mandarin which had not seeds. Relative to the juice characteristic, 'Nova' and 'Fina' clementine enhances by their high SST and low titratable acidity. Also 'Nova' showed the highest concentration of total phenols. The highest mineral content was observed in potassium, with a high concentration in 'Fina' clementine. Profile of organic acids, sugars and aromatic compounds are also reported.

S17P07

Evaluations of early satsuma selections for California

Kahn T., and Siebert T.

[1]University of California-Riverside (UCR), Botany and Plant Sciences, USA. tracy.kahn@ucr.edu

The global mandarin market continues to expand as consumers increasingly favor sweet, seedless, and easy-peeling varieties. According to the United States Department of Agriculture, acreage of mandarins in California has increased from 8,600 acres in 1998-1999 to 33,000 acres in 2010-2011. Satsumas are the earliest maturing of all mandarins, but competition in the marketplace with clementine mandarins has left growers seeking even earlier maturing satsuma selections. Our research program provides initial tree and fruit quality trait evaluations of citrus cultivars introduced into CA from other parts of the world to determine which early maturing selections should be considered for further multi-location trials to evaluate yield and commercial potential. This study summarizes the results of our fruit quality evaluations of 21 satsuma selections introduced into California including 'Owari' and 'Okitsu Wase' commercial standards between 2005 and 2011 sampled from two locations in California three to four times per season. Differences among selections were most pronounced for three of the fruit quality characteristics: solids: acid ratio, percentage of acidity based citric acid and rind color which are important measures of early maturity.

S17P08

Yield and fruit quality of two satsuma cultivars, 'Okitsu' and 'Iwasaki', in Andalusia (Spain) in 2010/11 season

Merino C., Hervalejo A., Salguero A., and Arenas Arenas F.J.

Instituto de Investigación y Formación Agraria y Pesquera (IFAPA), Cítricos, Spain. carlos.merino.luna.ext@juntadeandalucia.es

New citrus cultivars in Andalusia need to be evaluated in experimental fields under local conditions before been planted in a large scale as there isn't a citrus genetics and breeding programme in this region of Spain,

thus being most new plant material brought from other countries or other parts of Spain, with different climate and soil conditions. This experiment has been carried out to evaluate the horticultural performance of two early ripening mandarin cultivars, 'Okitsu' and 'Iwasaki', during the 2010/2011 season, the study being focused on both yield and fruit quality components: size, weight, shape and external fruit colour, juice content, total soluble solids concentration, total acids concentration and soluble solids/acid ratio. The results showed significant differences between 'Iwasaki' and 'Okitsu'. Regarding fruit ripening, 'Iwasaki' is about two weeks earlier. This cultivar had also greater fruit size, rind thickness and external colour.

S17P09

Behaviour of 'Parana' grapefruit in the Southeastern region of Paraguay

Wlosek Stañgret C.R.[1], and Canteros B.I.[2]

[1]Facultad de Ciencias Agrarias de la Universidad Nacional de Asunción, sede Santa Rosa (FCA UNA), Misiones, Paraguay; and [2]Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Bella Vista, (INTA), Corrientes, Argentina.
fruticultura@trociuk.com

Citrus cultivation in Southeastern Paraguay had increased in the last years. The region is located between 25° and 27°S, mean temperature 21°C, records of -2°C and 39°C, annual rainfall 1700 mm, 150-300 m over sea level. The weather is favorable to Canker disease that is endemic and limit the growth of grapefruit. The objective of this work was to study the adaptation and behaviour of the 'Parana' grapefruit, which is highly resistant to Citrus Canker. This poorly described variety is planted in the region since 2004. It is registered in Paraguay in SENAVE (Servicio Nacional de Calidad y Sanidad Vegetal y de Semillas) under Resolution 857-2011 in the National Registry of Commercial Cultivars (RNNC) as a grapefruit (*Citrus paradisi*) variety. More than 1,500 ha are cultivated and it is a good opportunity to small farmers due to the rusticity, productivity, and Canker resistance. The main use is for concentrated juice. The trees are medium size, smaller than 'Duncan'. The main rootstocks are 'Volkamer' lemon, 'Rough' lemon, 'Swingle' citrumelo, and 'Troyer' citrange. On 'Rough' lemon the production is 20 kg/tree at the third year and 140 kg/tree after sixth years. On other rootstocks the production is 30% less but the quality and quantity of juice is best. The variety is easy managed in nursery and orchard, few thorns, some resistance to frost damage, and precocity of fruit production, without alternative bearing. The harvest is from March to July, fruits are 8.0 to 8.5 cm in diameter, 280 g per fruit, mean of 40 seeds, juice yield of 40%, and high ratio (7.5 to 11.5) at harvest.

S17P10

Characterization of grapefruit varieties produced in Argentina and Paraguay

Sgroppo S.C.[1], Pereyra M.V.[1], Wlosek Stañgret C.R.[2], and Canteros B.I.[3]

[1]Facultad de Ciencias Exactas y Naturales y Agrimensura. Universidad Nacional del Nordeste (FCENA UNNE), Corrientes, Argentina; [2]Facultad de Ciencias Agrarias de la Universidad Nacional de Asunción, sede Santa Rosa (FCA UNA), Misiones, Paraguay; and [3]Instituto Nacional de Tecnología Agropecuaria (INTA EEA Bella Vista), Corrientes, Argentina. fruticultura@trociuk.com

Grapefruit (*Citrus paradisi)* is an important source of biactive compounds, largely due to the presence of flavonoids (naringin, rutin, narigenin, hesperidin and hesperedine), as well as ascorbic acid, carotenoids, etc. The naringin is the main flavonoid in grapefruit and is used as a marker of juices. This study has been addressed on 'Dalan dan' grafted on 'Troyer' (CT) and 'Carrizo' (CC) citranges and on 'Duncan' and 'Paraná' varieties. The first three plantations were from EEA INTA Bella Vista, Corrientes (Argentina); grapefruit 'Paraná' were produced in Paraguay. Values of pH were in the range of 3.41 to 4.15; acidity titratable higher than 1042mg/g tissue; total sugars between 71.65 to 99.70 mg glu/100mL (57% were sucrose) and ash between 0.279 and 0.993%. Naringin levels rose from 1.76 to 7.61 mg/100 mL, neohesperidin from 1.07 to 0.20 mg/100 mL for 'Dalan dan' (CT) and 'Duncan' respectively. Hesperidin was not detected in the grapefruits 'Dalan dan' (CC) and 'Duncan', but trace amounts were determined in the other 2 varieties. It was noted that the grapefruit 'Duncan' has more than twice of the naringin content and lower neohesperidin and sucrose content than other fruits analyzed. No significant differences were found between parameters of fruits 'Dalan dan' and 'Paraná'. On the other hand, the results of sensory evaluation showed that the fruits of ´Dalan dan´ are sweeter and with a flavor similar to ´Parana´, while ´Duncan´ fruits have the higher acidity.

S17P11

Evaluation of Lemon Selections for the Deserts of the United States

Wright G.C.[1], and Kahn T.[2]

[1]University of Arizona (Univ of AZ), Yuma Agriculture Center, USA; and [2]University of California Riverside, Department of Botany and Plant Sciences, USA. gwright@ag.arizona.edu

New lemon selections suitable for the US desert climate are needed to diversify production. Desert lemons occupy an early-season market niche and are a source of fruit for packinghouses located in the region and in cooler areas. This project was designed to evaluate 12 lemon selections under desert conditions. The objectives are to provide the lemon industry with information on tree growth, yield, packout, and fruit quality characteristics for selections. These include: 'Allen Eureka', 'Variegated Pink-Fleshed Eureka', 'Corona Foothills' (a bud sport of 'Villafranca'), 'Limoneira 8A' Lisbon', 'Walker Lisbon', 'Femminello Santa Teresa', 'Interdonato', 'Limonero Fino 49', Limonero Fino 95', 'Messina', 'Seedless' lemon and 'Yen Ben'. 'Corona Foothills', 'Limonero Fino 49', 'Walker Lisbon' and 'Femminello Santa Teresa' have heretofore had the greatest yields, while 'Messina', and 'Variegated Pink-Fleshed Eureka' have had the least yield. 'Yen Ben' had adequate yields, but had later maturing fruit than most of the other selections. With regards to fruit packout, 'Messina' had the largest size, followed by 'Corona Foothills', 'Interdonato', 'Limonero Fino 49' and 'Limonero Fino 95'. 'Variegated Pink-Fleshed Eureka' and 'Yen Ben' had the smallest sized fruit. There was little effect of selection upon fruit exterior quality. 'Variegated Pink Eureka', 'Yen Ben', 'Messina' and 'Seedless' had the least number of seeds per fruit, while 'Interdonato' had the most, followed by 'Walker Lisbon', 'Corona Foothills', 'Femminello Santa Teresa' and 'Limonero Fino 49'. 'Interdonato', 'Yen Ben', 'Messina' and 'Seedless' had the smoothest peel, while 'Variegated Pink Eureka' had the roughest. There were significant differences between the selections as to % juice, juice pH, acidity levels and peel thickness.

S17P12

Selection and field evaluation of three new cultivars of lemon in the South-east of Spain

Porras I., Pérez-Pérez J. G., García-Lidón A., Sánchez-Baños M., and Pérez-Tornero O.

Murcian Institute of Agriculture and Food Research and Development (IMIDA), Department of Citriculture, Spain. ignacio.porras@carm.es

South-east of Spain is the major exporter of lemon for fresh market, being 'Fino' the main group of lemon varieties with more than 80% of the total production. To continue leading the international lemon market it is necessary to incorporate new varieties which improve fruit quality and diversify supply. Three new selections of lemon have been evaluated in a citrus breeding program at IMIDA: 'Finolate', 'Fino Callosa', and 'Garpo', and were compared with 'Fino 49', the most widespread lemon variety in this region. These varieties were originated by spontaneous mutation. Morphological determination were carried out both in leaves and fruits, including leaf size and area, number stomata, petiole length, fruit weight, number of seeds and others. Fruit quality parameters from the three varieties were similar to 'Fino 49', except in rind colour of 'Garpo'. 'Fino Callosa' had the highest yield due to higher number of fruits per tree. 'Finolate' was classified as a late ripening cultivar and their fruits can be easily stored in tree.

S17P13

Preselection of promising triploid mandarin varieties in Corsica

Bouffin J.[1], Froelicher Y.[1], Luro F.[2], and Ollitrault P.[1]

[1]Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Bios / Agap, France; and [2]Institut National de la Recherche Agronomique (INRA), Gap, France. jean.bouffin@orange.fr

The selection of seedless mandarin varieties adapted to the European market is one of the main objectives of the CIRAD breeding program. Triploid hybrid creation is the method selected to develop seedless varieties. More than one thousand triploid progenies are currently evaluated at the San Giuliano Research Station in Corsica. There were created by 2x X 2x hybridization exploiting spontaneous 2n gametes. The objective of a first set of crosses with clementine as female parent was to select clementine-like varieties, in order to extend

the production period of this crop in Corsica. Seven hybrids were preselected at the end of the first level of evaluation according to the visual and organoleptic fruit characteristics, the period of production and the yield. There are currently under the second level of evaluation to analyse their agronomic behaviour. One of this hybrids is under D.U.S. examination for the grant of Community plant variety rights. The objective of a more recent second set of crosses is to develop late mandarin varieties. Several progenies, with different mandarins as female parent, present interesting fruit characteristics. One of them, with short juvenile period and very high yield, is preselected for the second stage of evaluation.

S17P14

Two new IVIA triploid hybrids of mandarin, 'IVIA-592' and 'IVIA-599'

Cuenca J., Aleza P., Juárez J., Pina J.A., and Navarro L.

Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain. jjuarez@ivia.es

In citrus there are two clearly differentiated markets: the fresh fruit and the processed juice. In the Mediterranean area, citrus fruits are primarily produced for the fresh fruit market and Spain is the principal producer of the area. Seedlessness is one of the most important characteristics for mandarin fresh fruit. Triploid hybrids allow implementing this trait in commercial varieties. In this work we described 'IVIA-592' and 'IVIA-599', two new triploid hybrids originated from the IVIA triploid breeding program that will be released to growers in 2013. IVIA-592 was obtained from an open pollinated 'Fortune' mandarin. Fruits reach optimum maturity at the beginning of January, although they can be harvested from December until the end of January. Fruits are easy-to-peel with a diameter between 55-60 mm and fruit rind is deep orange red in color. IVIA-599 was obtained from a cross between 'Fortune' and 'Kara' mandarins. Fruits reach optimum maturity at the beginning of February, although they can be harvested from January until second half of February. Fruits are easy-to-peel with a diameter between 60-70 mm and fruit rind is orange red in color. This variety has the same origin as 'Safor', a previously released triploid hybrid. The fruits of the two varieties are somewhat similar, but 'IVIA-599' matures one month earlier. With these two varieties it will be possible to supply similar fruits to the market from January to the end of March.

S17P15

Selection of new 'Ponkan' like mandarins

Bastianel M., Cristofani-Yaly M., Schinor E.H., Simonetti L.M., Manente K.K.M., de Negri J.D., Azevedo F.A., and Machado M.A.

Centro de Citricultura Sylvio Moreira, Instituto Agronômico, Brasil. mbastianel@centrodecitricultura.br

Although the citriculture is one of the most important economic activities in Brazil, it is based on a small number of varieties. To increase the number of varieties/genotypes with potential for commercial growing, either for the industry or fresh market, has been one of the main objectives of citrus breeding programs. There are few available commercial varieties of mandarins being the 'Rio' and 'Montenegrin' (*Citrus deliciosa*), the most produced in the South and Northeast regions of the country, and 'Ponkan' (*C. reticulata*) and 'Murcott' tangor are the most produced in the Southeast region. 'Ponkan' mandarin is preferred by the local market, mainly for easy-peeling greatly appreciated by the consumer. However, besides having a restricted period of harvest, it is susceptible to Alternaria Brown Spot (*Alternaria alternata*) which has hindered its maintenance in the orchards. Aiming to offer a larger number of commercial varieties with different harvest periods, more than 200 accessions of mandarins present in the Germoplasm Bank, and more than 200 hybrids generated in the breeding program are being evaluated. We selected about 50 mandarins accessions and two hybrids ('Ponkan' x 'Murcott') that have physical characteristics similar to 'Ponkan' fruits. These materials were evaluated for maturation curves and other characteristics of commercial interest (fruit size, skin color, number of seeds and disease resistance). Differences were observed among tested materials, as fruit characteristics and *in vitro* resistance to *Alternaria*. Promising materials will be established in regional trials for evaluation of production.

S17P16

New tangors for Brazilian citriculture

Schinor E.H., Pacheco C.A., Bastianel M., Azevedo F.A., and Cristofani-Yaly M.

Centro APTA Citrus Sylvio Moreira/IAC, Brazil. evandro@centrodecitricultura.br

Obtaining of hybrids is an important strategy for the improvement of citrus. Since 1997, the Centro de Citricultura Sylvio Moreira – Instituto Agronômico, has been conducting an extensive program of citrus breeding via hybridization, to explore sources of disease resistance. Among the hybrids obtained from crosses between 'Murcot't tangor (TM) and 'Pera' sweet orange (LP), two of them were visually selected as potential cultivars. The aim of this study was to characterize horticulturally these materials. The plants of the hybrid 'TMxLP 281' are medium-sized, with high production. The fruits had early harvest season between April and June, the fruits are flattened and they have intense orange color of the skin and pulp, with average weight of 145 g, 42.0% of juice content, º Brix of 12.4%, acidity of 1.02% and ratio of 12.1, indicating its potential for fresh fruit market. The plants of the hybrid 'TMxLP 16' are high, with good production. The 'TMxLP 16' showed fruit early ripening, with a harvest season between April and June, with fruits have a rounded shape, intense orange color of the skin and pulp, with average weight of 202 g, 56.9% of juice yield, 12.9% ºBrix, acidity 1.06% and ratio of 12.2, with the potential for fresh market and juice industry. The analysis of acceptability showed 67 and 71% of fruit approval and 96 and 98% of intent to purchase by the analysts, for hybrids 'TMxLP 281' and 'TMxLP 16', respectively. To the juice of the hybrid 'TMxLP 16' the acceptability and purchase intent were 83 and 94%, respectively. Financial support: FAPESP, CNPq, INCT-Citrus.

S17P17

Three new easy peeler varieties bred by the Agricultural Research Council released world wise.

van Rensburg P.J.[1], Sippel A.D.[2], Combrink N.K.[2], Maritz J.G.J.[2], and Bijzet Z.[2]

[1]LGS Exports (Sa), Po Box 12484, Stellenbosch, 7613, South Africa; and [2]Agricultural Research Council – Institute for Tropical and Subtropical Crops, Private Bag X11208, Nelspruit, 1200, South Africa (ARC/ ITSC), South Africa. pietvrensburg@worldonline.co.za

A brief description of the 'ARCCIT1614', 'ARCCIT1519' and 'Sonet', bred by researchers at the Institute for Tropical and Subtropial Crops (ITSC) of the Agricultural Research Council (ARC), will be provided, as well as performance data on these varieties. Both the 'ARCCIT1614' and 'ARCCIT1519' are 'Ellendale' x 'Robin' hybrids made by hand pollinations in 1982 by Dr H de Lange. Seeds were irradiated prior to planting (0.1 kgy gama radiation) in an attempt to induce seedlessness. Both these varieties have high internal quality and are high yielding mid season easy peeler varieties, maturing in June in South Africa. The 'Sonet' originated from seed from an open pollinated 'Miho Wase' satsuma tree, growing adjacent to 'Nova', 'Ellendale' and 'Novelty' pollen sources. The seed was harvested in 1996 from the 'Miho Wase' satsuma fruit. The 'Sonet' is a high yielding easy peeler variety, maturing at the end of March in South Africa. The IP management of these three varieties has been licensed to LGS Exports (SA) of South Africa for the EU, USA, Morocco and most of South America.

S17P18

A new potential elite citrus hybrid

Cheng C.F., Wei Z.X.[1] Hong L., Wu C.Q., and Qi J.R.

Chongqing Academy of Agricultural Sciences (CAAS), Fruit Research Institute, China. chengchf@hotmail.com

A new elite citrus type was generated which resulted from a hybrid progeny from crosses of 'Licheng' sweet orange and 'Chandler' red pummelo in 2004. The hybrid seedling was grafted on a three year old satsuma mandarin in the next year. In 2010, the grafted hybrid bloomed, and the tree had 49.5% flower branch with leaves, 50.5% without leaves, meanwhile it showed well fruit set. The smooth fruit, whose fruit shape index was 0.83, is aromatic, with a circular trace at the blossom end. The total soluble solid of the fruit was 14.0%, which contained 9.95% of total sugar. The content of vitamin C in fruit reached 46.45 mg/100 ml, and the total titrated acid was lower, only 0.79 g/100 mL, so TSS/TA ratio became higher and got to 17.6. Interestingly, only 1.2 monoembryonic seeds were found per fruit. So, this is a promising new citrus hybrid type.

S17P19

Acceptability of 'Fremont' mandarin in Brazil: a variety with agronomic performance and resistance to Alternaria Brown Spot.

Pacheco C.A., Schinor E.H., Moretti M.R., Bastianel M., Machado M.A., and Azevedo F.A.

Centro APTA Citros Sylvio Moreira/IAC, Brasil. camilla_andrade@yahoo.com.br

Evaluated the pattern of consumption of mandarin and acceptability of a new variety of this group, the 'Fremont' mandarin (*Citrus clementina* x *C. reticulata*), opposite the Brazilian population, represented by the inhabitants of Cordeirópolis, São Paulo state and region. Questionnaires were administered to a group of 50 subjects to evaluate the frequency and the consumption habits such fruit as well as the acceptability of the new variety under study, taking into consideration age, sex and street addresses of the participants. The consumer acceptance test was conducted in a laboratory with untrained, where samples of juice and fruit were served in disposable cups and plates, respectively, at room temperature in the presence of drinking water to rinse the palate between tasting and another sample. A nine-point hedonic scale to evaluate the external and internal characteristics of the fruit and the juice, as well as a five-point hedonic scale to analyze consumer purchase intent were used. Such evaluations have found that only 8% of the panelists consume mandarins every day, while 40% of the panelists reported consuming fruit at least 1-3 times per month. On the issue of acceptance, samples processed (juice) and fresh (fruit) were 79% and 69% approval, respectively, showing good acceptance by the judges. However, purchase intent among consumers was excellent, and 84% of the tasters would buy the juice and 70% buy the fruit of 'Fremont' mandarin.

S17P20

Scion and rootstock combinations for ornamental citrus in containers

Fadini M.[1], Girardi E.A.[2], Santos M.G.[2], Gesteira A.S.[2], Passos O.S.[2], Souza F.V.D.[2], and Soares Filho W.S.[2]

[1]Universidade Federal do Recôncavo da Bahia (UFRB), Centro de Ciências Agrárias, Ambientais e Biológicas - CCAAB, Brasil; and [2]Centro Nacional de Pesquisa de Mandioca e Fruticultura - Embrapa Mandioca e Fruticultura (CNPMF - Embrapa), Núcleo de Recursos Genéticos e Desenvolvimento de Variedades - NUGENE, Brasil. wsoares@cnpmf.embrapa.br

The selection of ornamental citrus genotypes has been carried out in Cruz das Almas, Bahia, Brazil, by the Citrus Breeding Program of Embrapa Cassava & Fruits in order to introduce this activity. Herein, we report results from two experiments evaluating the performance of scion and rootstock combinations for ornamental purposes. In the first trial the hybrid 'Rangpur' lime x *Microcitrus* 'Sydney Hybrid-004' ('LCR x MCSH-004') was budded and cleft grafted onto 'HTR-051' and 'HTR-010' dwarfing rootstocks in containers for home gardens. In the second trial *Severinia buxifolia*, *Triphasia trifolia*, *Fortunella japonica,* 'Variegated' calamondin, *Citrus amblycarpa*, 'Variegated Cara-Cara' sweet orange and 'LCR x MCSH-004' were budded onto 12 rootstocks in containers for landscaping. Budding is recommended for 'LCR x MCSH-004' propagation, and initial plant growth and branching were higher on 'HTR-051'. Budtake of *T. trifolia* was not successful on any rootstock. All scion varieties performed well in containers except to *C. amblycarpa* which was too vigorous. 'Flying Dragon' trifoliate orange and 'HTR-051 'rootstocks led to lower canopies, with 'San Diego' citrandarin, 'HTR-051', 'HTR-053', 'HTR-069' and 'TSKC x (LCR x TR)-059' inducing higher flower and fruit set. The hybrid 'LCR x MCSH-004' showed high potential for ornamental use. An adequate scion and rootstock combination is critical for ornamental citrus cultivation in containers to improve size control and other traits such as budtake and flowering.

S17P21

Current situation of the Chilean citrus industry

Castro M., Besoain X., Herrera V., and Cautin R.

Pontificia Universidad Catolica De Valparaiso (Pucv), Facultad De Agronomia, Chile. mcastro@ucv.c

Traditionally the Chilean citrus industry had been oriented to the local market; however, it has significantly changed to the fresh fruit export over the last years. The opening of the Japanese market for lemons and the opening of the US mandarin & orange market has had a mayor influence in this situation. The main citrus-

growing areas are from Coquimbo Region (29°SL) to O'Higgins Region (34.45° SL) where the dry summer and Mediterranean climate prevails. Presently 18,934 ha are planted in total, distributed in oranges (41.4%), lemons (37.5%), mandarins and clementines (19.1%), grapefruits (1.52%), limes (0.3%) and tangelos (0.2%). Regarding exports, a total 134.7 million dollars were exported, which included oranges (39.6%), lemons (27.2%), clementines (22%), mandarins (10.5%), and grapefruits (0.7%). Rootstocks have a strong influence on the variety grafted on citrus. The most used ones in the last four years are: 'C-35' (oranges, mandarins, grapefruit and lime), *Citrus macrophylla* (lemons and limes), 'Rubidoux' (oranges and grapefruits) and 'Carrizo' (oranges, mandarins, grapefruit and limes). With regard to varieties, the most used in lemons are: 'Fino 49', 'Eureka', and 'Messina'; in oranges, 'Fukumoto', 'Parent Washington' and 'Lane Late'; in mandarins, 'W. Murcott', 'Orogrande' and 'Clemenules'; and in grapefruit, 'Star Ruby'. Over the last years, the Chilean industry has been interested in introducing protected varieties into the varieties offered.

S17P22

Influence of gamma irradiation on seedless citrus production: pollen germination and fruit quality

Bermejo A., Pardo J., and Zaragoza S.

Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Citricultura y Producción Vegetal, España. bermejo_alm@gva.es

Gamma irradiation is widely used to obtain seedless citrus fruits. Herein, different new clones obtained by bud irradiation of several seedy citrus cultivars grown in the Mediterranean climate were studied to assess seedlessness, pollen germination, fruit characteristics and quality attributes. Findings indicate that irradiation altered aspects other than seedlessness, such as pollen germination, and some of the clones presented different size, acidity and maturity index. Fruit quality and nutritional bio-components also were affected. Our results indicated high contents in natural antioxidants as vitamin C, carotenoids and phenolic compounds in these citrus varieties, and provide a qualitative and quantitative survey of the fruit taste and organoleptic quality. High-performance liquid chromatographic methods were used to identify and quantify of these bioactive compounds. These aspects are necessary for the consumer that demands the prevention of health problems through nutrition and certain fruit quality traits including fruit size, internal quality, good rind colour and easy peeling. All irradiated clones examined presented lower seed numbers and reduced pollen germination compared to the corresponding controls, and some of these clones, which ripen late in the season and whose fruit quality is maintained or improved, are in the process of registration. In conclusion, budwood irradiation is a suitable technique to improve cultivars, produce seedless cultivars, adjust ripening time or raise the content of health-promoting compounds.

S17P23

Identification of new volatile compounds in a citrus hybrid mandarinquat 'Indio' (*Citrus japonica* × *Citrus reticulata*)

Delort E., Decorzant E., Casilli A., and Jaquier A.

Firmenich SA, Analytical Innovation, Switzerland. estelle.delort@firmenich.com

The volatile composition of mandarinquat peel extract was investigated for the first time. At first sight, GC/MS chromatograms appeared to be very complex, due to the probable addition of the traits of both parents. Consequently, many peaks could not be identified due to co-elutions. Further investigation based on more sophisticated processing procedure (deconvolution software) and advanced chromatographic techniques (two-dimension GC/MS) have been carried out. Compared to deconvolution software, the use of a two-dimension GC/MS allowed elucidating most of the co-elutions. Finally, our study showed that, even if the hybrid contained many of the compounds found in one or both parents, some compounds were lower-expressed; some others were even absent in the hybrid. Surprisingly, a few compounds, which have never been identified in any of the parents, were identified. The identification of new volatile compounds in hybrids may result from the interaction between the two genomes during hybridization, which may cause the activation of some genes by the other genome which are otherwise silent in the parent genome.

S17P24

Molecular characterization of 'Tahiti' lime selections by RAPD analysis

Bremer Neto H.[1], Mourão Filho F.A.A.[1], Stuchi E.S.[2], Soriano L.[3], Miyata L.[1], and Camargo L.E.A.[4]

[1]Universidade de São Paulo (USP), Crop Science, Brazil; [2]Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Centro Nacional de Pesquisa de Mandioca e Fruticultura, Brazil; [3]Universidade de São Paulo (USP), Centro de Energia Nuclear na Agricultura, Brazil; and [4]Universidade de São Paulo (USP), Plant Pathology and Nemathology, Brazil. horstbn@gmail.com

Genetic diversity of 'Tahiti' lime is restricted, and in Brazil most of the commercial orchards are predominantly based on only two selections. New 'Tahiti' lime selections were obtained and their horticultural performance was evaluated in the State of São Paulo, Brazil, during seven years. Anatomical, physiological, and biometric analyses indicated differences between the 'IAC 5', 'IAC 5-1', 'CNPMF/EECB', 'CNPMF 2000' and 'CNPMF 2001' selections, especially between 'CNPMF 2000' and 'CNPMF 2001', which showed lower drought tolerance and reduced fruit yield. RAPD markers were used to evaluate the genetic similarity of these selections. PCR reactions with primers A7, A10 and AA7 resulted in 23 fragments of which only four were polymorphic. Specific markers were found for 'CNPMF 2000' which had the lowest genetic similarity related to the other 'Tahiti' lime selections. Morphological analyses corroborated this result, since plants of 'CNPMF 2000' selection had distinct morphological and anatomical characteristics, such as longer thorns and higher number of stomatas.

S17P25

A natural mutant cultivar 'Zigui Shatian' pummelo (*Citrus grandis*) showing self-sterility due to abnormal post-zygotic embryo development not self-incompatibility

Chai L.J., Ge X.X., Biswas M.K., Xu Q., and Deng X.X.

Key Laboratory of Horticultural Plant Biology of Ministry of Education, Huazhong Agricultural University, China. xxdeng@mail.hzau.edu.cn

'Shatian' pummelo (*Citrus grandis*), a widespread citrus cultivar in China, was reported to be self-incompatible, and its pollen tube was believed to be arrested in style after self-pollination. Herein, we characterized one 'Shatian' pummelo mutant, called 'Zigui Shatian' pummelo. It showed similar morphology (leaf shape, stoma size and density, pollen shape and size), the same development progress of female and stamen organ, and same DNA ploidy level as the common 'Shatian' pummelo. However, in contrast with the common 'Shatian' pummelo, pollen tubes of self-pollinated 'Zigui Shatian' could grow normally in the stigma, style and ovary, and finally enter into the embryo sac resulting in successful fertilization. Further histological analyses verified that post-zygotic development was abnormal which caused the seed abortion in self-pollination. Simple sequence repeats (SSR) analysis revealed that one of the 120 primers showed polymorphism; it indicated that 'Zigui Shatian' pummelo and 'Shatian' pummelo were different at DNA level. All these data suggested that there is another way to block the self-pollination in 'Shatian' pummelo, not only in the style. Our research provided valuable information and material for the future in-depth study self-incompatibility mechanism in pummelo and other citrus species.

S17P26

Relation between temperature and the colour coordinate "a" during the development of the external colour of lemon fruits

Conesa A.[1], Manera J.[2], Brotons J.[3], and Porras I.[4]

[1]Miguel Hernandez University (UMH), Department of Vegetable Production and Microbiology, Spain; [2]Miguel Hernandez University (UMH), Department of Physics and Architecture of Computers, Spain; [3]Miguel Hernandez University (Department of Economic and Financial Studies), UMH, Spain; and [4]Murcian Institute of Agriculture and Food Research and Development (IMIDA), Department of Citriculture, Spain. agustin.conesa@umh.es

This study describes the evolution of the colour coordinate "a" in the peel of the lemon varieties 'Eureka Frost', 'Lisbon Frost' and the clone 'Fino 49', all on *Citrus macrophylla* rootstock, to ascertain the influence of temperature on the same. The study covered six campaigns (2003/04 and 2005/06 to 2009/10) with measurements of the colour coordinate "a", being made every week or fortnight. The results point to greater correlation between the colour coordinate "a" in peel and low temperatures than medium temperatures. The coordination between the colour coordinate "a" and low temperatures is greatest for the mean value

recorded during the previous 21 days. The correlation between the loss of greenness in lemon peel and temperature increases if the mean of the minimum temperatures is considered, rather than the average of the means. The negative values of the colour coordinate "a" decrease (decreased greenness of the peel) with temperatures below 15-17 ºC, increasing as the minimum temperatures fall until they reach zero. The colour coordinate "a" and the mean minimum temperature for the 21 days preceding the measurement provides excellent adjustments. The good values of R2 for the varieties 'Eureka', 'Fino' and 'Lisbon' show that the synthesis of the many carotenoids in the peel varies as a function of temperature. The model present a higher correlation considering not only temperatures but colour coordinate a in a previous observation. For this purpose we are going to use a fixed type model.

S17P27

Determination of the geographical origin of navel orange by near infrared spectroscopy

Su X.S.[1], Zhang X.Y.[2], Jiao B.N.[2], and Cao W.Q.[1]

[1]College of Chemistry and Chemical Engineering, Southwest University, China; and [2]Citrus Research Institute, Chinese Academy of Agricultural Sciences/Citrus Research Institute, Southwest University, China. suxuesu@163.com

The capacity to identify the geographical origins of navel orange samples obtained from Jiangxi, Chongqing and Hunan provinces, was studied by near infrared reflectance spectroscopy (NIRS). Three models of navel orange traceability were developed using the first derivative (9 points smoothing) of spectra at 1140-1170 nm combined with Soft Independent Modeling of Class Analogy (SIMCA). The three models were considered optimized when principal components were 2. Under the 5% significance level, the identification rates of the three models for sample calibration was all 100%, and the rejection rates were 85.7%, 83.3% and 100%, respectively; while the identification rates for sample validation were all 100%, and the rejection rates were 100%, 89.5% and 100%, respectively. Giving values 0, 1, -1 for Jiangxi, Chongqing and Hunan classes as category variable, partial least squares-discrimination analysis models(PLS-DA) were established to determine geographical origins of navel oranges. The best models were developed using raw spectra at 950-1650 nm when principal components were 13. The results showed that the correlation between the predicted category variable and the measured category variable was significant with high correlation coefficient (R2, 0.973), low root mean square error of calibration (RMSEC, 0.109) and root mean square error of prediction (RMSEP, 0.159). The identification rates of PLS-DA model for calibration and validation samples were both 100%. These results indicated that NIRS coupled with SIMCA and PLS-DA methods can be used for quickly and accurately discriminating geographical origin of navel orange samples.

S17P28

A new lemon genotype for ornamental use obtained by gamma irradiation

Uzun A.[1], Gulsen O.[1], Kafa G.[2], and Seday U.[2]

[1]Erciyes University, Department of Horticulture, Turkey; and [2]Alata Horticultural Research Station, Genetics and Breeding, Turkey. aydinuzun@erciyes.edu.tr

Some citrus species and cultivars with attractive appearance of fruits and leaves are used for ornamental purposes. Some of them have small fruit and plant size and mixed color of leaves or fruits. We report here a new lemon genotype with potential use for ornamental purposes. This genotype was obtained exposing budwood of the 'Kutdiken' lemon cultivar to gamma irradiation. This variety is widely grown in Turkey because of its high yield and good quality fruits. Fruits of the new genotype have attractive appearance with vertical brown lines and brown point heaps. Differences of fruit rind of this genotype have originated from mutation/mutations caused by gamma irradiation. This genotype has a good potential for ornamental use indoors or in gardens.

S17P29

New mandarin triploid hybrids `Aya´ and `Hana´ selected in Morocco

Handaji N.[1], Benyahia H.[1], Benaouda H.[1], Arsalane N.[1], and Srairi I.[2]

[1]Institut National de la Recherche Agronomique (INRA), Morocco; and [2]Domaine Kabbage, Morocco. citrusinra@yahoo.fr

Triploidy has played important role in development of new seedless mandarin cultivars for fresh fruit market and have received increasing attention for the consumers. Many series of diploid x diploid crosses were

assessed. Fruits were collected when ripe. Small embryos isolated from undeveloped seeds were cultured on Murashige and Skoog culture media supplemented with 1mg/L of gibberillic acid. Triploid plantlets selected by flow cytometry were grafted onto `Troyer´ citrange at INRA domain with spacing 6 x 3 meters. Two triploid mandarins, `Aya´ and `Hana´, were identified as the best triploid hybrids showing commercial interest. These clones were described and registered in the official catalogue as new mandarins cultivars.

S17P30

Mutation breeding for seedless cultivars in Argentina

Garavello M.[1], Anderson C.[1], Prina A.[2], and Martinez A.[2]

[1]Instituto Nacional de Tecnologia Agropecuaria (INTA), Concordia, Entre Ríos, Argentina; and [2]instituto de Genética "Edwald A. Favret" (IGEAF - INTA), Castelar, Buenos Aires, Argentina. mgaravello79@gmail.com

For many years the application of irradiation techniques on citrus buds has been used in producing low-seeded selections from existing high quality citrus cultivars. An irradiating program began in 2008 at Concordia Experiment Station using X-rays for the elimination of seeds in commercially important cultivars. The irradiations were carried out at the Institute of Genetics belonging to the National Agricultural Research Center of INTA in Castelar, BsAs. The first step was to adjust the dose and exposure time on material of different species of citrus. Doses of 2 and 4 kr on grapefruit hybrids, and doses of 2, 4, 6, 8 and 10 kr on `Bonelli´ lemon (*Citrus limon*), `Roble´ sweet orange (*C. sinensis*) and `Willow leaf´ mandarin (*C. deliciosa*) were used. The irradiated material was top-worked on sweet orange trees in a high density plot. The doses were 2 kr for mandarins, and 4 kr for lemons, grapefruits and oranges. There were two subsequent treatments on the varieties, one in November of 2008 and another in March of 2009. The selections obtained were: two of sweet orange (0-1 seed/fruit), two of lemon (2-3 seeds/fruit) and one of grapefruit (5 seeds/fruit). These selections were budded in a nursery to assess their stability, production and other characteristics of interest.

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Session 18

ROOTSTOCKS

S18O01

Reflections on a career in citrus rootstock evaluation and commercialization

Castle W.S.

University of Florida, Institute of Food & Agricultural Sciences (UF/IFAS), Citrus Research and Education Center, U.S.A.
bcastle@ufl.edu

The author undertook a 35-year career involved in the development and evaluation of new citrus rootstocks and commercialization of the most promising candidates. It was apparent at the close of that journey that rootstocks remained critical to successful citriculture, but improvements were still needed. Insights were gained along the way about the process starting with creating new rootstocks and ending with their adoption by growers. Many of the traditional objectives in creating new rootstocks remain in place, but new objectives related to tree size and disease have emerged. Thus, the history of rootstock development, use and importance in relation to other fruit crops is considered along with a particular interest of the author: the social component of rootstock research and commercialization and its consequences regarding the time course of evaluation and adoption of new rootstocks. Eventually, new rootstocks are released and the next step is commercialization. Evaluation of research data and the grower selection process are the ultimate human experience in the realm of rootstocks. Thus, because choosing rootstocks is a human endeavor, it is influenced by many factors. Among the selection criteria is the expected use of a new rootstock. Lastly, our reliance in Florida on cooperative research is emphasized.

S18O02

Citrus rootstocks in Morocco: Present situation and future prospects

El Guilli M.[1], Belmahdi I.[2] and Zemzami M.[3]

[1]Institut National de la Recherche Agronomique (INRA), Phytopathology, Morroco; [2]Office National de la Sécurité Sanitaire des Produits Alimentaires (ONSSA), Phytopathology, Morroco; and [3]Domaines Agricoles (DA), Virology, Morroco. mguilli@yahoo.com

Until the last decade, the Moroccan citrus industry has relied exclusively on sour orange as a rootstock. However, rootstock trials were initiated in the 1960's at the experimental farms of INRA. The major selection traits targeted at that time included yield and fruit quality, compatibility with clementine varieties and tolerance to *Phytophthora* Gummosis, salt and limestone soils. In spite of the outstanding results of some rootstocks revealed by these trials, sour orange continued to be the main rootstock of choice of citrus farmers. Not until year 2000, after a long and intense debate among citrus industry stockholders, was a real action initiated to diversify citrus rootstocks in new plantings. While sour orange remained in use, trifoliate hybrids ('Carrizo', 'Troyer', 'C-35'), *Citrus volkameriana*, and *Citrus macrophylla* started to increase in percentage, especially in large nurseries. In 2008, during a sanitary survey in the Loukous region in the North of Morocco, CTV was detected at high incidence in many orchards. A big effort is actually being done to promote the use of CTV tolerant rootstocks. Figures of how each of these rootstocks has developed during the last 6 years are presented. A preliminary assessment of the impact of the rootstock shift on yield, quality and market reaction is discussed.

S18O03

Seven hybrid citrus rootstocks released by USDA: comparison of characteristics and use

Bowman K.D. and Albrecht U.

United States Department of Agriculture, Agricultural Research Service (USDA, ARS), United States Horticultural Research Laboratory, USA. kim.bowman@ars.usda.gov

The USDA-ARS has supported a long-term citrus rootstock breeding program in Florida, and in the past two decades the Florida citrus industry has also provided strong financial support for USDA rootstock development. The result of this effort has been the development and release of seven hybrid citrus rootstocks that, to varying degrees, have achieved commercial success in Florida and in other citrus production areas around the world. During the 2010 propagation cycle, about 62 percent of all Florida nursery trees, or 1,855,798 trees, were propagated on the seven USDA rootstocks in Florida. These rootstocks, 'Carrizo' citrange (*Citrus*

sinensis × *Poncirus trifoliata*), 'Swingle' citrumelo (*Citrus paradisi* × *P. trifoliata*), 'US-852' (*Citrus reticulata* 'Changsha' × *P. trifoliata* 'English Large'), 'US-812' (*C. reticulata* 'Sunki' × *P. trifoliata* 'Benecke'), 'US-897' (*C. reticulata* 'Cleopatra' × *P. trifoliata* 'Flying Dragon'), 'US-802' (*Citrus grandis* 'Siamese' × *P. trifoliata*), and 'US-942' (*C. reticulata* 'Sunki' × *P. trifoliata* 'Flying Dragon'), each have some strong attributes that prompted their release and commercial interest, including outstanding performance in field trials with commercial scion cultivars. Although information about each rootstock was provided during release, in many cases only limited information was available comparing the seven rootstocks because they were developed in different eras or tested in different trial locations. In this study, we compare the nursery characteristics, susceptibilities to disease, and performance of the seven rootstocks under a range of different field conditions. Although all seven rootstocks induce good scion fruit productivity under some conditions, the large differences in effect on scion vigor and tolerance of biotic and abiotic stresses, result in each rootstock having a different ideal niche for commercial use.

S18O04

The evaluation of Chinese rootstock for tree growth, yield and quality of 'Lane Late' oranges grown in Australia

Khurshid T.[1], Sanderson G.[2] and Donovan N.[3]

[1]Dareton Primary Industries Institute (DPII), New South Wales Department of Primary Industries, Australia; [2]Dareton Primary Industries Institute (DPII), New South Wales, Department of Primary Industries, Australia; and [3]Elizabeth McArthur Agricultural Institute (EMAI), Biosecurity Research, Australia. tahir.khurshid@dpi.nsw.gov.au

This paper reports on the short term performance of a range of new rootstocks imported from China. The experimental program was conducted at the Dareton Primary Industries Institute, New South Wales Department of Primary Industries beginning in 1997. A detailed data is presented from a 'Lane Late' trial which was propagated on to a range of rootstock treatments including *Citrus reticulata*, *Poncirus trifoliata*, *P. trifoliata* hybrid, *Citrus junos*, *Citrus erythrosa*, *Citrus aurantium* hybrid, *P. trifoliata* ('Tri22' Australian strain), *Citrus sinensis* and (*C. sinensis* x *P. trifoliata*). The Australian standard 'Tri22' was used for comparison purposes. This trial was established in October 1999 to evaluate the horticultural performance of new rootstocks established from single-node cuttings with a 'Lane Late' scion. Six years of data (2002-2007) were collected on tree growth, fruit yield and quality to identify superior rootstocks for the next phase of semi commercial plantings. Chinese *P. trifoliata* type, 'Houpi' and 'Zao Yang' resulted in higher cumulative yields of 127 kg/tree and 115 kg/tree, respectively compared to 81 kg/tree for the control ('Tri22'), and yield efficiencies of 2.4 and 3.2 kg/cm2, respectively compared to 2.5 kg/cm2 were produced. 'Houpi' had a large trunk circumference of 26 cm compared to 'Tri22' while 'Zao Yang' had a similar trunk circumference of 20 cm compared to 'Tri22'. Data on tree growth, fruit quality and fruit size distribution are presented for all other rootstocks.

S18O05

Dwarfing rootstocks for 'Valencia' sweet orange

Ramos Y.C.[1], Stuchi E.S.[2], Girardi E.A.[2], Leão H.C.[3], Gesteira A.S.[2], Passos O.S.[2] and Soares Filho W.S.[2]

[1]Escola Superior de Agricultura "Luiz de Queiroz" - Universidade de São Paulo (ESALQ - USP), Departamento de Produção Vegetal, Brasil; [2]Centro Nacional de Pesquisa de Mandioca e Fruticultura - Embrapa Mandioca e Fruticultura (CNPMF - Embrapa), Núcleo de Recursos Genéticos e Desenvolvimento de Variedades - NUGENE, Brasil; and [3]Fischer S/A - Comércio, Indústria e Agricultura (Grupo Fischer), Departamento Técnico da Citrosuco, Brasil. wsoares@cnpmf.embrapa.br

The Brazilian and other citrus industries will likely require, among other technologies, the use of rootstocks that are suitable for higher planting densities with high production efficiency of high quality fruits and tolerant to abiotic and biotic stresses. Considering this approach, hybrid rootstocks have been obtained by the Citrus Breeding Program of Embrapa Cassava & Fruits, in Cruz das Almas, Bahia State, Brazil. One experiment evaluating the first three commercial crops of 'Valencia' sweet orange budded on several genotypes in Colômbia, São Paulo State, Brazil, indicated that the hybrids 'TSKC ('Sunki' mandarin) x [LCR ('Rangpur' lime) x TR (trifoliate orange)] – 059', 'TSKC x CTSW ('Swingle' citrumelo) – 033', 'TSKC x CTSW – 041', 'LCR x TR – 001', 'HTR (trifoliate hybrid) – 051', 'HTR – 053' and 'HTR – 069' allowed planting densities higher than those

attained with the use of the traditional rootstocks 'Rangpur' lime and 'Sunki' mandarin. They also induced higher production efficiency of fruits with higher or equivalent quality in comparison to fruits on 'Rangpur' lime, which is the usual rootstock in Brazil. Additionally, 'TSKC x (LCR x TR) – 059' and 'LCR x TR – 001' induced higher tolerance to drought, with results similar to 'Rangpur' lime, and the first hybrid also anticipated fruit bearing of the scion variety. In spite of not being dwarfing rootstocks, the hybrids 'TSKC x CTTR ('Troyer' citrange) – 002' and 'TSKC x CTSW – 028', as well as 'Rangpur' lime selection 'CNPMF-03', 'Sunki Tropical' mandarin and 'Indio' and 'San Diego' citrandarins performed well.

S18O06

The development of improved tetraploid citrus rootstocks to facilitate advanced production systems and sustainable citriculture in Florida

Grosser J.W., Barthe G.A., Gmitter Jr. F.G., and Castle W.S.

University of Florida, Citrus Research and Education Center (CREC), Horticultural Sciences, USA. jgrosser@ufl.edu

The 10 billion dollar/year Florida citrus industry is now under siege by an insect vectored bacterial disease Citrus Greening, also known as Huanglongbing (HLB). Genetic resistance to HLB is not present in commercial scion or rootstock cultivars. A possible mid-term solution to this problem is to shorten the grove rotation and time to profitability by adapting open hydroponics systems (OHS) or other similar evolving production systems to Florida conditions. Such systems that feature high density plantings being developed will require improved tree-size controlling rootstocks that bear early and produce good yields of fruit with juice of high quality. We have been exploring somatic hybridization of complementary diploid rootstocks via protoplast fusion to generate allotetraploid rootstock candidates, and more recently we have been conducting rootstock breeding at the tetraploid level by crossing superior somatic hybrids. Preliminary testing suggests that tetraploid rootstock candidates from both of these sources have potential to facilitate the success of new production systems, as selected allotetraploid rootstocks generally exhibit good soil adaptation, disease resistance, nursery and young tree growth, and also a strong tendency to reduce tree size. A major rootstock trial is underway that features high planting densities and a production system that mimics the principles of OHS, with a goal of identifying superior rootstocks for use in advanced production systems. This trial includes selected somatic hybrids, tetrazygs, diploid hybrids and controls, with trees being grown using slow release fertilizer and daily short-duration microjet irrigation. Yield and fruit quality data from this and other trials will be presented, as there are significant rootstock affects on both parameters. The best somatic hybrid and 'tetrazyg' rootstocks will be highlighted. The unexpected reduced impact of HLB in the trial will also be discussed.

S18O07

Citrus macrophylla rootstock improves the performance of 'Mexican' lime trees on citrus interstocks in calcareous soils

Medina-Urrutia V.M.[1], Robles-González M.M.[2], Virgen-Calleros G.[1] and Manzanilla-Ramírez M.A.[2]

[1]Universidad de Guadalajara (CUCBA-UDG), Centro Universitario de Ciencias Biologicas y Agropecuarias, Mexico; and [2]Instituto Nacional de Investigaciones Forestales Agricolas y Pecuarias (INIFAP-Colima), CIRPAC, Mexico. vmmedinau@gmail.com

Most lime orchards are planted using Mexican lime (ML) (*Citrus aurantifolia*) trees budded on 'Alemow' (ALE) (*Citrus macrophylla*) vigorous rootstocks on calcareous soils in México. Citrus tree size can be reduced using *Poncirus trifoliata* and hybrid rootstocks which are not well adapted to calcareous soils. The effect of different citrus rootstocks used as interstocks on growth and yield of ML trees was determined during 10 years in Tecomán, Col., México. The purpose was to reduce the tree size of ML/ALE combinations to increase the plant densities on calcareous soils to mitigate the impact of HLB disease. Twenty one different interstocks between ML scion and ALE rootstock and the control (ML/ALE) were evaluated. Sixteen trifoliate genotypes, four mandarins types and *Severinia buxifolia* (SB) were included as interstocks. ML trees on FDT and 'Hiryu' trifoliate orange interstocks reduced by over 40% the canopy volume compared to the control. Also FDT and 'Hiryu' showed the greatest interstock tree trunk, followed by many citranges. Average ML yield during the first five years ranged from 80.8-117.1 kg/tree/year. SB and 'Morton' citrange as interstocks obtained the

lower and higher ML yields/tree, respectively. Most interstocks produced similar yields to the control ML/ALE (108.1 kg/tree/year). 'Hiryu' and FDT interstocks that significantly reduced the tree size of limes produced 100 kg/tree. In the second period of five years, ML yields ranged from 96.8- to 184.7 kg/tree/year. 'Hiryu' and 'C-35' citrange interstocks produced the lower and higher yields, respectively. Seven trifoliate yield higher than control. Estimated tree density using dwarfing combinations was around 600 trees/ha, which produced more than 60 t/ha-1 under HLB-free conditions.

S18O08

Performance of 'Valencia Late' orange on 14 rootstocks in the Gharb region in Morocco

Benyahia H.[1], Benazzouz A.[1], Talha A.[1], Beniken L.[1], Omari F.E.[1], Handaji N.[1], Jacquemend E.[2] and Srairi I.[3]

[1]National Institut for Agricultural Research (INRA), Department of Plant Breeding and Phytogenetic Ressources Conservation, Laboratory of Citrus Breeding, Kenitra, Morocco, BP 257; [2]Agricultural Research Station of San Giuliano (INRA/CIRAD), Corse, F-20230, France; and [3]Domaines Abbès El Kabbage (DAK), Agadir, Morocco. hamidbenyahia2002@yahoo.fr

Citrus is a major and valuable crop in Morocco and it is by far one of the most important export crops. The most important orange variety grown commercially in Morocco is the 'Valencia Late Nucellar' and, as for all other citrus cultivars, the main rootstock used is sour orange. This study was conducted in order to evaluate the effect of various rootstocks on yield, yield efficiency, tree size and fruit quality of the 'Valencia Late' orange in the Gharb region in Morocco and to identify rootstocks that may replace sour orange. Total cumulative yield over 5 years of production was the highest on 'Swingle' citrumelo and *Poncirus trifoliata* 'B6Z13'. In terms of productivity, 'Swingle' citrumelo, *Citrus volkameriana*, the hybrid 'Sunki' mandarin x *P. trifoliata*, *Citrus macrophylla* and the hybrid 'Sunki' mandarin x *P. trifoliata* 16 seem far rootstocks promoters. This study revealed that some rootstocks have made a significant improvement in the rate of juice of 'Valencia Late' variety. These rootstocks are: *C. volkameriana*, 'PT 6 B CZ 24', 'PT 6 B CZ 13', 'Cleopatra' mandarin x 'Carrizo' citrange, 'Swingle' citrumelo and citrumelo '1452'. In terms of acidity and Brix, this study has identified rootstocks that have made a significant improvement in both parameters of fruit quality. These rootstocks are 'Carrizo' citrange, 'Sunki' mandarin, *P. trifoliata*, Citrumelo '1452', 'Swingle' citrumelo and 'Sunki' mandarin x *P. trifoliata* 16.

S18O09

Results from a three years field trial planted at Concordia, Argentina using transgenic rootstocks potentially resistant to Citrus Tristeza

Anderson C.[1], Plata M.I.[1], Garavello M.[1], Costa N[1] and Peña L.[2]

[1]Estacion Experimental Agropecuaria Concordia, Instituto Nacional de Tecnología Agropecuaria (EEA INTA), Concordia, Entre Rios, Argentina; and [2]Centro de Protección Vegetal y Biotecnología, Instituto Valenciano de Investigaciones Agrarias (IVIA), Moncada, Valencia, España. anderson.citrus@gmail.com

Citrus Tristeza Virus (CTV) causes one of the most devastating diseases of citrus worldwide. It induces the death of sweet orange, mandarin, lime and grapefruit trees budded on sour orange, which has determined its replacement by other CTV-tolerant rootstocks. The availability of a CTV-resistant rootstock with the sour orange attributes of productivity, fruit quality and tolerance to abiotic stresses would be a major benefit to the citrus industry worldwide. The objective of the field trial was to evaluate the response to CTV of 10 sour orange (*Citrus aurantium*) transgenic lines carrying CTV-derived sequences. They were obtained in the laboratories of IVIA, Spain and planted at INTA Experiment Station in Concordia, Argentina, where CTV is endemic and efficiently transmitted by the Brown Citrus Aphid (*Toxoptera citricida*). Trees were obtained by budding rooted cuttings of transgenic sour orange lines with non-transgenic and virus-free 'Valencia Late' sweet orange (*Citrus sinensis*). 'Valencia' trees budded on tolerant rootstocks as well as non-transgenic sour orange were planted as controls. Trees were planted in a complete randomized design with two trees per plot and 5 replications. Every six months imprints were taken to determine the progress of CTV infection in each tree. Three and half years after planting, 47.3 % of the trees are CTV infected, showing stunting growth and yellowing of foliage.

S18O10

'Forner-Alcaide 5': a citrus rootstock released in Spain

Forner-Giner M.A., and Forner J.B.

Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Citricultura y Producción Vegetal, Spain. forner_maginer@gva.es

'Forner-Alcaide 5' is an interspecific hybrid rootstock between 'Cleopatra' mandarin (*Citrus reshni*) and *Poncirus trifoliata* obtained in a program for breeding citrus rootstocks at the Instituto Valenciano de Investigaciones Agrarias (IVIA). This rootstock is commercial in Spain since 2005. 'Forner-Alcaide 5' is resistant to Citrus Tristeza Virus (CTV) and citrus nematode (*Tylenchulus semipenetrans*). 'Carrizo' citrange (*Citrus sinensis* x *P. trifoliata*), the most used rootstock in Spain, frequently shows iron chlorosis and salinity problems. 'Forner-Alcaide 5' shows a high resistance to salinity, similar to 'Cleopatra' mandarin and it is more tolerant to iron chlorosis than 'Carrizo' citrange. The behavior of this rootstock in water stress and flooding screening tests is good, as well as its *Phytophthora* spp. tolerance. Agricultural performance of 'Forner-Alcaide 5' has been evaluated with navel oranges, satsumas and clementines, compared to the traditional rootstocks used in Spain. This rootstock always shows a higher productivity than 'Carrizo' citrange, a good fruit size and fruit quality.

S18P01

Rootstocks affect fruit yield and some fruit quality traits of 'Star Ruby' grapefruit in Adana, Turkey

Yeşiloğlu T., İncesu M., Yılmaz B., Çimen B. and Tuzcu Ö.

University of Cukurova (CU), Horticulture, Turkey. turgutyesiloglu@gmail.com

In this study, the effects of local sour orange (*Citrus aurantium*), 'Taiwanica' (*Citrus taiwanica*), 'Yuzu' (*Citrus junos*), 'Troyer' and 'Carrizo' citranges (*Citrus sinensis* x *Poncirus trifoliata*), 'Cleopatra' mandarin (*Citrus reshni*) and also *Citrus ampullacea*, 'Kinkôji' (*Citrus obovoidea*) and 'Sanbôkan' (*Citrus sulcata*) on some pomological characteristics of 'Star Ruby' grapefruit were evaluated under Adana ecological conditions. Fruit yield (kg), fruit weight (g), height (mm), diameter (mm), index, juice quantity (%), rind thickness (mm), total soluble solids (TSS, %), total acid content (TA, %), TSS/TA ratio, fruit peel and juice colour (hueo) were evaluated. Yield was the highest on *C. sulcata* followed by that on 'Cleopatra' and sour orange. The largest fruit size was obtained from the trees on 'Carrizo' citrange, followed by 'Troyer'. The thickest fruit rind was showed by fruits from the trees on 'Carrizo' and the thinnest on 'Taiwanica' and sour orange. However, trees on the other rootstocks produced similar rind thickness and did not showed significant difference from each other. There were significant differences between rootstocks as regards TSS/TA ratio. It was the highest for fruits from the trees on 'Carrizo'. According to the hueo data, red colour in both peel and juice were the highest in the fruits on 'Carrizo' and 'Troyer' citranges.

S18P02

Rootstock effects on photosynthetic performance of young 'Valencia' orange trees

Yeşiloğlu T., Çimen B., İncesu M. and Yılmaz B.

University of Cukurova (CU), Department of Horticulture, Turkey. bcimen@cu.edu.tr

Iron deficiency is a major problem for many fruit crops grown on calcareous soils. Citrus production in the Mediterranean region is often made in high pH calcareous soils, therefore, nutrient imbalances regarding iron deficiency often occur. In this study, 'Valencia' orange budded on five rootstocks ('Carrizo' citrange, 'Swingle' citrumelo, 'Rangpur' lime, 'Antalya Cleopatra' mandarin, 'Tuzcu 31-31' sour orange) were evaluated in terms of their photosynthetic performances in calcareous soil. Thus leaf chlorophyll concentration, chlorophyll fluorescence parameters in light-adapted (FT: Continuous fluorescence yield, QY: FV' /FM': Photosystem II quantum yield) and leaf gas exchange parameters (photosynthesis rate, transpiration rate, stomatal conductance and leaf water use efficiency) were measured. Scions which were grown on the rootstocks 'Rangpur' lime and 'Antalya Cleopatra' mandarin had the highest chlorophyll contents while 'Swingle' citrumelo had the lowest. However, leaves of scions did not differ in terms of transpiration rate, leaves of shoots on 'Swingle' citrumelo displayed a significant reduction in photosynthetic rate, stomatal conductance and quantum yield of photosystem II. The highest photosynthetic rate and leaf water use efficiency were obtained from the leaves of scions grafted on sour orange.

S18P03

Effect of the rootstock on some agronomic traits of 'Nules' and 'Nour' clementines in the Souss valley in south Morocco

Benyahia H.[1], Benazzouz A.[1], Beniken L.[1], Handaji N.[1], Kabbaj T.[2], Essajid A.[2], Chahidi B.[2], Jacquemend E.[3] and Srairi I.[1]

[1]National Institut for Agricultural Research (INRA), Department of Plant Breeding and Phytogenetic Ressources Conservation, Laboratory of Citrus Breeding, Kenitra, Morocco; [2]Domaines Abbès El Kabbage (DAK), Agadir, Morocco; and [3]Agricultural Research Station of San Giuliano (INRA/CIRAD), Corse, France. hamidbenyahia2002@yahoo.fr

In Morocco, citrus cover about 105,000 ha. Clementine, a species concentrated in Souss and Moulouya regions, accounts for 24% of citrus. Sour orange, the dominant rootstock in Morocco citrus groves, is sensitive to Tristeza disease. Recently, many interesting rootstocks have been introduced from abroad. An experimental trial was initiated in 1997 in the Souss Valley in South Morocco. It was aimed at evaluating vegetative development, production and fruit quality of two clementine varieties ('Nour and 'Nules) grafted on 22 different rootstocks. The trial was set on and soil composed of sand/loam with a moderate pH (8,5) at a planting density of 6x3m. Plants grafted on *Citrus macrophylla* and *Citrus volkameriana* had the highest canopy volume and exceeded significantly all the other combinations. These two rootstocks had also the best yields over a period of 10 years. 'Troyer' citrange ranked third for 'Nules' and an even lower production for 'Nour'. For 'Nour', the second and third productive rootstock was 'Cleopatra' mandarin. Some hybrids of *Poncirus trifoliata* improved significantly fruit quality (% of juice, Brix and acidity) in comparison to sour orange. Fruits size was more improved by *C. macrophylla* and *C. volkameriana* especially for 'Nour'. The most efficient production was obtained with a dwarfing hybrid ('Sunki' mandarin x *P. trifoliata*), *C. volkameriana* for 'Nules', followed by 'Nour'. We conclude that there are some advantages conferred by some new rootstocks in comparison to sour orange. They permit a high productivity, earliness in fruit set and improved fruit quality.

S18P04

Behavior of 'Navelina ISA 315' on 15 rootstocks in Metaponto area, Basilicata, Italy

Mennone C.[1], Ippolito A.[2] and Reforgiato Recupero G.[3]

[1]Azienda Agricola Sperimentale Dimostrativa Pantanello, Italy; [2]Università degli Studi "Aldo Moro", Bari, Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Italy; and [3]CRA- Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee (CRA-ACM), Italy. giuseppe.reforgiato@entecra.it

With the epidemic spread of Citrus Tristeza Virus (CTV) in Italian citriculture, sour orange, the most used rootstock, has been almost completely abandoned, because of its susceptibility to the disease. Even if a number of alternative rootstocks tolerant to CTV are available, their adaptability in specific conditions needs to be tested. At the Pantanello research Station, Metaponto, Basilicata Region, Italy, *Citrus tachibana*, trifoliate oranges 'Yamaguchi', 'Argentina', 'Roubidoux' and 'Flying Dragon', tangelo 'Orlando', sour oranges 'S. Marina' and 'Platania', *Citrus macrophylla*, 'Ichang' pummelo, citranges 'Troyer' and 'Carrizo', citrumelo 'Swingle', 'Siamelo', and 'Poorman's' orange have been planted using as scion 'Navelina ISA 315' sweet orange. Since 1983 fruit production and quality were evaluated. In each combination ten trees were arranged in a complete randomized block design. Among the tested rootstocks, interesting results in relation to fruit production and quality were obtained with citranges, showing a vigour comparable to sour orange. Some plants grafted on 'Swingle', showing a severe symptom of iron chlorosis, died. Trifoliate orange selections, mainly 'Flying Dragon', induced a reduced canopy and low production.

S18P05

Influence of rootstock on the characteristics of clementine in the plain of Sibari, Italy

Russo G.[1], Reforgiato Recupero G.[1], Recupero S.[1], Pietro Paolo D.[1], Di Leo A.[2] and Filippelli S.[2]

[1]Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee (CRA-ACM), Italy; and [2]Centro Sperimentale Dimostrativo (ARSSA), Mirto Crosia Italy. giuseppe.russo@entecra.it

In May of 2002, a trial was started in the plain of Sibari, a typical area for clementine in Italy, using four varieties of clementines grafted on five rootstocks (sour orange, 'Carrizo' citrange, 'Swingle' citrumelo, 'Flying Dragon' trifoliate orange and 'Alemow'). The clementine varieties were two early maturing

selections ('Caffin' and 'Spinoso VCR'), a medium ('SRA 63') and a late ('Rubinio VCR'). The trial was conducted in a sandy soil with pH 8.26 (Total lime 6%, active lime 1.6 %). The planting distance was 5x4 m using a randomized complete-block design. The four selections of clementine on 'Alemow' showed over the years 2006-2011 the highest cumulative yield, followed by 'Carrizo' and 'Swingle'. Sour orange induced a reduction of 50% on cumulative production than 'Carrizo'. The most productive variety was clementine 'SRA 63' followed by 'Spinoso'. In terms of qualitative characteristics, 'Alemow' induced a reduction of total soluble solids concentration, and 'Flying Dragon' induced the highest content. Based on the induced production characteristics and tolerance to tristeza, 'Carrizo' and 'Swingle' are confirmed as subjects able to replace the sour orange. Even if clementine 'SRA 63' was the most productive variety, is problematic the evaluation of a variety within the clementines, without taking into account the ripening period, for the enormous influence of this factor on crop profitability.

S18P06

Is there a relationship between ferric-chelate reductase activity in roots of *Poncirus trifoliata* and leaf chlorophyll contents?

Correia P.J., Lopes D., Duarte A., Gama F., Saavedra T. and Pestana M.

Institute of Mediterranean Agricultural and Environmental Sciences - University of Algarve (ICAAM/UAlg), Faculty of Sciences and Technology, Portugal. aduarte@ualg.pt

Poncirus trifoliata is a citrus rootstock very sensitive to Fe deficiency which is very common in crops grown in calcareous soils due to the detrimental effect of bicarbonate ion. Higher plants have distinct behaviors when faced with bicarbonate-induced Fe chlorosis, and several mechanisms may be activated under Fe shortage. The aim of this study was to investigate the activity of ferric-chelate reductase (FC-R), a key enzyme in Fe uptake, and to establish relationships with leaf chlorophyll. Plants were grown in nutrient solutions without Fe (0 µM Fe), with 1 µM Fe, with 120 µM Fe and with 120 µM Fe plus CaCO3 (1 g L-1). Total leaf chlorophyll in young and mature leaves was determined using a calibration model based on a relationship between SPAD readings and concentration of chlorophyll (r2=0.95; $P<0.01$). The activity of FC-R was determined in roots apexes and several biomass parameters in shoots (number of leaves, height, dry and fresh weight) and roots (dry and fresh weight) were evaluated at the end of the experiment. Biomass was negatively affected by Fe deficiency in the nutrient solution. The activity of FC-R increased in plants grown without iron (0 µM Fe). The results about the relations between root FCR and leaf chlorophyll are discussed.

S18P07

Influence of six citrus rootstocks on fruit quality of 'Lane Late'

Hervalejo A. [1], Cardeñosa V.[2], Forner-Giner M.A.[3], Salguero A.[1], Pradas I.[2], Moreno J.M.[2] and Arenas Arenas F.J.[1]

[1]Instituto de Investigación y Formación Agroalimentaria y Pesquera (IFAPA Las Torres), Consejería de Agricultura, Pesca y Medio Ambiente. Junta de Andalucía, Sevilla, Spain; [2]Instituto de Investigación y Formación Agroalimentaria y Pesquera (IFAPA Alameda del Obispo), Consejería de Agricultura, Pesca y Medio Ambiente. Junta de Andalucía, Córdoba Spain; and [3]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Citricultura y Producción Vegetal, Spain. fjose.arenas@juntadeandalucia.es

Seven-year-old trees of 'Lane Late' grafted on three new citrus rootstocks, 'Forner-Alcaide nº5' (FA5), 'Forner-Alcaide nº13' (FA13) and 'Forner-Alcaide nº41' (FA41) (hybrids of 'Cleopatra' mandarin x *Poncirus trifoliata*), and three traditional rootstocks, 'Carrizo' citrange (CA) (*Citrus sinensis* x *P. trifoliata*), *Citrus macrophylla* (MP) and 'Cleopatra' mandarin (*Citrus reshni*) (CL), cultivated in Andalusia (Spain), were evaluated in the season 2010–2011. They were characterized as to their effects on fruit appearance, taste and nutritional compounds. FA5 and CL preserved fruit quality longer, showing them as the most suitable for late harvested cultivars such as 'Lane Late'. MP and CA showed a loss of organoleptic quality faster due to their lower acidity of the juice. MP produced larger fruits than CL and FA13, nevertheless MP had the lowest juice content and the poorest organoleptic quality. In relation to the remaining quality parameters, MP and CA had the lowest values in fruit firmness, total polyphenols content and antioxidant activity. CL, FA13 and FA5 had the highest antioxidant activity, while FA13 showed the highest fruit firmness, similar to FA41 and FA5.

S18P08

Agronomic performance of 'Nules' clementine and 'Navelate' orange on citrus rootstocks 'F-A 5', 'F-A 13' and 'F-A 418' in Northeastern Spain

Fibla J.M.[1], Pastor J.[1], Martinez-Ferrer M.T.[1], Campos Rivela J.M.[1], Forner-Giner M.A.[2] and Forner J.B.[2]

[1]Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Fruit Production, Spain; and [2]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Citricultura y Producción Vegetal, Spain. teresa.martinez@irta.cat

The agronomic performance in northeastern Spain (Tarragona) of three new rootstocks reducing canopy volume 'Forner Alcaide 5' (F-A 5), 'Forner Alcaide 13' (F-A 13) [hybrids of 'Cleopatra' mandarin (*Citrus reshni*) and *Poncirus trifoliata*] and 'Forner Alcaide 418' (F-A 418) [hybrid of 'Troyer' citrange (*Citrus sinensis* X *P. trifoliata*) x common mandarin (*Citrus deliciosa*] was studied. In May 2000, trees were planted including 'Carrizo' citrange as a standard. Rootstocks were grafted with 'Nules' clementine (*Citrus clementina*) and 'Navelate' orange (*C. sinensis*). Vegetative growth, yield and fruit quality was determined from 2004 until 2012. In 'Nules' clementine, F-A 5 rootstock did not reduce tree growth. Yield and canopy volume were higher than with the other rootstocks. Trees on F-A 13 had a similar growth and yield than 'Carrizo' citrange, and F-A 418 induced a dwarfing effect and both growth and yield were lower than in the other rootstocks. F-A 5 showed a 'Navelate' growth and yield performance similar to 'Carrizo' citrange, and both were higher than the other two rootstocks tested. 'Navelate' trees on F-A 13 showed an intermediate response between F-A 5 and F-A 418. The latter induced a significant reduction in size and deficient vegetation. At harvest, no significant differences on fruit quality were found between rootstocks. However in trees on 'Carrizo' citrange rootstocks, a delay in the peel-colour index was found respect to F-A 5.

S18P09

New rootstocks for the high density plantings in citrus

Bordas M.[1], Torrents J.[1] and Forner-Giner M.A.[2]

[1]Agromillora Research S.L. El Rebato s/n. 08739 Subirats (Barcelona)-Spain; and [2]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Citricultura y Producción Vegetal, Spain. mbordas@agromillora.com

The general trend in fruit crops is the use of dwarfing/semi-dwarfing rootstocks to control vigour, allowing higher planting densities, enabling earlier production and increasing productivity per unit land area. The future development of the citrus industry is betting on the incorporation of dwarfing rootstocks, which offer alternative systems of production. In 2006, Agromillora and IVIA began a cooperative program with the aim to reduce size and to find suitable candidates for higher density system plantings. All the rootstocks included in this program are monoembryonic or seedless. Thus, micropropagation allows new options to produce new rootstocks that are difficult or impossible to propagate by traditional techniques, and to make plants available quickly. 35 hybrids between different parental species are being evaluated. We are testing for the tolerance/resistance to CTV, iron chlorosis, salinity, water stress and flooding. As a result of this research and preliminary field results, we selected 4 dwarfing/ semidwarfing rootstocks, three hybrids 'Cleopatra' mandarin x *Poncirus trifoliata* ('CIVAC03013', 'CIVAC03014', 'CIVAC030119') and one hybrid 'King' mandarin x *P. trifoliata* ('CIVAC050122'), all tolerant to CTV. 'CIVAC03014' stands out for its high resistance to salinity and water stress tolerance; 'CIVAC050122' for resistance to iron chlorosis. These materials are being micropropagated for the establishment of experimental plots. It is intended to test their adaptation to a higher density production system and determine the most appropriate management for total mechanization using commercial scion varieties for the citrus industry.

S18P10

Performance of 'Nules' clementine grafted on twelve rootstocks in ANECOOP

del Pino A.[1], Martínez M.[1] and Forner-Giner M.A.[2]

[1]ANECOOP, Spain; and [2]Instituto Valenciano de Investigaciones Agrarias (IVIA), Departamento de Citricultura y Producción Vegetal, Spain. adelpino@anecoop.com

The aim of this trial was to study the performance of 'Clemenules' mandarin (*Citrus clementina*) on twelve rootstocks: 'Carrizo' and 'C-35' citranges [*Citrus sinensis* x *Poncirus trifoliata*], 'Cleopatra' mandarin (*Citrus*

reshni), *Citrus volkameriana*, *Citrus macrophylla*, 'Swingle' citrumelo [*Citrus paradisi* x *P. trifoliata*], and six new hybrids obtained at the Instituto Valenciano de Investigaciones Agrarias (IVIA): 'Forner-Alcaide 5', 'Forner-Alcaide 13', 'Forner-Alcaide 41' and 'Forner-Alcaide 31' are hybrids of 'Cleopatra' mandarin and *P. trifoliata*, 'Forner-Alcaide V17' is a hybrid of *C. volkameriana* and *P. trifoliata*, and 'Forner-Alcaide 2324' is a hybrid of 'Troyer' citrange and 'Cleopatra' mandarin. The plot is located in the Experimental Field of ANECOOP, the "Masia del Doctor", sited ten km north of Valencia (Spain). The trial was planted in 2004 and yield has been weighed during the first five harvests. In the first yield recorded, 'Forner-Alcaide 5' was the rootstock which produced more (80% higher than 'Carrizo' citrange), and after five years, it has the highest cumulative yield of all the rootstocks tested. 'Carrizo', 'Forner-Alcaide V17' and 'Forner-Alcaide 13' showed a high productivity and 'C-35', 'Cleopatra' mandarin, 'Swingle' citrumelo and *C. volkameriana* had the lowest. Fruit quality was analysed in the last two harvests. Rootstock significantly affected fruit quality variables. 'Forner-Alcaide 5', 'Former-Alcaide 13' and 'Forner-Alcaide V17' would appear to induce higher fruit size, juice content and fruit quality.

S18P11

Influence of rootstock hydraulic conductance in the response to water stress and its relationship to aquaporin expression in citrus

Rodríguez-Gamir J.[1], Ancillo G.[2], Bordas M.[3], Primo-Millo E.[1] and Forner-Giner M.A.[1]

[1]Instituto Valenciano e Investigaciones Agrarias (IVIA), Centro de Citricultura y Producción Vegetal, Spain; [2]Instituto Valenciano e Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain; and [3]Agromillora Research S.L, Spain.
forner_margin@gva.es

Recent climate projections by the International Panel for Climate Change (IPCC, 2007) predict that water scarcity will increase in the near future in many regions of the globe. Therefore, climate change poses an acute problem to the citrus crops because the sector is expected to maintain its yield/quality standards under more adverse climate conditions. Agronomic strategies such as the use of new rootstocks better adapted to dryer conditions are needed. Plant water relations may be affected by drought stress and root hydraulic conductance determines the ability of the rootstock to supply water and nutrients to the plant. This ability could be the main factor influencing plant tolerance to abiotic stresses. Aquaporins are involved in regulating root hydraulic conductance by mediating water flow through the cell membranes. Hypothesizing that aquaporins could play a role in citrus response to water stress, responsiveness of photosynthetic parameters and water balance to moderate water stress in different citrus rootstocks was evaluated. The plant materials used were the rootstocks *Poncirus trifoliata*, 'Cleopatra' mandarin (*Citrus reshni* and '030115' (a hybrid of the two former rootstocks), all grafted with the citrus variety 'Valencia Late' (*Citrus sinensis*). Results showed that down-regulation at transcriptional level results in decreased root hydraulic conductance. This facilitates water maintenance in the cells and adequate levels of leaf water potential and photosynthesis in plants under water stress. Thus, the decrease of aquaporin expression could be a water stress tolerance mechanism in citrus.

S18P12

Micropropagation for evaluation of new citrus somatic hybrid rootstocks

Bordas M.[1], Torrents J.[1] and Navarro L.[2]

[1]Agromillora Research, S.L, Spain; and [2]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain. mbordas@agromillora.com

In citrus, virtually no rootstocks have tolerance or resistance to all the main biotic and abiotic stresses present in a given growing area of the world and at the same time induce high yields and fruit quality. Although several breeding programs are currently active to produce such rootstocks, propagation and evaluation of new hybrids continues to be an important component of the development process. The development of somatic hybridization by protoplast fusion in citrus has helped reach improvement goals that seemed unattainable by conventional methods. It is possible to add the genomes of the parents in the hybrids without or with very little recombination. At IVIA, a collection of somatic hybrids between 'Carrizo' citrange (*Citrus sinensis* x *Poncirus trifoliata*) and *Citrus macrophylla* and *Citrus taiwanica* have been obtained. These hybrids are

potentially very interesting for the Spanish citrus industry, but they are juvenile and do not produce seeds for conventional evaluation. In 2011, a collaboration between Agromillora and IVIA began for the multiplication and evaluation of new citrus somatic hybrid rootstocks. We will present the development of a protocol for the micropropagation of three allotetraploid somatic hybrids. We have determined the conditions for the *in vitro* introduction and the establishment of lines in the laboratory, obtaining highly efficient multiplications rates. The environmental conditions in the greenhouse for the successful acclimatization and growth have also been set. Finally, we have accomplished the large-scale adaptation for efficient commercial propagation. This is allowing a very quick process to start evaluation at large scale.

S18P13

Performance of 'Forner-Alcaide 5' and 'Forner-Alcaide 517' with 'Loretina' clementine.

Molina-Nadal M.D.[1], De Miguel A.[1], Valero J.L.[1], Ripoll F.[2] and Forner-Giner M.A.[1]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Citricultura y Producción Vegetal, Spain; and [2]Cooperativa de Algemesí (COOPAL), Spain. molina_marnad@gva.es

The influence of two citrus rootstocks, 'Forner-Alcaide 5' [*Citrus reshni* x *Poncirus trifoliata*] and 'Forner-Alcaide 517' ['King' mandarin (*Citrus nobilis*) x *P. trifoliata*] a semidwarfing rootstock, on growth, yield and fruit quality on 'Loretina' clementine (*Citrus clementina*) has been analysed. Trees were grown in a plot in Algemesi, near Valencia (Spain) and had been planted in 2000. Yield was weighed during five harvest, and fruit quality and tree size determined in 2011. Tree size of plants grafted on 'Forner-Alcaide 517' grow 40% less than 'Forner-Alcaide 5'. Yield efficiency (kg/m3) in 2011 between rootstocks has no differences. Fruit size is smaller in 'Forner-Alcaide 517' than in 'Forner-Alcaide 5', but sugar content and acidity is higher in 'Forner-Alcaide 517' than in 'Forner-Alcaide 5'.

S18P14

Performance of three rootstocks with 'Clemenrubi' and 'Orogros' clementines cultivated under screen.

Molina-Nadal M.D[1], de Miguel A.[1], Bonafé C.[1], Valero J.L.[1], Ripoll F.[2] and Forner-Giner M.A.[3]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Servivio de transferencia de tecnología de Carcaixent, Spain; [2]Cooperativa Agraria de Algemesí (COOPAL), Spain; and [3]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Citricultura y Producción Vegetal, Spain. molina_marnad@gva.es

In a screenhouse trial planted in 2004 and grafted in 2005 with two precocious clementines, 'Clemenrubi' and 'Orogros' (*Citrus clementina*), three citrus rootstocks: 'Carrizo' and 'C-35' citranges [*Citrus sinensis* x *Poncirus trifoliata*] and 'Forner-Alcaide 5' [*Citrus reshni* x *P. trifoliata*] were evaluated in an orchard near Valencia. Yield, fruit size and fruit quality were evaluated for the last four years. Tree size has been evaluated the last year. In the last yield, 'Forner-Alcaide 5' and 'C-35' showed no differences, but 'C-35' is the rootstock that showed higher cumulative yield of the three rootstocks involved in the experiment. Citrange 'Carrizo' presented smaller fruit size, juice content in both varieties, than the other rootstocks, but higher peel thickness. Fruits on 'Forner-Alcaide 5' had higher maturity index. All the plants in the experiment showed multiple shooting alterations in the truck of the variety. For this reason, 30% of fruits of 'Clemenrubi' and 7% on 'Orogros' grafted on 'Carrizo' citrange had dry juice sacs. 'Forner-Alcaide 5' presented only 1% of fruits affected and 'C-35' 3%, in both cultivars.

S18P15

Rootstock influence on the incidence of fruit splitting in navel oranges

Mesejo C., Reig C., Martínez-Fuentes A. and Agustí M.

Instituto Agroforestal Mediterraneo. Universitat Politècnica de València (IAM-UPV), España. carmeco@upv.es

This study aimed to determine the influence of four rootstocks, 'Cleopatra' mandarin (*Citrus reshni*), 'Carrizo' and 'C-35' citranges [*Citrus sinensis* X *Poncirus trifoliata*], and 'FA-5' [*C. reshni* X *P. trifoliata*], on the incidence of fruit splitting in 'Chislett' and 'Lane-late' navel oranges (*C. sinensis*). 'Carrizo' and 'C-35' citranges showed

higher proportion of split fruits (15%) than 'Cleopatra' mandarin and 'FA-5' (5%). On the other hand, 'Chislett' orange showed higher proportion of split fruits (10%) than 'Lane-late' orange (< 2%). Peel resistance to puncturing was not significantly modified by the rootstock. However, fruits from trees budded onto 'Carrizo' and 'C-35' citranges presented a thinner peel. On the other hand, cryo-scanning electron microscopy (SEM) of fruit peduncles showed that average area of xylem vessel were larger in fruits from trees budded onto 'Carrizo' and 'C-35' citranges.

S18P16

Rootstocks for 'Lane Late' orange in Valencia

del Pino A.[1], Martínez M.[1] and Forner-Giner M.A.[2]

[1]ANECOOP, Spain; and [2]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Citricultura y Producción Vegetal, Spain. adelpino@anecoop.com

The performance of 'Lane Late' orange on twelve rootstocks has been evaluated: 'Carrizo' and 'C-35' citranges [*Citrus sinensis* x *Poncirus trifoliata*], 'Cleopatra' mandarin (*Citrus reshni*), *Citrus volkameriana*, *Citrus macrophylla*, 'Swingle' citrumelo [*Citrus paradisi* x *P. trifoliata*], and six new hybrids obtained at the Instituto Valenciano de Investigaciones Agrarias (IVIA): 'Forner-Alcaide 5', 'Forner-Alcaide 13', 'Forner-Alcaide 41' and 'Forner-Alcaide 31' which are hybrids of 'Cleopatra' mandarin and *P. trifoliata*, 'Forner-Alcaide V17' that is a hybrid of *C. volkameriana* and *P. trifoliata* and 'Forner-Alcaide 2324' that is a hybrid of 'Troyer' citrange and 'Cleopatra' mandarin. The plot is located in the Experimental Field of ANECOOP, the "Masia del Doctor", sited ten km north of Valencia (Spain) and planted in 2005. Five first yields have been determined and fruit quality has been analysed. Regarding to the yield, *C. volkameriana* is the rootstock that showed higher cumulative yield, followed by 'Forner-Alcaide 5', 'Forner-Alcaide 13' and 'Carrizo' citrange. The lowest yield was in 'Lane Late' orange grafted in 'Forner-Alcaide V17' and 'Forner-Alcaide 2324'. Differences in fruit quality have been found.

S18P17

Citrus rootstock trials on calcareous soils in California

Roose M. L., Kupper R.S. and Federici C.T.

University of California (UCR), Botany and Plant Sciences, Riverside, CA 9252 U.S.A. mikeal.roose@ucr.edu

Calcareous soils are challenging for citrus production because relatively few rootstocks are well-adapted to such soils and also tolerant to Citrus Tristeza Virus and common soil pathogens. Many rootstocks derive disease resistance from trifoliate orange which is very susceptible to iron chlorosis and poor growth on calcareous soils. We report results of four rootstock trials on calcareous soils in California that were planted in 1997 and 2001 with 26 to 32 experimental and standard rootstocks budded with navel orange, 'Moro' blood orange, and 'Lisbon' lemon. Data on tree size, health, and chlorosis were collected several times from 1997 to 2011. Tree survival varied among sites and rootstocks but was nearly 100% for 'Carrizo', a fairly susceptible standard rootstock. Trees on trifoliate orange had moderate or high chlorosis and poor growth at all sites. Rootstocks with low chlorosis overall included 'ASRT' ('African shaddock' x 'Rubidoux' trifoliate), 'Bitters (C22)', and several unreleased hybrids. At some sites, tree size was not correlated to chlorosis suggesting that other factors limited tree performance.

S18P18

Potential of 'Tahiti' acid lime and 'Flame' grapefruit in the Brazilian semiarid

Bastos D.C.[1], Passos O.S.[2], Nascimento F.S.S.[1] and Nascimento S.S.[1]

[1]Embrapa Semiarido (Embrapa CPATSA), Fruticultura - Fitotecnia, Brasisl; and [2]Embrapa Mandioca e Fruticultura Tropical (Embrapa CNPMF), Fruticultura - Fitotecnia, Brasil. debora@cpatsa.embrapa.br

The citrus industry of the Brazilian notheastern region is concentrated mainly in the states of Bahia and Sergipe, accounting for 10% of the national citrus production. This region has peculiar characteristics

favorable for the citrus industry expansion. The climate is very hot and semiarid. The rainfall is less than 600 mm and the average annual temperature is 26.2º C, relative humidity of 67% and solar radiation around 3,000h/year, constituting a comparative advantage with regard to biomass production and crops, especially fruits such as acid lime, lemon and grapefruit, supplemented by water via irrigation. This work was planned in order to evaluate the behavior of acid lime 'Tahiti' and 'Flame' grapefruit grafted on different rootstocks in the semiarid northeast. The experiments were conducted at the Experimental Station of Embrapa Semiarid in Juazeiro, Bahia, with 'Tahiti' and 'Flame' scions on twenty rootstocks mostly hybrids of *Poncirus trifoliata*. The rootstocks 'Cleopatra mandarin x 'Swingle trifoliate-288', 'Rusk' citrange, 'English trifoliate x Sunki mandarin-26 and 256', 'Cleopatra mandarin x Swingle trifoliate-294', 'Swingle trifoliate x Sunki mandarin-314' proved to be most promising rootstocks for 'Flame' and 'Rusk' citrange, 'Volkameriana' lemon, citrange 'C35', 'English trifoliate x Sunki mandarin-264 and 256', citrange 'Troyer' and 'Swingle trifoliate x Sunki mandarin-314' for 'Tahiti', both in production and productivity under semiarid conditions.

S18P19

Selection of new citrandarins for citrus rootstocks

Cristofani-Yaly M.[1], Schinor E.H.[1], Bastianel M.[1], Nascimento A.L.[1], Stuchi E.S.[2] and Machado M.A.[1]

[1]Centro de Citricultura, Instituto Agronômico (IAC), Brazil; and [2]Embrapa Mandioca e Fruticultura and Estação Experimental de Citricultura de Bebedouro (Embrapa and EECB), Brazil. mariangela@centrodecitricultura.br

In the 1990s, the Centro de Citricultura Sylvio Moreira / IAC launched a wide program of genetic breeding of citrus rootstocks through controlled crosses. From the hybridizations using several rootstocks varieties, we selected to evaluate 73 citrandarins (hybrids from 'Sunki' mandarin x *Poncirus trifoliata* 'Rubidoux'). The hybrids were grafted with 'Pera' sweet orange and the trials were established in two regions of the São Paulo State. The experimental design was a randomized complete-block with three replications and one plant per plot without irrigation. Our results, in 5-yr-old plants, showed that the citrandarins influenced various horticultural traits of the scion as harvest season, yield, juice quality, and drought tolerance. The ºBrix ranged from 8 to 17, the canopy volume ranged from 3.51 to 11.30 m3 and the yield efficiency varied from 3.47 to 18.94 kg/m3. Graft incompability with 'Pera' sweet orange was observed in six hybrids among the 73 evaluated. Among the tested hybrids, 19 induced dwarfing into the scion variety. Financial support: FAPESP, CNPq, INCT-Citrus.

S18P20

Initial production of 'Valencia' sweet orange on 40 rootstocks in Northern São Paulo State, Brazil.

Fadel A.L.[1], Mourão Filho F.A.A.[1], Stuchi E.S.[2] and Ramos Y.C.[1]

[1]Universidade de São Paulo (USP), Departamento de Produção Vegetal, Brazil; and [2]Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Estação Experimental de Citricultura de Bebedouro, Brazil. afadel@usp.br

Due to increasing demand for diversification of rootstocks in Brazilian citrus industry, a research work was installed in March 2007 to evaluate 40 different rootstocks for 'Valencia' sweet orange (*Citrus sinensis*), in the Northern region of São Paulo State, Brazil. The experiment has a randomized block design with three replications and five plants per plot. The data regarding accumulated fruit production in 2010 and 2011 were submitted to variance analysis and the means were compared by Scott-Knott test ($P<0.05$). The ten most productive combinations were those that included the rootstocks 'Rangpur' lime (*Citrus limonia*), 'Malvasio SRA 115' mandarin (*Citrus reticulata*), 'Sunki' mandarin x 'Benecke' trifoliate (*Citrus sunki* x *Poncirus trifoliata*) 'C-13' citrange (*C. sinensis* x *P. trifoliata*), 'C-54-4-4 SRA 337' mandarin (*C. reticulata*), 'Peau Lisse SRA 267' mandarin (*Citrus deliciosa*), 'Cleopatra' mandarin x 'Rubidoux' trifoliate (*Citrus reshni* x *P. trifoliata*), ['Rohde Red' 'Valencia' sweet orange plus 'Volkamer' lemon (*C. sinensis* plus *Citrus volkameriana*) 4x], 'East India SRA 414' mandarin (*C. reticulata*), and 'Sunki' mandarin x 'English Palmira' trifoliate hybrid (*C. sunki* x *P. trifoliata*). Plants on these rootstocks will be further evaluated regarding other important traits such as tolerance to water deficit and to Citrus Sudden Death disease.

S18P21

Crop production of 'Okitsu' satsuma mandarin on nine rootstocks in Southern Brazil

Tazima Z.H.[1], Neves C.S.V.J.[2], Yada I.F.U.[3] and Leite Junior R.P.[4]

[1]Instituto Agronômico do Paraná (IAPAR), Ecofisiologia, Brazil; [2]Universidade Estadual de Londrina (UEL), Agronomia, Brazil; [3]Instituto Agronômico do Paraná (IAPAR), Biometria, Brazil; and [4]Instituto Agronômico do Paraná (IAPAR), Proteção de Plantas, Brazil. zuleide@iapar.br

This study reports the performance of 'Okitsu' satsuma mandarin (*Citrus unshiu*) trees grafted on nine different rootstocks and evaluated for nine harvests, in Southern Brazil. The field plot was established in the Experimental Station of Instituto Agronômico do Paraná (IAPAR), located in the municipality of Londrina, State of Paraná, Brazil, and planted in July 2000. The experimental design was randomized blocks with nine treatments, six replications, and two trees per plot. Trees on 'Swingle' citrumelo had the highest yield in all the nine years of evaluation. In contrast, trees on 'Volkamer' lemon had the lowest yield in seven of nine years of harvest. 'Swingle' rootstock induced both, the highest cumulative yield (1.568.7 kg/tree) and the highest average yield per tree. According to the relative yield, 'Swingle' citrumelo induced a production index of 58% higher than trees on 'Rangpur' lime, the most important citrus rootstock in Brazil. Furthermore, trees on 'Rangpur' lime had a performance similar to the ones on Trifoliate orange, in regard to crop production. In relation to yield efficiency, the trees on 'Swingle' were similar to the ones on Trifoliate orange, 'C-13', and 'Rangpur' lime. No alternate bearing was observed for the 'Okitsu' satsuma mandarin trees in any of the nine different rootstocks studied. The highest yield efficiency was presented by trees on Trifoliate orange. Financial support: Instituto Agronômico do Paraná and Fundação Araucária

S18P22

Effect of rootstock on yield of 'Okitsu' satsuma mandarin on sandy soils in Southern Brazil

Tazima Z.H.[1], Neves, C.S.V.J.[2], Yada I.F.U.[3], Stenzel N.M.C.[4] and Leite Junior R.P.[5]

[1]Instituto Agronômico do Paraná (IAPAR), Ecofisiologia, Brazil; [2]Universidade Estadual de Londrina (UEL), Agronomia, Brazil; [3]Instituto Agronômico do Paraná (IAPAR), Biometria, Brazil; [4]Instituto Agronômico do Paraná (IAPAR), Fitotecnia, Brazil; and [5]Instituto Agronômico do Paraná (IAPAR), Proteção de plantas, Brazil. nstenzel@iapar.br

Trees of 'Okitsu' satsuma mandarin grafted on nine different rootstocks were evaluated for eight harvests, in Southern Brazil. The field plot was established on Oxisol (Typic Haplorthox) at the Experimental Station of the Instituto Agronômico do Paraná (IAPAR), located in the Paranavaí municipality, State of Paraná, Brazil, in January 2001. The experimental design was randomized blocks with nine treatments, three replications, and two trees per plot. In regard to annual crop production, no statistical differences between treatments were observed in five of the eight years of harvest. The highest cumulative fruit yield and average yield per trees of 'Okitsu' satsuma mandarin were determined for the trees on 'Volkamer' lemon, 'Rangpur' lime, 'Caipira DAC' sweet orange, 'Cleopatra' mandarin and 'Carrizo' citrange, while trees on 'Sunki' mandarin, 'C 13' citrange, 'Swingle' citrumelo and trifoliate orange showed the lowest yield. Trees on 'Volkamer' lemon presented similar relative yield to the ones on 'Rangpur' lime, the main rootstock used in Brazil. Financial support: Instituto Agronômico do Paraná and Fundação Araucária.

S18P23

Scion rooting and its discriminance in 'Shiranuhi' mandarin hybrid in plastic film house in Korea

Moon Y.E.[1], Kang S.B.[2], Kim Y.H.[2] and Han S.G.[2]

[1]Agricultural Research Center for Climate Change, NIHHS, RDA, Republic of Korea; and [2]Citrus Research Station, NIHHS, RDA, Republic of Korea. yimoon@korea.kr

Citrus is usually propagated by grafting onto rootstock. In Korea, as trifoliate orange (*Poncirus trifoliata*) has dwarf and strong cold hardness, it is widely used as the rootstock of satsuma mandarin. 'Shiranuhi' [(*Citrus unshiu* × *Citrus sinensis*) × *Citrus reticulata*)], a kind of citrus, also, generally grafted onto trifoliate orange, and most of farmers have recognized that 'Shiranuhi' root is trifoliate orange naturally. Meanwhile, reduction of flowering in 'Shiranuhi' orchards has been issued among farmers and researchers over the past few years, and they guessed that it was occurred by severe prunning, oversupply of fertilization,

overfruiting and temperature during growth periods. However, few researchers strongly assumed that it was caused by scion rooting of 'Shiranuhi'. So, this study was carried out to find out relationships between reduction of flower and scion rooting, discriminance methods and the ecology of scion root of 'Shiranuhi' in Korea. From our results, when we conducted the DNA analysis, fourteen trees in less bloomed twenty trees was proved as tree with the scion rooting of 'Shiranuhi' and this results was firstly identified in Korea. Scion root of 'Shiranuhi' is tended to be 30% thicker and more vigorous in root growth than in the case of trifoliate orange. Scion root of 'Shiranuhi' was usually observed in a deeply planted tree, and xylem of 'Shiranuhi' root indicated more white color than a case of trifoliata orange. 'Shiranuhi' tree by scion rooting was more vigorous but less flowering than trees grafted onto trifoliata orange. When we used F2/R15, F4/R15 and F5/R15 primer for discriminance of 'Shiranuhi' root and trifoliate root, it was possible for us to discriminate scion roots of 'Shiranuhi'.

S18P24

Effects of scion root on flowering, fruit quality and yield of 'Shiranuhi' mandarin hybrid in plastic film house in Korea

Moon Y.E[1], Kang S.B.[2], Han S.G.[2], Ko S.W.[2], Chae C.W.[2], Lee D.H.[3] and Choi Y.H.[2]

[1]Agricultural Research Center for Climate Change, National Institute of Horticultural & Herbal Science. R.D.A., Korea, Republic of KR; [2]Citrus Research Station, National Institute of Horticultural & Herbal Science. R.D.A., Korea, Republic of, KR; and [3]Planning Coordination Division, National Institute of Horticultural & Herbal Science. R.D.A., Korea, Republic of, KR. yimoon@korea.kr

Most of citrus is usually propagated by grafting, and trifoliate orange (*Poncirus trifoliata*) is widely used as rootstock of satsuma mandarin since it has dwarf and cold hardness in Korea. 'Shiranuhi' mandarin [(*Citrus unshiu* × *Citrus sinensis*) × *Citrus reticulata*)] mainly needs trifoliate orange (*P. trifoliata*) as its rootstock. However, recently, scion root was observed in 'Shiranuhi' mandarin trees grafted onto the trifoliate orange. This study was carried out to find out effects of outbreak of scion root on the fruit quality, flowering and yield of 'Shiranuhi' mandarin in Korea. For the experiment, we selected three farmers who have outbreak of scion root in their 'Shiranuhi' mandarin orchards and surveyed the difference of growth and fruit quality of 'Shiranuhi' mandarin between scion root and rootstock root (trifoliate orange root). In our experiment, fruit size and weight of scion root of 'Shiranuhi' mandarin were smaller than rootstock root. Flesh ratio, also, showed decrease in scion root of 'Shiranuhi'. Moreover, flowering was severely decreased in scion root trees than normal trees. Among survey of 174 'Shiranuhi' mandarin trees, 160 trees were proved to be scion root, and they had less flowering. Yields of scion root (18.2 kg) decreased 30% compared to rootstock root (25.9 kg). However, there was no difference on the TSS, acidity and soluble solid-acid ratio between scion root tree and rootstock root tree. From the results, we concluded that scion root had negative influence on flowering, fruit size and the yields of 'Shrinanuhi' mandarin. Therefore, we suggest finding a solution to improve flowering, fruit size and fruit quality in 'Shiranuhi' mandarin which has outbreak of scion root.

S18P25

Evaluation on drought resistance traits of citrus rootstock seedlings based on multivariate statistics

Xu X.B., Yang Y.L., Gu Q.Q., Huang C.H. and Qu X.Y.

College of Agronomy,Jiangxi Agricultural University (JAU), P.R.China. xiaobiaoxu@hotmail.com

The drought resistance of nine citrus rootstocks was comprehensively evaluated by determining the physiological indexes such as relative water content (RWC), the activity of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), malondialdehyde (MDA), proline (Pro) and soluble sugar content (SSC) under artificial controlling water condition by taking the seedlings of nine citrus rootstocks from the local citrus rootstock resources of Jiangxi province in China, named 'Sanhu Red' tangerine (*Citrus reticulata*), 'Choupi' mandarin (*Citrus aurantium*), 'Sanhu' hybrid mandarin (*C. reticulata* × *C. aurantium*), 'Chongyi' wild tangerine (*C. reticulata*), 'Citrumelo' (*Citrus grandis* × *Poncirus trifoliata*), 'Trifoliate orange' (*P. trifoliata*), 'Goutou' orange (*C. aurantium*), 'Carrizo' citrange (*Citrus sinensis x P. trifoliata*), 'Sour' pummelo (*C. grandis*) as tested materials through the analysis of principal components and subordinate function values. The results

were found that the order of drought resistance was as follow: 'Sanhu Red' tangerine > 'Goutou' orange > 'Carrizo' citrange > 'Choupi' mandarin > 'Trifoliate' orange > 'Citrumelo' > 'Chongyi' mandarin > 'Sanhu' hybrid mandarin > 'Sour' pummelo. Furthermore, the 'Sanhu Red' tangerine is the local citrus variety of Jiangxi province and it has stronger drought resistance among the nine citrus rootstocks, which could provide a solid basis for exploiting rootstock resources of drought resistance. The result of the study could provide theoretical foundation for germplasm improving and reference for reasonable development of germplasm resource in citrus rootstocks.

S18P26

Performance of three sweet orange varieties grafted on different rootstocks

Yan S.T.[1], Cheng C.Z.[2], Ma Y.Y.[2], Wang X.[2] and Zhong G.Y.[3]

[1]Citrus Research Institute, Southwest University, China; [2]Southwest University, College of Horticulture and Landscape Architecture, China; and [3]Fruit Tree Research Institute, Guangdong Academy of Agricultural Sciences, China. shutangyan@sina.com

The performance of three sweet orange (*Citrus sinensis*) varieties 'Hamlin', 'Tongshui 72-1 Jincheng' and 'Troivita', were studied on 11 rootstock varieties 'Rangpur' lime (*Citrus limonia*) (RL), 'Volkamer' lemon (*Citrus volkameriana*) (VL), 'Carrizo' citrange (*C. sinensis × Poncirus trifoliata*) (CC), 'Smooth Flat Seville' (*Citrus aurantium* hybrid) (SFS), 'Goutoucheng' Sour Orange (*C. aurantium*) (GSO), 'Sunchusha' tangerine (*Citrus reticulata*) (ST), 'Rich 16-6' trifoliate orange (*P. trifoliata*) (R16-6), 'Rubidoux' trifoliate (*P. trifoliata*) (RT), 'Pomeroy' trifoliate (*P. trifoliata*) (PT), citrange (*C. sinensis* 'Ruby' × *P. trifoliata*) (C-35), and 'Cleopatra' mandarin (*C. reticulata*) (CM) in Chongqing, China during 2007-2009. Tree vigor, yield and quality were investigated and compared for three consecutive years. The experiment was arranged in randomized block design, three replications and five trees per plot. The scion cultivars 'Hamlin' orange, 'Tongshui 72-1 Jincheng', and 'Trovita' orange grafted on 'R 16-6' all showed excellent performance as did 'Tongshui 72-1 Jincheng' and 'Trovita' orange on 'Rangpur' Lime (RL), with strong or medium tree growth vigor, highest yield, yield efficiency, and best fruit quality. 'Hamlin' orange and 'Tongshui 72-1 Jincheng' on 'Pomeroy' trifoliate (PT) and 'Volkamer' lemon (VL) also showed good productivity and fruit quality with a smaller tree canopy, and could be planted more densely. 'Rich 16-6' trifoliate, PT, VL, and RL are the most suitable rootstocks for 'Hamlin', 'Tongshui 72-1 Jincheng' and 'Trovita' orange. Additionally, PT and VL can be used for high-density cultivation.

S18P27

Effects of six rootstocks on tree growth, fruit quality and N, P, K content of lemon

Hong L., and Xie Y.H.

Chongqing Academy of Agricultural Sciences (CAAS), Fruit Research Institute, China. cqxieyh@hotmail.com

The present experiment was carried out to screen proper rootstock for 'Fino 47' lemon. We had analysed tree growth, fruit quality and annual dynamic changes of N, P, K content of leaves using 'Fino 47' lemon grafted with six different rootstocks. Differences were found in both the vegetative and reproductive growth of different treatments. The trees on 'Carrizo' citrange had the highest tree height and crown diameter. Whilst, growth of new branches on 'Carrizo' citrange and 'Swingle' citrumelo became more than other treatments. The trees on 'Volkamer' had largest number of terminal branches. In addition, the flowering behavior of different treatments was not the same. Spring blossom of three-year-old trees on 'Swingle' citrumelo was highest of all, however 'Junos' with highest autumn blossom. In the aspect of fruit quality, the largest fruit weight was gotten from trees on 'Swingle' citrumelo. The largest content of total acids and soluble solids were gotten from trees on 'Volkamer'. The pericarp was thinner and oil cells smaller using 'Trifoliate' and 'Junos' as rootstocks. Fruit on 'Junos' had highest juice content, while lowest on 'Red' tangerine. The rootstocks did not influence the annual dynamic changes of N, P and K in leaves, only influenced the contents of mineral nutrient in leaves at different stages. We came to the conclusion primarily that 'Volkamer' and 'Swingle' citrumelo were suitable rootstocks for 'Fino 47' lemon, therefore may be extended for demonstration and production.

S18P28

Tree performance and fruit quality evaluation of 'Shatangju' mandarin grafted on four rootstocks in South China

Liu X.[1], Huang M.[1], Li J.[2], Chen J.[1] and He Y.[1]

[1]College of Horticulture, South China Agricultural University, China; and [2]Zhongkai University of Agriculture and Engineering, China. willderliu@gmail.com

'Shatangju' (*Citrus reticulata*), a delicious fresh fruit variety of citrus, is native to South China. Its fruit is orange red, thin skin and easy peeling, and now it's one of the largest economic benefit fruits in South China. But very few studies have been conducted on rootstocks for 'Shatangju' in China. So, a field experiment with 'Shatangju' grafted onto 4 rootstocks was carried out in Guangdong Province, to compare rootstock performance and to make its comprehensive evaluation by principal component analysis (PCA). The evaluated rootstocks were: 'Trifoliate' orange (*Poncirus trifoliata*), 'Canton' lemon (*Citrus limonia*), 'Red' tangerine (*C. reticulata*) and 'Sour' tangerine (*C. reticulata*). The trees, planted in 2002, were arranged in a randomized block design, with five replicates and one plant per plot. After 2 successive years of evaluation, results showed that trees grafted on 'Trifoliate' orange and 'Canton' lemon were dwarfing and the others were vigorous. Fruits from 'Canton' lemon rootstock were largest and had lowest edible rate, while fruits on 'Trifoliate' orange were smallest but had the reddest color. The effects on fruit internal quality were irregular. The results from PCA by SPSS showed that the score of 'Canton' lemon rootstock was highest in both years. PCA proved to be a very effective procedure to obtain a synthetic judgment of rootstocks.

S18P29

Influence of different rootstock type on chlorophyll content and mineral elements concentration in scion of 'Kinnow' mandarin

Hosseini Farahi M.[1], Aboutalebi Jahromi A.[2] and Hasanzada H.[3]

[1]Young Researcherch Club, Yasouj Branch, Islamic Azad University, Yasouj, Iran; [2]Department of Horticulture Science, Jahrom Branch, Islamic Azad University, Jahrom, Iran; and [3]M.Sc in Horticulture and Specialist of Minab Agricultural Research Station, Iran . m.hosseini.farahi@gmail.com

The aim of this work was evaluated the effect of the 4 citrus rootstocks: sour orange (*Citrus aurantium*), 'Bakraei' (*Citrus reticulata* × *C. aurantium*), 'Mexican' lime (*Citrus aurantifolia*) and 'Volkamer' lemon (*Citrus volkameriana*) on chlorophyll content and mineral elements (N, P, K, Ca, Mg, Na, Fe, Zn, Cu, Mn, Cl and B) in scion's leaf of 'Kinnow' mandarin *(C. reticulata)* grown in pot containing calcareous soil in greenhouse as completely randomized design with 4 replications. Results showed that rootstocks had significant effects on leaf chlorophyll content. The greatest chlorophyll content obtained on 'Volkamer' lemon rootstock. The rootstock types had significant effect on leaf mineral concentrations except of Cl, P, Cu, Mn and Zn. Leaf N and Mn concentration was lower than optimum range on all rootstocks, K on 'Mexican' lime rootstock and Ca on all rootstocks except 'Mexican' lime. The scions on sour orange had the highest Cl. Na concentrations was the highest on 'Mexican' lime rootstock.

S18P30

Preliminary studies on high density planting of some citrus cultivars to obtain a more rapid recovery of investment capital

Bassal M.A.

Suez Canal University, Faculty of Agriculture, Horticulture Department, Egypt. magdy_bassal@yahoo.com

'Clemenules', 'Hernandina' and 'Nova' mandarins budded on sour orange rootstock were planted at two planting spacing of 2x5 m and 5x5 m to obtain economic yields earlier in the lifespan of the orchard. Annual fruit production plus cumulative and average yield per tree and per acre were estimated during five production seasons, from 3rd to 7th years after planting (YAP). In addition, tree height, canopy diameter and circumference, tree volume, and trunk girth were determined in the 6th and 7th YAP. Trees planted at 2x5 m were significantly taller than those planted at 5x5 m, but the canopy volume of trees planted at 5x5 m was

larger, while trunk girth was not affected by tree spacing in the 6th and 7th YAP. On the other hand, trees of 'Nova' mandarin had the smallest canopy diameter and circumference compared to those of 'Hernandina' and 'Clemenules' clementine, while 'Clemenules' trees had the greatest height and volume. The yield per acre from trees planted at 2x5 m was consistently higher than those planted at 5x5 m. Yields from the higher density planting were about 454%, 221%, 137%, 76% and 90% greater than those from the lower density, in the 3rd to 7th YAP, respectively. Cumulative yield (over 5 years) per acre from closely planted trees was 40 tons (117%) more than that from widely spaced trees. This means that growers can obtain about 117% more economic return from the same land area with approximately the same cost of production. Cumulative and average annual yield per tree indicated that 'Hernandina' and 'Nova' cultivars were significantly more productive than 'Clemenules' under the conditions of this study.

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CITRUS AND HEALTH

S20O01

Flavanones are involved in the cardiovascular protective effects of citrus fruits

Morand C.

Human Nutrition Unit, UNH-UMR 1019, INRA, Centre de Recherche Clermont/Theix, France. christine.morand@clermont.inra.fr

Although it is widely known that consuming fruits rich in polyphenols has a beneficial effect on health, it is more difficult to attribute a protective effect to a particular compound. As suggested by several epidemiological studies citrus fruits could be largely involved in the beneficial effect of fruits consumption on cardiovascular health. Citrus fruits provide vitamin C, carotenoids and they are also exclusive and abundant sources of flavanones, a particular class of dietary polyphenols. Until recently the role of these bioactive compounds in vascular protection was still far from being elucidated. By performing clinical trials, preclinical studies and *in vitro* experiments, we have examined the specific effects of the major flavanones in orange (hesperidin) and grapefruit (naringin) on vascular protection. In healthy overweight men, we showed that a 4 week consumption of orange juice or hesperidin significantly reduced diastolic blood pressure and both significantly improved postprandial vascular reactivity. Both also significantly affected leukocyte gene expression with over 1.000 genes up-regulated by both orange juice and hesperidin, involved in chemotaxis, adhesion, infiltration and lipid accumulation, processes involved in atherosclerosis development. In mice, naringin consumption protected from diet-induced atherosclerosis with a marked reduction of lipid deposit and modulated expression of genes in aorta implicated in cell adhesion and transendothelial migration. *In vitro*, flavanone metabolites significantly attenuated monocytes adhesion to TNFα-activated endothelial cells and decreased the expression of some atherogenic genes, like cell adhesion molecules or chemokines. In conclusion, taken together, these results suggest a beneficial impact of citrus flavanones on vascular health that could be highly mediated through genomic effects.

S20O02

Health benefits of citrus: recent advances and future outlook

Patil B.S., Jayaprakasha G.K., and Uckoo R.M.

Texas A&M University (TAMU), Vegetable and Fruit Improvement Center, Department of Horticultural Sciences, USA. b-patil@tamu.edu

Citrus fruits have a multitude of health promoting properties and in the past few decades, research has made significant contributions in connecting specific health benefits to a group of secondary metabolites called bioactive compounds. These compounds have been isolated and investigated for their potential roles in improving human health, such as in reducing the risk of chronic diseases including cancer, cardiovascular diseases and osteoporosis. Also, in recent years, a worldwide surge in the consumer demand for these delicious and healthy fruits has led to increased production. However, different production systems and post-harvest factors may affect citrus bioactives, thus altering the healthfulness of the fruit. Here, the influence of specific pre-and post-harvest factors on the levels of bioactives and their relevant biological activities will be discussed. Research at the Vegetable and Fruit Improvement Center on “farm to consumer” aspects of citrus, specifically in grapefruit, will be presented, including varying production systems, testing biological activities using cell culture and animal models and conducting human intervention studies. -The present research report is based upon work supported by USDA-NIFA #2010-34402-20875 “Designing Foods for Health” through the Vegetable & Fruit Improvement Center.

S20O03

Orange and grapefruit bioactive compounds, citrus consumption and health benefits

Cancalon P., and King D.

Florida Department of Citrus (FDOC), Scientific Research, USA. dking@citrus.state.fl.us

Some of the health benefits of citrus fruits may be attributed to specific bioactive compounds. Citrus are rich in flavonoids, the two more prominent being hesperidin in oranges and naringin in grapefruit. The citrus phytochemical matrix also contains other potentially bioactive compounds such as carotenoids, limonoids

and ascorbic acid, acting individually or in concert. Recent human clinical studies suggest that citrus bioactives provide most of their beneficial effects via processes targeting one or more steps of chronic inflammation processes. Long term inflammation is considered to be a key element in the development of many chronic diseases: cardiovascular diseases, metabolic syndrome, osteoporosis, dementia and some forms of cancer. Citrus phytochemicals appear to act through their metabolites by modulating complex biochemical pathways, signalling pathways, or differential expression of specific disease-related genes. The establishment of health-beneficial 'causes and effects' associated with consumption of citrus fruit and juice products has been the focus of a significant number of recent clinical studies. A review of human clinical studies, and relevant supportive evidence, will be reported.

S20O04

Effects of a long-term grapefruit juice consumption on vascular protection and bone metabolism: a controlled randomized cross-over study in post-menopausal women to determine the specific role of naringin

Habauzit V[1], Verny M.A.[2], Milenkovic D.[2], Pickering G.[1], Duale C.[1], Bayle D.[2], Thien S.[2], Rambeau M.[2], Mazur A.[2], Horcajada M.N.[2], Dubray C.[1], and Morand C.[2]

[1]Centre Investigation Clinique, CIC-CPC INSERM 501, Centre Hospitalier Universitaire, Clermont-Ferrand, France; and [2]INRA, UMR 1019, Unité de Nutrition Humaine, France. christine.morand@clermont.inra.fr

The consumption of some specific polyphenol-rich foods (cocoa, green tea or soya products) has been associated with improvement in several intermediate markers of risk related to cardiovascular disease (CVD) and osteoporosis. Citrus fruits also constitute dietary polyphenol-rich sources; however clinical evidence of the role of polyphenols in citrus health effects is less consistent.

We aimed at 1) characterizing the effect of a long-term consumption of grapefruit juice on both vascular and bone protections in humans and 2) assessing the specific role of its main bioactive phenolic (naringin) in the observed effects. Fifty-two healthy post-menopausal women (non-smoking, aged 50-65 years, 3-10 years since menopause, waist size > 88 cm) were enrolled in a randomized, controlled cross-over trial. For two periods of 6 months, subjects consumed 340 ml/d of either a grapefruit juice (providing 100 mg naringin per serving) or a control beverage mimicking the composition of juice but without naringin. Beyond the monitoring of the effect of treatments on conventional risk factors for CVD, the main outcomes were the evaluation of endothelium-dependent vasoreactivity, in both macro- and microcirculation (brachial FMD and PAT signal), and arterial stiffness (carotid-femoral PWV). Regarding bone metabolism, the main outcome measured was impact on bone mineral density at multiple sites. The statistical analyses of the obtained data are currently going on.

S20O05

Effect of technological treatments on the bioavailability of flavanones from orange

Vallejo F.[1], Tomás-Navarro M.[1], Carbonell J.V.[2], Navarro J.L.[2], and Tomás-Barberán F.A.[1]

[1]Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC), Ciencia y Tecnología de Alimentos, España; and [2]Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Ciencia de los Alimentos, España. fvallejo@cebas.csic.es

The effect of solubility and concentration of citrus juice flavanones on their bioavailability was evaluated. Fresh squeezed orange juice (FS) and orange juice produced by two different technological processes (High Pressure Homogenization (HPH) and Pasteurization (P)) were compared. Dissimilar flavanone precipitations among treatments were observed. Thus, HPH treatment kept in suspension through soluble part higher amount of flavanones increasing therefore their bioavailability. The cultivars studied were 'Ortanique' and 'Lane Late'. The study was carried out in healthy volunteers (n=18) following the administration of 400 mL of orange juices and 24-h urine collection. The determination of the total amount of flavanones present in the supernatant and pellet of juices was measured by HPLC-ESI-MSn. The total excretion of Phase II metabolites in all volunteers was quantified by UPLC-QqQ using the relevant standard metabolites produced by organic synthesis (glucuronidated and sulfonated flavanone metabolites).

S20O06

Evaluation of the anticancer activity on prostate cancer by low molecular weight citrus pectin

Lu S.[1], Chen J.[1], Yin Y.[2], Xia Q.[1], Zhang J.[1], and Yang Y.[1]

[1]Zhejiang Academy of Agricultural Sciences (ZAAS), Institute of Food Science, China; and [2]Zhejiang Normal University (ZNU), College of Chemistry and Life Science, China. lushengmin@hotmail.com

Low molecular weight citrus pectin (LMCP) was prepared by treating commercial citrus pectin (CP) at 121ºC for 30 min and then lyophilized. The molecular properties including weight, galacturonic acid content and degree of esterification (DE) of LMCP and CP were determined, and their resistance capacity on hormone-independent prostate cancer PC3 cells *in vitro* was investigated as well. Galatin-3 levels in PC3 cells were detected using Northern blotting. The results indicated that the average molecular weight (Mp), galacturonic acid content and DE of LMCP were 6685u, 78% and 38% respective in LMCP compared to 55,654 u, 64% and 59% respectively in CP. Both CP and LMCP had inhibitive activities to PC3 vitality in a dose-dependent manner, however, LMCP had stronger inhibition capacity against PC3 cells. The inhibition of LMCP in middle dose (2.5 mg/mL) to PC3 cell viability was 38%, and the inhibition rate increased to 80% ~90% when treated with LMCP in high dose (5-10 mg/mL). The IC50 for inhibiting PC3 cells proliferation was 3.55±0.44 mg/mL. Galactin-3 level in PC3 cells treated with 10 mg/mL LMCP had no significant difference with that in the control cells, which indicated that the anticancer activity of LMCP on PC3 cells might have no relation with galactin-3 levels.

S20O07

A molecular approach to characterize the accumulation of ascorbic acid in citrus fruits

Alós E, Legaz P., Rodrigo M.J, and Zacarías L.

Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Food Science, Spain. ealos@iata.csic.es

Citrus fruits are widely recognized by their concentration of vitamin C and therefore as one of the major sources of ascorbic acid (AsA) for human nutrition and health. Moreover, AsA is the most abundant antioxidant present in plant cells and plays important roles in many developmental and stress processes. The biosynthesis of AsA in higher plants has been elucidated recently and to date four, somehow interlinked, AsA biosynthetic pathways have been proposed; namely the L-Galactose, the L-Gulose, the *Myo*-inositol and the D-Galacturonic acid pathways. Despite the relevance of AsA in citrus fruits, the biochemical and molecular bases of its synthesis and accumulation are almost unknown. Therefore, the objective of the present work has been to initiate a molecular characterization of the metabolic pathways involved in the synthesis and accumulation of AsA in citrus fruits in order to understand its regulation and to identify potential key steps of the pathway. Nine genes belonging to the four biosynthetic pathways and also to the degradation and recycling of AsA have been selected and their expression characterized in peel and pulp of fruit during development and maturation. Results in fruits of selected citrus varieties indicate that changes in AsA content appear to be regulated not only by *de novo* biosynthesis of AsA but also by degradation, recycling and transport, and that different mechanisms may operate in peel and pulp.

S20P01

Protective effect of grapefruit naringin on atherosclerosis development and identification of the underlying mechanisms

Milenkovic D.[1], Chanet A.[1], Habauzit V.[2], Bennetau-Pelissero C.[3], Berard A.M.[4], Mazur A.[1], and Morand C.[1]

[1]INRA, UMR1019, Unité Nutrition Humaine, France; [2]Centre Investigation Clinique, CIC-CPC INSERM 501, Centre Hospitalier Universitaire, Clermont-Ferrand, France; [3]Université de Bordeaux, ENITA Bordeaux 1, France; and [4]ERU « Facteurs de risque vasculaires », CHU-Université de Bordeaux, France. christine.morand@clermont.inra.fr

The aim of this study was to investigate the anti-atherogenic effect of naringin in a mouse model of hypercholesterolemia (mice fed a high-fat/high-cholesterol diet) and decipher its molecular targets in aorta using nutrigenomic approach. Dietary naringin supplementation (0.02% wt/wt) for 18 weeks reduced plaque progression by 41% compared to controls as well the concentrations of several biomarkers of endothelial

dysfunction. Microarray studies performed on aortas demonstrated differentially expressed genes encoding proteins involved in cell adhesion, actin cytoskeleton organization or focal adhesion. Expression profile of these genes suggested limited immune cell adhesion to endothelial cells (ECs) and infiltration in the intima of vascular wall. This hypothesis was strengthened by *in-vitro* experiments on ECs using naringin metabolites at physiologically relevant concentrations. These metabolites significantly reduced monocyte adhesion to TNFα-activated ECs. Exposure of both monocytes and ECs to naringin metabolites potentiated their inhibitory effect on monocyte adhesion, suggesting that monocytes may also be targets for naringenin metabolites. In conclusion, this study revealed that the atheroprotective effect of dietary naringin could be linked to its effect on gene networks and cell functions related to leukocyte adhesion and transendothelial migration, processes highly involved in the early steps of atherosclerosis development.

S20P02

Effect of membrane processing on the radical scavenging activity of juice from IGP product *Citrus limon* from "Rocca imperiale" (south Italy)

Loizzo M.R.[1], Tundis R[1], Bonesi M.[1], Pugliese A.[1], Di Sanzo G.[2], Balducchi R.[2], Verardi A.[3], and Calabro V.[3]

[1]University of Calabria (UNICAL), Department of Pharmacy, Health Sciences and Nutrition, Italy; [2]ENEA C.R. Trisaia (ENEA), Laboratorio Biotecnologie (UTTRI-BIOTEC), Italy; and [3]University of Calabria (UNICAL), Department of Engineering Modeling, Italy.
vincenza.calabro@unical.it

Citrus limon from "Rocca imperiale" was awarded the IGP (Protected Geographical Indication) denomination in 2012. The juice is characterized by yellow colour and a sweet taste. Juice obtained by manual pressure *of C. limon* was firstly centrifuged and then concentrated with ultrafiltration process (carried out on Osmonics® module, by using a polysulphone membrane, cut off 150 kDa, under d transmembrane membrane pressure ranging from 0.5 to 2 bar) with the aim to concentrate and separate the antioxidants. Samples of permeate and retentate were continuously collected and analysed in order to measure the amount of antioxidant in each flow stream. Considering that antioxidant compounds may act *in vivo* through different mechanisms of action, no single method can fully evaluate the antioxidant capacity of food since levels of single antioxidant in food do not necessarily reflect their antioxidant activity. For this reason permeate and retentate juices were evaluated for their radical scavenging activity through DPPH and ABTS assay. Permeate samples showed a percentage of inhibition of ABTS· from 35.97 to 54.27 using pure products while retentate samples exhibited a percentage of inhibition 22.67 to 43.66 using pure products. In DPPH assay IC50 values v/v -ranging from 3.46.64 to 10.64 for permeate and from 7.64 to 8.70 for retentate, respectively.

S20P03

The effect of time and refrigeration on bioavailability of flavanones in healthy volunteers after fresh squeezed orange juice consumption

Tomas-Navarro M., Vallejo F., and Tomás-Barberán F.A.

Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC), Ciencia y Tecnología de los Alimentos, Spain.
mtomas@cebas.csic.es

The effect of both temperature and time is unclear yet on flavanones bioavailability. A comparison between ingestion of freshly squeezed orange 'Lane Late' juice and the same juice after a 24h refrigeration period (8 ºC) in the fridge was carried out. This study was developed in healthy volunteers (n=7) following the administration of 400 mL of freshly squeezed orange juice (FS) and 24 h urine collection. The same operation was done with the refrigerated one (FR). The determination of the total amount of flavanones present in the supernatant and pellet of both juices was measured by HPLC-MS. Significant differences were observed in flavanones precipitation (pellet) after 24 h refrigeration (FR) compared to the freshly squeezed one (FS). The total excretion of flavanone metabolites in all volunteers decreased between 2 and 5-fold after storage for 24 h at 8 ºC (FR). The results show that research on technological and biotechnological methods to preserve the bioavailability of the freshly squeezed juice are necessary in order to obtain processed juice with the highest bioavailability.

S20P04

The leaves of Mediterranean mandarin could be used as an important source of hesperidin, nobiletin and tangeretin.

Durand M.V.S.[1], Fanciullino A-L.[2], Jay-Allemand C.[3], Ollitrault P.[1], Froelicher Y.[1], and Bidel L.P.R.[4]

[1]UMR AGAP, CIRAD, F-20230 San Giuliano, France.; 2UR 1103 GEQA, INRA, F-20230 San Giuliano, France; [3]UMR DIADE, Université Montpellier II, F-34095 Montpellier, France.; and 4UMR DIADE, INRA, F-34060 Montpellier, France. durand@corse.inra.fr

Citrus are rich in flavonoids such as flavones and flavanones. Recent studies have shown that these phytonutrients play a beneficial role for human health. Citrus plants can contain high levels of hesperidin, a flavanone which is effective in the treatment of chronic venous insufficiency. Nobiletin and tangeretin are two polymethoxylated flavones. Nobiletin has been shown to increase hepatic insulin sensitivity and decrease atherosclerosis. Tangeretin may have antiproliferative and anticarcinogenic effects by blocking MAPKs activation and ROS generation. Studies on flavonoids from citrus are generally focussed on ripe fruits and on one group of metabolites. The aim of this study is to quantify accurately all groups of flavonoid from leaves of mandarin (*Citrus deliciosa*). Flavones and flavanones analysis was carried out by liquid chromatography and mass spectrometry (LC-MS) using a new generation column. Moreover, a specific gradient was designed to evaluate both flavanones and methoxylated flavones. This method was applied to leaves from mandarin tree. All leaves were fully-expanded, and were about one-year-old from the spring flush of the previous season. They were of similar height above the ground, had the same east orientation and experienced similar exposure to light. Comparison of metabolite levels revealed that mandarin leaves were rich in hesperidin, nobiletin and tangeretin. Interestingly, nobiletin and tangeretin also accumulated in high level.

S20P05

Comparative study of fruit flavonoids in ten citrus hybrids

Ran Y.[1], Zhao Q. Y.[2], Su X. S.[3], Chen W. J.[2], Jiang D.[2], and Jiao B. N.[1]

[1]Citrus Research Institute, Southwest University; College of Food Science, Southwest University, China; [2]Citrus Research Institute, Southwest University, China; and [3]School of Chemistry and Chemical Engineering, Southwest University, China. bljiao@tom.com

Citrus species are a rich source of flavonoids. Numerous epidemiological, clinical, and preclinical studies have shown that citrus flavonoids have an anti-inflammatory, anti-cancer, anti-atherosclerosis, antioxidant and anti-bacterial activities. Many relevant research reports about citrus flavonoids were focused on the determination of flavonoids in different orange and mandarin varieties, and a little about citrus hybrids. In this study, 10 citrus hybrids cultivated in Chongqing city, China, were analyzed for 8 flavanone glycosides (FGs) and 3 polymethoxylated flavones (PMFs) by the ultra performance liquid chromatography (UPLC). The results showed that the total flavonoids in juice are much lower than those in peel. Of all the samples, the total flavonoids in 'Kiyomi' tangor fruit was the highest, followed by 'Wanmi No.3' and 'Wanmi No.1'. The range of the total flavonoid concentration in all juice was from 0.13 to 0.42 mg/g, and from 4.8 to 20.0 mg/g in peel. The most abundant flavonoids in 10 citrus hybrids were hesperidin, narirutin, didymin and polymethoxylates flavones. The largest amount of narirutin was found in peel of 'Kiyomi' tangor, which was 3.87 mg/g; while the largest amount of hesperidin and didymin were found in peel of 'Wanmi No.3', with the concentration of 16.07 mg/g and 1.89 mg/g, respectively. Furthermore, neohesperidin was only found in 'Niushen' hybrid; the content in Niushen's peel was 4.88 mg/g. The content of total polymethoxylated flavones in 'Jucheng 1-1232' was the highest of the 10 analysed citrus hybrids, which was 0.94 mg/g.

S20P06

Over-consumption of satsuma mandarins is associated with "Shang huo" reaction: results from case-control studies

Ma Z.C.[1], and Deng X.X.[2]

[1]Huazhong Agricultural University (Hzau), College of Horticulture and Forestry Sciences, China; and [2]Key Laboratory of Horticultural Plant Biology of Ministry of Education, Huazhong Agricultural University, China. mzhaocheng@gmail.com

Satsuma mandarin accounted for a large proportion of citrus varieties. It is rich in carotenoids, limonoids and some other important functional components, potantially benefitial for human health. Over-consumption

of satsuma, however, (even some people just eat one) induces “Shang huo”. This reaction is an itching or burning sensation in the lips, mouth, ear canal, and/or pharynx. Sometimes other reactions can be triggered in the eyes, nose, and skin. “Shang huo” make people afraid of eating satsuma and have negative impact on the citrus planting efficiency. Litchi and longan fruit also produce this kind of effect. This study was focused on the analysis of the mechanism of “Shang huo” by a case-control study. 200 volunteers participated in the project. In the first night, from 7 pm to 9 pm, male volunteers ate more than 1.5 Kg satsuma at a time, and female volunteers ate 1 Kg. This consumption was 2-3 times above normal consumption. Individual reactions were carefully observed and recorded for continuous 3 days. Statistically significant differences were found.

S20P07

Dissipation curves of cypermethrin in whole fruit and mandarin pulp in Argentina

Kulczycki Waskowicz C.[1], Sosa A.[1], Beldoménico H.[2], García S.[2], and Repetti M.R.[2]

[1]National Institute of Agricultural Technology, Concordia Agricultural Experiment Station (INTA, EEA Concordia), Pesticides Residues Plant Protection, Argentina; and [2] School of Chemical Engineering, Santa Fe (UNL), Central Laboratory, Pesticide Division, Argentina. ckulczycki@correo.inta.gov.ar

Cypermethrin is a pyrethroid insecticide used for pest control in several crops. The objective was to determine a degradation curve of cypermethrin in whole fruit and mandarin pulp. The assay was performed in a plot of satsuma mandarin located at INTA’s Agricultural Experiment Station in Concordia during summer 2011. The treatment used 25% of cypermethrin at a dose of 60 cm3/hl commercial formulation. The treatment was performed with a high volume spray under Good Agricultural Practice. Volume applied was 2916 l/ha. Pesticide degradation in fruit was studied for a 61-day period since insecticide application and included periodic samplings. The residue determinations were performed by gas chromatography with ECD detector. The quantification limit was 6 µg/kg and the detection limit 3 µg/kg. Fruit diameter and internal quality was also determined: juice percentage and maturity rate (ºBrix, % titratable acidity). The first elimination phase was fast, from an initial deposit of 0.51 mg/kg to 0.15 mg/kg after 21 days. The second elimination phase was slower reaching at the end of the experiment levels of 0.06 mg/kg. No residues were detected in pulp. Fruit diameter remained constant throughout the assay; therefore, the dilution factor due to growth of plant tissue was not considered. In whole fruit, even though residue levels of cypermethrin were low, presence at the end of the assay showed great persistence.

S20P08

Dissipation and residue of forchlorfenuron in citrus fruits

Chen W. J.[1], Su X. S.[2], Zhao Q. Y.[1], and Jiao B. N.[1]

[1]Citrus Research Institute, Chinese Academy of Agricultural Sciences, China; and [2]Chemistry and Chemical Engineering, Southwest University, China. suxuesu@163.com

The dissipation and residue of forchlorfenuron in citrus fruits under field conditions were studied. The field trial was conducted in Guangzhou, Zhejiang and Hainan provinces, China. An analytical method using QuEChERS cleanup and ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) was successfully applied to analyze forchlorfenuron in citrus fruits. The recoveries of forchlorfenuron were found in the range of 82.8%-95.5% at three spike levels, with relative standard deviations of 2.1-%-8.0-%. The results showed that the degradation rate of forchlorfenuron in citrus fruits followed the first-order kinetics equation C=A·eBt.The half-live of forchlorfenuron were 15.8-23.0 days. The degradation rate of forchlorfenuron in citrus at 42 days (2010) and 52 days (2011) were 82.4-86.4% and 80.6-88.3%. The results of final residual trial revealed that when the citrus plants were sprayed at 1-2 times successively at the concentration of 20 mg ai/L and 30 mg ai/L, the residues in pulp of citrus fruits were lower than 0.003 mg/kg and most of the residues were concentrated in the peel. The results could give a reference for MRL setting of forchlorfenuron in citrus fruits in China.

S20P09

Dissipation and residue of 2,4-D in citrus fruits

Chen W. J.[1], Su X. S.[2], Zhao Q. Y.[1], and Jiao B. N.[1]

[1]Citrus Research Institute, Chinese Academy of Agricultural Sciences, China; and [2]School of Chemistry and Chemical Engineering, Southwest University, China. bljiao@tom.com

The dissipation and residue of 2,4-D in citrus fruits under field conditions were studied. The field trial was conducted in three provinces of China. An analytical method using QuEChERS cleanup and ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) was successfully applied to analyze 2,4-D in citrus fruits. The recoveries were in the range of 85.0-%-107.8-% at three spike levels with relative standard deviations of 1.0-%-4.6-%. The results showed that the dissipation kinetics of 2,4-D in citrus fruits did not follow with the first-degree reaction pattern. The degradation rate of 2,4-D in citrus at 65 days (2010) and 75 days (2011) were 42.7-77.3% and 31.2-59.1%. The terminal residues in pulp peel and whole fruits were all bellow the maximum residue limit (MRL) value set by European Union (EU) (1 mg/kg). Two supervised trials on the post-harvest use of 2,4-D on orange and 'Ponkan' mandarin were also conducted. The residues of 2,4-D at day 0 in orange samples treated with the 200 mg ai/L 2,4-D solution and in 'Ponkan' samples treated with the 250 mg ai/L 2,4-D solution were 0.122 mg/kg and 0.265 mg/kg, respectively, both bellow the MRL set by EU. The degradation rate of 2,4-D in orange and 'Ponkan' fruit after 96 days and 93 days were 55.6% and 76.5%. Hence it was safe for the use of 2,4-D and the results could give a reference for MRL setting of 2,4-D in citrus fruits in China.

S20P10

Effect of pesticide spray volumes and doses on the production of marketable lemons in Tucumán, Argentina

Salas López H.[1], Mansilla C.[2], Figueroa D.[1], Carrizo B.[1], Rojas A.[1], Campos A.[1], and Goane L.[1]

[1]Estación Experimental Agroindustrial Obispo Colombres (EEAOC), Fruticultura, Argentina; and [2]CITRUSVIL, Argentina. hsalas@eeaoc.org.ar

Current management of lemon trees includes different pesticide volumes and doses. Field trials were conducted in lemon orchards located in two climatically and ecologically different regions throughout three years to evaluate the impact of three spray volumes (10000, 5000 and 2000 L/ha) and three doses of pesticides (high, medium and low) on lemon fruit health. The highest pesticide dose consisted of 15 kg/ha of a commercial product of copper hydroxide (53.8%), 100 L/ha of mineral oil and 2 L/ha of a commercial product of abamectin (1.8%); medium and lowest doses had the same components, but different quantities: the medium 7.5 kg, 50 L and 1 L, respectively, and the lowest 3 kg, 20 L and 0.4 L per hectare respectively. Trials were conducted in a randomized complete block design with four replicates. Healthy fruit percentage was estimated at harvest from approximately 2.000 fruits per plot. Damaged fruits were separately analyzed to discriminate the main cause for rejection: wind, pests or diseases. Independently of doses, healthy fruit proportion was significantly higher in plots sprayed with high and medium pesticide volume. Such treatments were more efficient for controlling pests (mealybugs, mites and thrips) mainly, although they resulted in a higher proportion of fruit damaged by wind. The study demonstrated that a reduction of pesticide spray volume (to 5000 L/ha) and doses is possible without affecting fruit quality.

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POSTHARVEST AND JUICE PROCESSING TECHNOLOGY

S21O01

Biochemical and molecular mechanisms involved in mandarin flavor deterioration after harvest

Tietel Z., and Porat R.

ARO (The Volcani Center), Dept. of Postharvest Science, Israel. rporat@volcani.agri.gov.il

The sensory preference of mandarins declines during storage and transport mainly due to a decrease in perception of acidity and typical mandarin aroma and accumulation of off-flavors. Aroma profiling analysis of homogenized segments of Or and Mor mandarins conducted by gas chromatography mass spectrometry (GC-MS) revealed that the contents of some aroma volatiles, mainly aldehydes and terpenes, somewhat decreased during storage, whereas contents of other volatiles, especially the ethanol fermentation metabolites ethanol and acetaldehyde, and various ethyl esters which are fatty acid and amino acid catabolism products significantly increased during storage. These biochemical observations were further supported by complimentary genome-wide transcript profiling analysis studies conducted using the Affymetrix Citrus Genome Array, which revealed correlative increases in gene expression patterns related to ethanol fermentation metabolism and lipid and amino acid catabolism. Overall, we propose that simultaneous induction of ethanol fermentation metabolism and lipid and amino acid catabolism, most probably for energy production means, are involved in causing off-flavors in mandarin fruit after harvest. Furthermore, we propose that high levels of ethanol serve as substrates for subsequent downstream esterification reactions with acyl-CoA's derived from fatty acid and amino acid catabolism; a reaction catalyzed by alcohol acyl transferases (AAT's), which results in accumulation of various ethyl esters volatiles imparting undesired off-flavor odors.

S21O02

Traceability of citrus fruit using isotopic and chemical markers

Fabroni S., Amenta M., and Rapisarda P.

CRA Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee (CRA ACM), Italy. simona.fabroni@entecra.it

Sicilian citriculture is currently involved in a marked crisis mainly related to the lack of a commercial strategy along the whole chain of production. This crisis can be overcome focusing on typical citrus productions, which have been awarded PGI (Protected Geographical Indication) status by the European Commission. Thus it comes the need of a system of traceability to verify the authenticity of these productions. The aim of this research was to classify, by a chemical and chemometric approach, Sicilian typical citrus productions (Arancia Rossa di Sicilia and Limone di Siracusa) whose geographical origin was authenticated by samplings made in PGI or not-PGI areas. The joint use of chemical (quality parameters), spectral (NIR spectral patterns) and isotopic (13C/12C, 18O/16O, 2H/1H) markers has revealed that a representative database of each geographical area can be used for the development of a traceability system of such typical productions. Besides, a growth potential for the Sicilian citriculture is the conversion to organic production. Thus, the question of the authenticity of foods labeled as organic becomes an imperative requirement. Our recent researches, aimed at identifying new markers by monitoring the δ15N and other components derived from primary and/or secondary metabolism in citrus fruits from organic and conventional commercial farms and experimental fields, have shown that δ15N analysis may contribute to the differentiation between organic and conventional fruit. Moreover, in case of supply of organic fertilizers in conventional regime, a model of multivariate analysis, including δ15N and other quality parameters (TSS, TA, ascorbic acid, total polyphenols, ORAC units), can contribute to a reliable discrimination between organic and conventional fruit.

S21O03

Fluctuation of limonin and nomilin content in different tissues during fruit development of three sweet orange varieties

Wu Houjiu, Tan Anqun, Wang Hua, Ma Yaqing, Sun Zhigao, Huang Xuegen, and Guo Li

National Research Center for Citrus Engineering Technology Citrus Reseach Institute, Southwest University (NRCCET), China. wuhoujiu@126.com

In this study, limonin and nomilin content in different tissues of fruit at different developmental stages of three sweet orange varieties (*Citrus sinensis*), 'Xuegan' 'No 7 Zhongyu Jincheng' and 'Fengqi' navel were

determined by HPLC. Results indicated that the limonin level in the peel of the three sweet oranges at different developmental stages was lower than that of nomilin, but limonin levels in sacs were higher than that of nomilin. The nomilin content was three folds that of limonin in seeds of 'Xuegan'. The limonin content in the 'Xuegan' peel gradually decreased with the fruit development. The nomilin content dropped from 2.090 mg.g-1 in August to 0.326 mg.g-1 in December. Over the same period of time, the limonin content declined from 3.288 mg.g-1 to 0.894 mg.g-1. The trend of limonin and nomilin levels in peels of the other two orange varieties were the same as in 'Xuegan'. Following fruit development, limonin and nomilin contents in sacs of the three sweet oranges showed a downward trend, but the limonin content was much higher than the nomilin content. The contents of the two limonoids in 'No 7 Zhongyu Jincheng' were the lowest among all the varieties. The limonin content in sacs of 'Fengqi' navel was the highest, and remained 0.211 mg.g-1 in November when close to harvesting, which gave a more bitter taste 42 times than the threshold (0.005 mg.g-1). The results suggested that the limonin was transferred to the sacs during fruit development in 'Fengqi' navel fruit, but accumulated in the seeds in 'Xuegan'.

S21O04

Post-harvest management of citrus fruit in South Asian countries

Ladaniya M.

National Research Centre for Citrus (NRCC), ICAR, India. msladaniya@gmail.com

South Asian countries including: India, Pakistan, Bangladesh, Nepal, Bhutan and Sri Lanka produce about 11.10 million tons of citrus fruits, contributing to roughly 11.0% of world output. Citrus is among the top three fruits in the region. With a population of 1604 million, the per capita /annum citrus fruit availability is 6.90 kg. Mandarin and sweet orange are major citrus fruits constituting 84%, followed by acid lime, lemon and other citrus (16%). Postharvest management is mostly on conventional lines. In North-west India and Pakistan, 'Kinnow' is sorted, washed, waxed and packed in modern packing houses. Harvesting is done by clippers for 'Kinnow', by snapping for 'Nagpur' mandarin and by bamboo harvesters for 'Khasi' mandarin. Total losses from farm to retail level in the region are 25-35% and vary with season and commodity. Acid lime losses increase with onset of monsoon. Wooden boxes are used for mandarins. Oranges and acid limes are packed in jute bags. Bamboo container 'doko' is used in Nepal, Bhutan and NEH region of India for 'Khasi' mandarin. Transportation of loose fruit in trucks is common. Refrigerated storages and modern packinghouses are being established. Citrus fruits are being sold in supermarkets. Modified atmosphere packaging increased shelf life of mandarins up to 3 weeks. Evaporative cool chamber (1.5 -8 tons capacity and very low energy consumption) extended storage life of 'Nagpur' mandarin, 'Mosambi' orange, acid lime, 'Kinnow' and grapefruit up to 21, 40, 30, 60 and 77 days as against 10,14, 5, 14, and 27 days at ambient condition, respectively. Cellar stores are used in Nepal and Bhutan. Export of 'Kinnow' from Pakistan is increasing. India exported nearly 30000 tons of citrus fruits in 2008-09.This paper discusses value chain and post-harvest overview in south Asia including postharvest research, conventional and innovative handling, storage structures, modern infrastructure and future challenges and prospects.

S21O05

A technological platform for the valorization and recovery of outsize and defective PGI citrus fruits from South Italy.

Mazzuca S.[1], Spadafora A.[1], Serra I.A.[1], Bernardo L.[1], Balducchi R.[2], Di Sanzo G.[2], Maccioni O.[2], Verardi A.[3], and Calabro V.[3].

[1]University of Calabria (UNICAL), Department of Ecology, ITALY; [2]ENEA C.R. Trisaia (ENEA), Laboratorio Biotecnologie (UTTRI-BIOTEC), Italy; and [3]University of Calabria (UNICAL), Department of Engineering Modeling, Italy. vincenza.calabro@unical.it

Clementine represents a special kind of citrus fruit, mainly grown in the south of Italy and characterized by sweetness, flavour and agreeable taste. The Ionian area of Calabria (Italy), offers a perfect climate for lemon crops, like the PGI (Protected Geographical Indication) Lemon of Rocca Imperiale. Selected genotypes 'Femminello' have given well adapted ecotypes and their fruits have excellent fragrance, the finest essential oil and sweet juice. Marketing and commercialization of organic fruit present specific and restrictive rules, in relation to size, color and taste of the fruit. Usually more than 20% of the organic fruit produced cannot

be directly commercialized and represents a waste for producers, despite their high quality and richness in health benefits. A valid alternative is represented by the processing of outsize and defective fruits to produce juices as well as to extract high value active compounds from various parts of the fruits. In this paper the authors suggest a technological platform devoted to the recovery and valorization of PGI citrus fruits from the South of Italy in order to obtain high added value products that can be distributed and commercialized in the organic market and health industry. The technological platform could operate with the production of fresh and pasteurized citrus juice from less quality citrus fruits. The authors put their attention to the process and technology required to guarantee high quality, taste and stability of juices. They studied also the methodologies that could be used for the extraction of active compounds in mild conditions, as i.e. membrane processes or supercritical extraction, in order to recovery active substances (proteins, antioxidant, terpens) for food, pharmaceutical and chemical industries. The platform will also be used for the characterization of fruit anatomy, structure, texture and physiology features, in order to well predict the quality of fruit.

S21O06

Industrial orange juice (var. 'Salustiana') debittering: effects on sensory properties

Fernández-Vázquez R.[1], Stinco C.M.[1], Hernanz D.[2], Heredia F.J.[1], and Vicario I.M.[1]

[1]Food Colour & Quality Laboratory (FCQL), Department Nutrition & Food Science. Universidad de Sevilla, Spain; and [2], Department Analytical Chemistry. Universidad de Sevilla, Spain. rociofernandezv@us.es

Debittering is an industrial process that removes bitter taste compounds in juices, which are

mainly limonoids. This process can be done by physical adsorption on a resin, which can also remove other interesting compounds related to the sensory properties. This study was aimed to analyse this effect in orange juice (OJ) of 'Salustiana' (*Citrus sinensis*). Samples (n = 9) of the industrial squeezed OJ and corresponding OJ after the debittering process (DOJ) were taken in an orange juice factory. Acidity, pH and total soluble solids were measured according to AOAC methods. No significant difference between OJs and DOJs were found. Colour was evaluated by image analysis (DigiEye System). Hue and lightness were lower (more reddish and darker) after debittering ($p<0.05$). Volatile profile (limonene, α-pinene, ethyl butanoate, octanal, linalool, citral and terpineol) were analysed by gas chromatography. All the aroma compounds decreased ($p<0.05$) (from 15.94 % to 61.16 %) after debittering as well as the total phenolic compounds measured by Folin Ciocalteu ($p<0.05$). Sensory analyses were conducted by 12 untrained panelists to determine the influence of the debittering on the perceived colour, smell and taste by paired comparison tests. The panelists did not find significant differences ($p>0.05$) in the colour, however aroma and taste were significantly ($p<0.05$) different in OJs vs DOJs.

S21P01

Anthocyanins in citrus

Fabroni S., Amenta M., Rizza G., and Rapisarda P.

CRA-Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee (CRA ACM), Italy. paolo.rapisarda@entecra.it

Anthocyanins are water-soluble pigments belonging to the flavonoid family. Their color ranging from red to blue to purple shades according to vacuole pH, copigmentation with other polyphenols and complex with metal ions. They are synthesized by organisms of the plant kingdom and bacteria, and have been observed to occur in all tissues of higher plants, providing color in leaves, stems, roots, flowers, and fruits. Blood oranges (*Citrus sinensis*) contain anthocyanins almost exclusively in the flesh and only in some cases in the rind. As regards the distribution of anthocyanins in other citrus tissues, they are present in the young shoots and flowers of citron (*Citrus medica*) and some lemon varieties (*Citrus limon*). The same plant tissues of orange species don't accumulate anthocyanins. Therefore, the biosynthesis of anthocyanins in citrus is tissue-specific and dependent on genotype. This study aims at providing a scientific contribution to the knowledge of anthocyanins contained in fruit and other plant tissues of different citrus species. Anthocyanins of blood orange fruits ('Moro', 'Tarocco', and 'Sanguinelli') and some of their hybrids, as well as of young leaves and

flowers of different cultivars of citron and lemon were analyzed by HPLC/MS-MS. The dominant pigments in fruits were cyanidin-3-glucoside and cyanidin-3-(6" malonil-glucoside) while in young leaves and flowers a different pattern was observed. This is the first report of patterns of anthocyanins in the different organs of a large number of genotypes of citrus.

S21P02

Organic acids metabolism in postharvest citrus fruit

Cheng Y.J., Sun X.H., Zhu A.D., Xu J., and Deng X.X.

Huazhong Agricultural University (H), Key Laboratory of Horticultural Plant Biology, Ministry of Education, China.
yjcheng@mail.hzau.edu.cn

Organic acids in citrus fruits play critical roles in their edible quality and sensibility to storage environments. However, very limited information on postharvest acid metabolism in citrus fruit is available yet. In the present study, organic acids metabolism in the pulp of 3 main pummelo cultivars (*Citrus grandis*), widely planted in China, including 'Guanximiyou', 'Shatianyou', and 'Hirado Bontan', were systematically analyzed in 3 successive growing seasons. Capillary electrophoreses, high performance liquid chromatography and gas chromatography mass spectrometry were used for physiological and metabolic analyses. Moreover, an 8×15K mitochondrial microarray, composing of 1569 functional gene probes was designed. Based upon the acid metabolic profiles, RNA was extracted from four key stages for the microarray assaying. Meanwhile, the damage of mitochondrial membranes, as well as the contents of reactivate oxygen species was examined. Our results demonstrated that during citrus fruit postharvest storage phase: 1) The metabolic profiles of organic acid differed among cultivars; 2) Fluctuations of acid contents were inherent properties of in all sound citrus fruits; 3) The dynamic equilibrium of organic acid content was determined by the status of acid synthesis, degradation, transportation and storage, which were resulted from the synergistic actions of mitochondria, vacuoles and peroxisomes; 4) Except tricarboxylic acid cycle (TCA), glutamine and γ-aminobutyric acid (GABA) pathway, which linked the primary and secondary metabolisms, also played significant role in citrus fruit acid regulation.

S21P03

Ethylene degreening treatment under commercial conditions does not cause loss of nutritional and aroma quality of citrus fruit.

Sdiri S., Navarro P., and Salvador A.

Instituto Valenciano de Investigaciones Agrarias (I.V.I.A.), Centro de Tecnología Postcosecha, Spain. salvador_ale@gva.es

A significant volume of Spanish citrus fruit production is commonly submitted to degreening with ethylene exposure prior to be exported to overseas countries under cold quarantine conditions. Many studies have reported the effect of postharvest application of ethylene on internal quality fruit. Nevertheless, information about the effect of degreening and quarantine treatments on the nutritional and aroma volatile compositions in citrus fruit is scarce. This study aimed to evaluate the effect of degreening with and without ethylene exposure followed by cold quarantine treatment on vitamin C, hesperidin, narirutin, didymin, sinensetin, nobiletin, tangeretin content as well as antioxidant activity of 'Navelina' sweet orange and 'Clemenules' and 'Oronules' mandarins. The levels of the most important aroma active compound in citrus fruit (ethanol, acetaldehyde, ethyl butanoate, hexanal, α-pinene, myrcene, ethyl hexanoate, octanal, limonene, linalool, nonanal and decanal) were also determined. The response to degreening process and the posterior quarantine treatment depended on the variety. Although some changes were observed in individual flavonoids compounds of 'Clemenules' mandarins and 'Navelina' oranges, the degreening treatments followed by cold quarantine storage did not induce dramatic changes in nutritional and aroma compounds. Nevertheless, 'Oronules' mandarins exhibited the highest response to ethylene exposure and quarantine treatment with an increase in the level of flavonoids components as well as the level of some volatile compounds.

S21P04

Change in nonvolatile flavours of blood and common orange fruits during cold storage

Fabroni S.[1], Amenta M.[1], Todaro A.[2], and Rapisarda P.[1]

[1]CRA-Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee (CRA ACM), Italy; and [2]Università degli studi di Palermo (UNIPA), Dipartimento dei Sistemi Agro-Ambientali, Italy. paolo.rapisarda@entecra.it

Prolonged cold storage may cause relevant changes in volatile and non-volatile components of orange fruit [*Citrus sinensis*], probably associated with the decrement of characteristic fruit flavour and sensory acceptability. The aim of this work was to evaluate the changes of nonvolatile components of two blood varieties ('Tarocco' and 'Moro') and a common variety ('Washington navel') of orange fruits stored at 6 ± 1 °C and 90-95 % RH for 60 days. Taste components such as total and individual sugars (TSS, glucose, fructose and sucrose) and acids (titratable acidity, ascorbic acid) were determined during fruit storage. In addition, other compounds influencing the sensory characteristics of the fruit such as total anthocyanins, putrescine and limonin were evaluated. Sensory assessments were performed by a trained sensory panel at 15 days intervals. TSS and TA did not show significant variations during storage in all varieties. Such a trend was confirmed by evaluating glucose, fructose and sucrose. Total anthocyanins significantly increased during storage while ascorbic acid content remained almost unchanged in all varieties. Limonin significantly decreased in all varieties, reaching the lowest values at the end of cold storage. A consistent and significant raise of putrescine occurred during storage in the fruit of the blood varieties, while in the common orange variety no significant difference was observed. These results are in accordance to those obtained by the sensory evaluation that has shown an increase of the descriptor "off-flavour" in the last period of storage only for blood orange fruits.

S21P05

Antioxidant capacity and total phenolic contents of bergamot (*Citrus bergamia*)

Yilmaz K.U.[1], Uzun A.[1], Ercisli S.[2], Sengul M.[2], Yildiz H.[2], and Uysal O.[3]

[1]Horticulture Department of Agriculture Faculty of Erciyes University, Kayseri, Horticulture Department, Turkey; [2]Horticulture Department of Agriculture Faculty of Ataturk University, Erzurum, Turkey; and [3]Alata Horticultural Research Station Erdemli- Mersin, Genetic and breeding, Turkey. onuruysal33@gmail.com

Bergamot grown in East Mediterranean Region of Turkey is cultivated mostly for its essential oil produced from its peel. Except the oil used in fragrant tea (earl grey) making and pharmaceutical industry, bergamot peel is also used in jam making. Usually only calcium citrate and citric acid are obtained from bergamot juice. With this study, some of the biochemical contents of bergamot juice were determined and its usage ways (patterns) were discussed. Two methods, namely β-carotene bleaching and DPPH (α,α-diphenyl-β-pycrylhydrazyl) assay were used to determine total antioxidant capacity, while Folin–Ciocalteu reagent was used to determine total phenols. Bergamot had high total phenolic (80.06 µg GAE/mg DW) and also high total anthocyanin content with β-carotene bleaching (60.12%) and with DPPH (180.07 mg/100ml). The study demonstrates the potential of bergamot juice for nutritional value through other fruit juices.

S21P06

Simulation of cold treatment during a cargo shipment of citrus fruits

Tauriello A.[1], Di Renzo G.C.[1], Altieri G.[1], Strano M.C.[2], Genovese F.[1], and Calandra M.[2]

[1]Università degli Studi della Basilicata (UNIBAS), Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali, Italy; and [2]Centro di ricerca per l'agrumicoltura e le colture mediterranee (CRA ACIREALE), Italy. antonella.tauriello@unibas.it

Commercial agreements for citrus shipment outside EU countries (Canada and USA) require both the respect of the fruit quality and the restriction of parasites spread (Medfly larvas). Both of these requirements are satisfied with the correct use of cold treatment during the shipment. This paper reports the results of a simulated refrigerated transport of citrus fruits in a 40ft container. Fruit temperature distribution in a refrigerated container (MSC 40ft) has been monitored in order to verify the temperature distribution in different positions of the load. A refrigerated container placed in the Oranfrizer s.r.l (Scordia-CT Italy) packing house, was loaded in 2 hours, starting with pallets placed in the end side of the reefer. For temperature measurements (in the air, in the

pallet and in the fruits) 40 digital probes were used. In order to optimize air circulation across fruits boxes, an innovative package (with a new designed vented surface) was compared to the standard boxes. Results show that the mean temperature difference between the probes placed in the head space was less than 0,5°C. Graphic peaks showed the on/off cycle of the automatic defrosting system. The probes placed on the container floor demonstrated a good result in terms of temperature homogeneity, but the temperature reached a minimum level of about -0,8°C which could be considered too low for citrus fruits storage. The innovative vented boxes allowed a more rapid cooling and a more accurate temperature control.

S21P07

Application of 3,5,6-TPA under commercial conditions to control calyx senescence associated to degreening treatment

Tormo D.[1], Conesa E.[1], Sdiri S.[2], Navarro P.[2], and Salvador A.[2]

[1]Fomesa Fruitech (FOMESA), Technical Department, Spain; and [2]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Tecnología Postcosecha, Spain. econesa.fruitech@fomesa.net

Postharvest degreening treatment with exogenous ethylene application is widely used in Mediterranean growth area to improve the external coloration of the early-season citrus varieties in which internal maturity is achieved when the peel is still green. Nevertheless this treatment can induce physiological disorders overall related to calyx senescence. In previous studies has been reported that the application of 3,5,6-trichloro-2-pyridyloxyacetic acid (TPA) reduces calyx senescence associated to ethylene exposure. However it is important to know the effect of this auxin under commercial industrial conditions.

The purpose of this study was to evaluate the effect of postharvest application of TPA on reduction disorders associated to degreening in clementines ('Clemenpons' and 'Clemenules') in different commercial postharvest applications (in drencher and on-line). The application of TPA in drencher very significantly reduces the disorders of calyx senescence associated to degreening treatment in clementines, recommending 40 ppm of TPA as the most effective dose. The application of TPA 'on-line' treatments were effective on reduction of calyx disorders, obtaining the best results with 100 ppm of TPA; nevertheless in the trials carried out clementines exhibited low susceptibility to calyx disorders after degreening process. The results suggest that this auxin could be a good option to control disorders of citrus fruit during degreening treatment.

S21P08

Postharvest behavior of new mandarins cultivars obtained at the IVIA

Sdiri S.[1], Navarro P.[1], Cuenca J.[2], Pardo J.[3], and Salvador A.[1]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Tecnología Postcosecha, Spain; [2]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain; [3]Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Citricultura y Producción Vegetal, Spain. salvador_ale@gva.es

A triploid breeding program has been carried out in Plant Protection and Biotechnology Center in the Instituto Valenciano de Investigaciones Agrarias (IVIA) based on sexual hybridization, embryo rescue and ploidy analysis by flow cytometry in order to obtain new mandarin varieties with late harvest. 'Safor' and 'Garbí' mandarins are new triploid hybrids recently released characterized by their high quality and seedless fruits. In the Citrus Center of Citriculture and Vegetable Production (IVIA) other breeding program based on budwood irradiation is being conducted in search of new seedless cultivars. At this moment two clones obtained from irradiated budwood of the seedy 'Moncada' mandarin ('Moncalina-6S17' and 'Manrad-2L09') are in process of being registered and exhibit excellent fruit quality. This study aimed to test the postharvest behavior of these four new cultivars. After harvest the fruit were waxed and stored up to 30 days at 1ºC, 5ºC, or 9ºC in order to evaluate the sensibility to chilling injury and the changes in quality parameters that occur during the cold storage. 'Safor' mandarins did not show chilling injury symptoms during 30 days at any temperature studied. In contrast, 'Garbí' mandarins exhibited susceptibility to chilling injury after 15 days of storage at 1ºC and 5ºC; during this period this cultivar showed disorders related with peel dehydration that were aggravated when the fruit were late harvested. 'Moncalina' and 'Manrad' did not show relevant symptoms related to cold-induced damage or dehydration at 1ºC, 5ºC or 9ºC for 30 days of storage. For all mandarin cultivars assayed, the storage at low temperature was not a limitation for the changes observed in fruit quality parameters.

S21P09

Characterization of fruits of four different lemon cultivars, collected in the Northern coast of Sicily

Cupane M.[1], Guarrasi V.[2], Palazzolo E.[3], San Biagio P. L.[2], and Germanà M. A.[1]

[1]Dip. DEMETRA, Università degli Studi di Palermo, Facoltà di Agraria, Italy; [2]Istituto di BioFisica, Consiglio Nazionale delle Ricerche, Italy; and [3]Dip. Sistemi Agro–Ambientali, Università degli Studi di Palermo, Facoltà di Agraria, Italy. valeria.guarrasi@pa.ibf.cnr.it

The present work investigates the properties of the fruits of four main lemon (*Citrus limon*) varieties cultivated in Sicily: 'Monachello', 'Zagara Bianca', 'Femminello Santa Teresa', and 'Femminello Comune'. Physical and chemical properties as well as aroma compounds were analyzed as discriminate factors. Also the effect of the storage conditions was verified. Standard experimental techniques were used to determine: weight, longitudinal and transverse diameter, pH, titratable acidity, total soluble solids, juice percentage, total polyphenols and color index. The volatile components were analyzed by i) gas chromatography with a mass spectrometer detector and ii) an electronic olfactory system ("e-nose") equipped with an array of six MOS sensors. Moreover, the composition of peel essential oils was examined. The results suggest that most of the analyzed factors allow a good discrimination among cultivars particularly regarding total polyphenols content. Also, all the cultivars, especially the 'Femminello Comune', maintain the fruit quality, even when stored at room temperature, a finding of potential industrial relevance. The present work shows the feasibility of a storage protocol with minimal impact allowing energy saving without compromising the physical or chemical properties and the aroma characteristics.

S21P10

Postharvest green mold control by hot water dips in kumquats

Vázquez D., Cocco M., Bello F., Panozzo M., and Meier G.

Instituto Nacional de Tecnología Agropecuaria (INTA), Argentina. fbello@correo.inta.gov.ar

Kumquats are eaten as a fresh fruit, pulp and peel together. Therefore, non-synthetic products for decay control should be applied. This study was conducted to evaluate postharvest green mold (*Penicillium digitatum*) control by alternative methods other than chemicals like application of hot water treatments with sodium bicarbonate. Fruit were harvested in Entre Ríos, Argentina, in August 2011. Kumquats were wound-inoculated (0.8 mm long, 0.11 mm in diameter) with a conidia suspension (106 spores/ml) and dipped in hot water (45ºC, 50ºC, 53°C) or water at room temperature (20°C) for 2 min, with or without the addition of 2% sodium bicarbonate and stored at 20°C, 85-90% RH. The counting of rotted fruit was performed after 5 and 12 days. Non-inoculated fruit were waxed after water treatments and stored at 5°C, 34 days (transport simulation) and then at 20°C, 4 days (marketing conditions). Weight loss, firmness and physiological disorders were evaluated. In hot water dips at 50 –53ºC, 85% green mold control was observed. The addition of 2% sodium bicarbonate to the water showed no significant effect in controlling green mold. Weight loss increased with the addition of bicarbonate to the bath and water temperature. However, no differences were detected in firmness after the trial. No damage in fruit was observed by hot water dips. The use of heat treatments such as hot water dips enables control of green mold in kumquats without altering the quality of the fruit.

S21P11

Fate of foodborne pathogens in minimal processed orange and reduction of their growth using UV-C illumination

Lourenço A.[1], Salazar M.[2], Graça A.[2], Quintas C.[3], and Nunes C.[2]

[1]Universidade do Algarve. (UAlg), FCT, Portugal; [2]Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM), Universidade do Algarve. FCT, Portugal; and [3]Universidade do Algarve (UAlg), CIQA.IST, Portugal. miguel.salazar@agro-on.pt

The consumption of minimally processed products increased in recent years and with it the concern of the occurrence of outbreaks due to foodborne pathogens. Chlorine is the most commonly used disinfectant, however there are concerns about the formation of carcinogenic chlorinated compounds and the limited

effectiveness or completely ineffectiveness of chlorine solutions. The aim of this work was to study the survival and growth of *Escherichia coli*-O157: H7, *Salmonella* spp. and *Listeria innocua* and to evaluate the effects of two doses of UV-C illumination on reducing their populations in minimally processed 'Valencia late' oranges.

The results indicate that minimally processed oranges were a good substrate for the growth of foodborne pathogens. The three pathogens were able to grow in oranges when stored at temperatures of 10 ºC or higher. When stored at 5 °C the population remained constant over time, indicating the importance of maintaining a cold chain and avoiding contamination. Concerning the use of UV-C as an eco-innovative disinfection system, the results showed that the efficacy of UV-C at 3.0 and 7.5 $kJ.m^{-2}$ and sodium hypochlorite at 100 ppm is low for this type of product. The most effective treatment, 30 minutes after application, was (in general) sodium hypochlorite and after 6 days the best result was obtained with UV-C at 7.5 $kJ.m^{-2}$, indicating the importance of good manufacturing practices in processing.

S21P12

Evaluation of the effect of ultrasonic variables at locally ultrasonic field on extraction of hesperidin from 'Penggan' mandarin (*Citrus reticulata*) peels

Ma Y.Q[1], Wu H.J[1], Ye X.Q[2], Wang H[1], and Sun Zh.G.[1]

[1]Southwest University, Chinese Academy of Agricultural Sciences (CRI), Citrus Research Institute, China; and [2]Zhejiang University (ZJU), Department of Food Science and Nutrition, China. myaya211@163.com

Ultrasonic power is an important parameter among ultrasonic variables. However, our previous study showed that ultrasonic power has a little effect on the yields of hesperidin from 'Penggan' peels. Moreover, it has been reported that the maximum ultrasonic power depended on the distance of ultrasonic irradiation surface. Therefore, to confirm this point, an experiment in ultrasound-assisted extraction (UAE) of hesperidin from 'Penggan' peels at locally ultrasonic field was performed by response surface methodology (RSM). A three-level three-factor Box–Behnken design was applied to evaluate the effects of three independent variables (ultrasonic power, time and temperature) on the yields of hesperidin at high and low ultrasonic irradiation surface. The results showed that the coefficients of two mathematical-regression models obtained at high and low ultrasonic irradiation surface was 0.9742 and 0.9745, respectively, indicating that quadratic polynomial model could be used to estimate the ultrasound-assisted extraction of hesperidin. From response surface plots, increases of the yields of hesperidin could be significantly dependent on increase of ultrasonic power at near ultrasonic irradiation surface. Such properties of ultrasonic power will require further exploration and study of highly efficient effects, which can be important for industrial use.

S21P13

Industrial orange (var. 'Salustiana') juice debittering: effects on the color and bioactive compounds

Stinco C.M.[1], Fernández-Vázquez R.[1], Hernanz D.[2], Heredia F.J.[1], Meléndez-Martínez A.J.[1], and Vicario I.M.[1]

[1]Food Colour & Quality Laboratory (FCQL), Department Nutrition & Food Science. Universidad de Sevilla, Spain; and [2], Department Analytical Chemistry. Universidad de Sevilla., Spain. rociofernandezv@us.es

Debittering is a usual practice in the citrus industry worldwide. This process is aimed to remove the bitter taste compounds in the juices that are mainly the "limonoids" (limonin, and nomilin). However, the resin used for this purpose can also adsorb other interesting compounds from the nutritional and sensory point of view. This study was aimed at assessing the influence of industrial debittering process on the colour and bioactive compounds of orange juice (*Citrus sinensis*, var. 'Salustiana'). Samples (n = 9) of the industrial squeezed orange juice (FOJ) and corresponding orange juice after the debittering process (DOJ) were taken in an orange juice factory. Carotenoids and vitamin C were analyzed by HPLC and total phenols by the Folin-Cicalteu method. Antioxidant activity was determined by TEAC method (Trolox Equivalent Antioxidant Capacity Assay for lipophilic and hydrophilic fractions). Results showed that the debittering process had a significant effect ($p<0.001$) on the total phenolic content and vitamin C. In accordance, the antioxidant activity in the hydrophilic fraction was significantly lower in DOJ ($p<0.0001$). On the contrary, this industrial process did not

have any effect on the content of pro-vitamin A carotenoids, nor on the total carotenoid content. Accordingly, the total color difference (ΔE*ab) between FOJ and DOJ was 3.28 ± 1.64 CIELAB units, thus slightly appreciable (visual discrimination threshold ΔE*ab >3).

S21P14

The effect of fermentation on antioxidant content (ascorbic acid, carotenoids and flavonoids) and antioxidant activity (abts and dpph values) of orange juice

Escudero B.[1], Cerrillo I.[1], Hornero-Méndez D.[2], Herrero-Martín G.[1], Berná G.[1], Martín F.[1], and Fernández-Pachón M.S.[1]

[1]Universidad Pablo de Olavide (UPO), Biología Molecular e Ingeniería Bioquímica. Área de Nutrición y Bromatología, Spain; and [2]Instituto de la Grasa (CSIC), Biotecnología de Alimentos, Spain. icergar@upo.es

Numerous epidemiological and intervention studies have shown an inverse association between consumption of fruits and vegetables and mortality from cardiovascular diseases and Cancer. Antioxidant compounds in these foods are responsible for these beneficial effects on health. Orange juice is known to be rich source vitamin C, carotenoids and flavonoids. On the other hand, numerous studies have shown the direct relationship between moderate alcohol consumption and a decrease in the risk of coronary heart diseases. Both effects could join in a novel drink created by alcoholic fermentation of orange juice. The objective of the present study is to analyze the effect of fermentation process on antioxidant content of orange juice. The new orange drink is produced from commercial orange juice by a controlled alcoholic fermentation process (20 ºC–15 days). Collection of samples was made daily during the fermentation. Samples were processed to determine total flavonoids content, ascorbic acid content, total carotenoids content and antioxidant activity using ABTS and DPPH assays. The results show that total flavonoids content significantly increase with the fermentation process (day 0 versus day 15). Ascorbic acid and total carotenoids content did not undergo significant variations throughout this process. ABTS and DPPH values on day 15 of fermentation are similar to those of the orange juice (day 0). The alcoholic fermentation of the orange juice produces a drink with similar ascorbic acid, total carotenoids content and antioxidant activity to the substrate. However, a significant increase in total flavonoids content was observed. They are necessary intervention studies to evaluate the potential healthy effect of the new orange drink.

S21P15

Study on microwave sterilization characteristics of NFC orange juice

Wang H., Ma Y.Q., and Wu H.J·

Citrus Research Institute, SouthWest University, Chinese Academy of Agricultural Sciences (CRI), Citrus Research Institute, China. wanghua40@126.com;myaya211@163.com

The effects of microwave sterilization on total number of colonies, yeast, mold and *Escherichia coli* in orange juice were investigated. The optimum conditions of microwave sterilization by the orthogonal test were determined as: sterilization time of 50 s, microwave power of 800W and orange juice volume of 50 mL. The results of the analysis of variance showed that the effects of sterilization time, microwave power and orange juice volume on sterilization rate are significant ($p<0.01$). Furthermore, the time required for microwave sterilization was shorter as compared to heat sterilization, which indicated that microwave sterilization could be beneficial for keeping the nutritional quality of orange juice.

S21P16

Equipments automation in citrus canned processing line

Zhang J., and Lu S.

Zhejiang Academy of Agricultural Sciences (ZAAS), Institute of Food Sciences, China. lushengmin@hotmail.com

China is the largest citrus canned producer, whose exports account for more than 70% of world trade. However, citrus canned processing industry in China is still a labor intensive one, e.g. there are more than one thousand workers needed in a ten thousand ton class factory. Short process season, high labor intensity, low skill and long work time in the industry have posed a big problem to both workers enrollment and administration in factories. Thus, automated production lines and equipments are urgently needed. Funded by China Ministry

of Agriculture for agricultural technology research system, we are carrying out research and development of automated, labor-saving and comprehensive utilization machinery to solve problem in Chinese citrus canned industry. The machinery includes automated peeling, segment defect detector, segment filling machine, cyst lossless pump systems, peel layer separator and low bubble filling equipment for citrus thick drinks.

S21P17

Extension of the project for obtaining bioethanol from citrus waste

Conesa C., Fito P., and Fito P.J.

Instituto Universitario de Ingeniería de Alimentos para el Desarrollo (IuIAD), Universidad Politécnica de Valencia (UPV), Spain. clcodo@etsia.upv.es

The production of citrus fruit in Spain generates more than 0.5 million tons of waste. This is mainly due to rejects when packing fresh fruit, withdrawing fruit under agricultural policies and the waste generated by processing industries. That is the reason why an important challenge for the citrus sector is the use of this waste to unlock its economic value and eliminate the environmental problems that it may generate. The Instituto de Ingeniería de Alimentos para el Desarrollo (IIAD) at the Universidad Politécnica de Valencia (UPV) has developed a system for reusing waste from citrus production and processing to make bioethanol and high value-added niche products such as essential oils. The purpose of this study was to find the best sites for obtaining bioethanol from citrus waste. This meant beginning with the world's largest citrus fruit producers and then calculating the waste produced by rejects, withdrawals and processing industry waste. The fermentable sugar content of the waste was determined and the countries where it would be feasible to introduce technology similar to that developed by the IIAD in Spain were identified. The candidate countries were Brazil, the USA, China, Mexico and Italy.

S21P18

Sensory analysis as an instrument for colour measurement in satsuma and 'Nova' mandarin fruits

Bello F., Vázquez D., Almirón N., Meier G., and Cocco M.

Instituto Nacional de Tecnología Agropecuaria (I.N.T.A.), Argentina. fbello@correo.inta.gov.ar

Colour index (CI) of early maturing citrus fruits is a parameter for correct fruit degreenig process. For this reason the objective of this study was to develop a colour card for satsuma and 'Nova' mandarins fruits and analyze the relationship of sensory panel assesses with the measuring instrument. Fruits of both varieties were classified and selected to take pictures with different colour indexes using the range of -24 to 7 for satsuma and -21 to 10 for 'Nova'. Charts were developed with 25 cm^2 and 100 cm^2 size photos for satsuma and 'Nova' respectively. The IC was determined as the average of 4 measurements in the middle of 25 fruits per variety with Minolta CR 300. Mandarins were then presented to 12 judges to indicate the IC using the charts. Correlation analysis was used for the relationship between values indicated by the judges and the colorimeter measurements. Satsuma card was r = 0.982 and r = 0.978 for 'Nova'. The high correlation between colour index values measured by instrumental and sensory analysis indicates that these cards are useful and accurate for measuring colour in satsuma and 'Nova' mandarin fruits.

S21P19

Discrimination of three *Citrus paradisi* cultivars by electronic nose.

Guarrasi V.[1], Bernik D.[2], Bulone D.[3], San Biagio P. L.[3], Germanà M. A.[1], and Negri R. M.[2]

[1]Dip. DEMETRA, Università degli Studi di Palermo, Facoltà di Agraria, Viale delle Scienze 11, 90128, Palermo, Italy; [2]Departamento de Química Inorgánica, Analítica y Química Física, Facultad de Ciencias Exactas y Naturales, Instituto de Química Física de Materiales, Ambiente y Energía, Universidad de Buenos Aires, Ciudad Universitaria (INQUIMAE), Buenos Aires, Argentina; and [3]Istituto di BioFisica, Consiglio Nazionale delle Ricerche, Via La Malfa 153, 90146, Palermo, Italy. valeria.guarrasi@pa.ibf.cnr.it

In this work the aromatic pattern of grapefruit (*Citrus paradisi*) juices has been evaluated by an electronic nose (e-nose) device, developed at the University of Buenos Aires. The core of this device is an array of 11 nonspecific metal oxide semiconductor (MOS) sensors, capable of changing their electrical conductivity

when exposed to the aroma. A non-supervised multivariate data analysis, the principal component analysis (PCA), was applied to data derived from sensor's responses. The fresh hand-squeezed juices of three different grapefruit cultivars grown in Argentina have been analyzed (two white pulp grapefruit cultivars: 'Marsh Seedless' and 'Thompson', and a pigmented pulp cultivar: 'Star Ruby'). The effect of the storage time on the aroma has been also taken into account by storing the fruits at room temperature for 10 days. The results show the capability of the e-nose to distinguish the aroma of juices of different grapefruit cultivars and, also, to detect the differences of juice aroma extracted from fruits stored for many days. The characteristics of grapefruit juice as flavor and smell are extremely important in conferring value to the product. It is possible to conclude that the e-nose can be successfully used both in the storage industries to monitor the fruits shelf-life and in the processing industries to control the product quality.

S21P20

Effect of several clay content in chitosan-clay nanocomposite coating on chemical and mechanical properties of orange fruit comparison with conventional coating during storage

Torabi A.R.[1], Hashemi S.J[1], and Hashemi Z.S.[2]

[1]Sari Agricultural Sciences & Natural Resources University, Biosystem and Agricultural Machinery Eng, Iran; and [2]Khouzestan Agriculture & Natural Resources University, Agricultural Engineering Faculty, Iran. fatemeh_hashemi48@yahoo.com

Water loss and respiration of citrus fruits can be reduced using Nano-composite coatings in combination with nano-clay and natural biopolymer during storage. To determine the effect of these coatings on the quality of orange fruits during storage, a study was conducted in the Ramsar county of Iran in 2012 using a factorial complete randomized design with 8 and 4 replications for chemical and mechanical properties, respectively. Coating conditions were: without coating, conventional coating and nanocomposit coating with 1, 2 or 3 percent of clay content. Storage periods were: first day, 40, 80 and 120 days. Results showed that differences of the weight loss, peel moisture, TA, TSS, TSS/TA and pH of fruits were in the range of 3.98% 12.02%, 70.43-77.66, 1.01-1.51, 9.54-11.54, 6.35-11.13, 3.06-3.42 and 2-2.34 per treatment, respectively The mechanical properties, namely bioyield point, firmness, cutting force and fruits punch were in the range of 153.75-240.56, 47.55-52.73, 21.25-69.44 and 13.46-20.23, respectively. Values were found to be significant for different coatings and storage days. These findings suggest that the chitosan-clay nanocomposite coatings displays a good potential for use in citrus coating intended for preserving the natural qualities of fresh and maintaining the marketability of the fruits during storage.

S21P21

Reducing decay and physiological disorders in oranges stored at suboptimal temperature by combined treatments

Artes Calero F., Velázquez P., and Artés Hernández F.

Universidad Politécnica de Cartagena (UPCT), Postharvest and Refrigeration Group, Spain. fr.artes@upct.es

The consumer's concern for chemical residues is raising the option of physical methods to control decay and physiological disorders in citrus. Middle-late season 'Navelate' oranges were stored for 7 weeks at 2ºC and 90-95 % RH plus 1 week at 20 ºC as shelf-life. Before storage fruit were subjected to several treatments, combined or not with 1 g/L of thiabendazole (TBZ): conditioning in air for 3 days at 36ºC, dipping for 2 min in water at 53ºC (HW) or individually wrapping in polyvinyl chloride. Also an intermittent fruit warming for 1 day at 20 ºC after every week at 2ºC (IW) was applied. No detrimental effect on quality attributes was detected. After of cold storage slight weight losses were reached, whereas neither decay nor cracking occurred. All treatments were very effective in reducing chilling injuries (CI) during storage (particularly HW TBZ wrapping, and IW). After shelf-life, weight losses and CI increased while decay and cracking developed. In control 27.62% decayed and 25.1% suffered CI (pitting and rind scald). Curing in air was the only treatment that increased cracking (5.5%) and decay (5.5%). Despite the low dose, TBZ prevented decay (1.36%) and reduced CI (12.53%). HW alone strongly reduced CI (5.5%) and when combined with TBZ or wrapping a synergistic effect was found. TBZ IW avoided decay and CI. After shelf-life no differences in total losses (between 3.94 and 5.39%) among TBZ wrapping, HW TBZ, HW TBZ wrapping, and IW TBZ were found.

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S22O01

The development of citrus industry in China during the last decade

Deng X.X.

Key Laboratory of Horticultural Plant Biology of Ministry of Education, Huazhong Agricultural University, China.
xxdeng@mail.hzau.edu.cn

Citrus industry developed very quickly during the last decade in China. The total production increased from 8.78 MMT (million metric tons) in 2000 to 26.45 MMT in 2010; during the same period, the import and export volume reached 105.2 and 931.6 thousand tons respectively. The distribution and the variety structure also changed. The scattering citrus production over the southern provinces has been concentrated to the advantageous areas and formed 4 dominant belts. More sweet oranges are grown and their proportion increased quickly even though mandarin still accounts for two thirds of total citrus acreage. Among the new plantation of sweet oranges, navel oranges are the main types for the newly developed orchards. It is remarkable that the exploring of late harvest mandarin of local varieties including 'Shatangju', 'Nianju', etc., has extended the fresh market supply. The promotion of late varieties, coupling with the extension of late harvest technology in the areas without frost in the winter, has efficiently improved the fresh market. More packinghouse and packing lines have been invested, especially in the navel orange producing areas. Unfortunately, the orange juice production in China is still a short leg. Huanglongbing, an old disease in south part of China, has been a problem for Chinese citrus industry. However, there are also areas with good control of this disease since the integrating protocol has been adopted efficiently. Chinese citrus industry is encountering the challenge of quickly increasing labor cost and the lack of young workforce. A national system for citrus under the CARS (China Agricultural Research System) project sponsored by the central government was set up in 2008. A total of 24 scientists have been stably financial-sported and 26 stations in the main producing areas have been set up by the CARS, aiming at germplasm exploring, new variety development, cultural practices, post-harvest and processing, mechanization and economy of citrus.

S22O02

Risk assessment of citrus yield based on yield minus deviate index and risk probability

Huang S.

Citrus Research Institute of Chinese Academy of Agricultural Science and School of Economics & Management of Southwest University; (CRIC), China. huangsen01@sina.com

Using the citrus yield minus deviate index and citrus risk probability, the citrus yield risk of the whole nation of China, Zhejiang province, Jiangxi province, Hunan province and Chongqing municipality were evaluated. The citrus yield minus deviate index showed the deviate trend on citrus yield, caused by harmful environment conditions, such as bad climate and diseases, etc. The citrus risk probabilities represent the frequency of the citrus yield minus deviate trend. The results demonstrated that the order of citrus yield minus deviate index, from high to low were Zhejiang, Hunan, Jiangxi, Chongqing and the whole nation of China. The study also indicated that citrus risk probability of Hunan was the highest and Chongqing was the lowest.

S22O03

Recent trends of the citrus industry in Chile

Ortúzar J.E., and Valenzuela M.

Chilean Citrus Committee, Chile. jortuzar@agricom.cl

The Chilean Citrus industry has undergone a major transformation over the last 15 years. A shift from a domestic to an export market oriented industry encouraged a wide renovation of plantings, a significant change of production and postharvest practices and a steady improvement of fruit quality. Total planted area, currently estimated at 19,000 ha, has not increased significantly. Orange is the main species with 8,000 ha, followed by lemon with 7,090 ha and easy peelers with nearly 4,000 ha. Navel oranges and easy peelers exports grow steadily as new plantings come into bearing, while lemon plantings and exports have

slowed down. USA is currently the main market, followed by Japan. Citrus exports in 2011 to all markets reached 155,000 T, valued at US$ 138 million FOB. Main varieties of orange are 'Lane Late', 'Fukumoto' and 'Washington' Navel budded on 'Swingle', 'C-35', 'Carrizo' and 'Rubidoux' trifoliate rootstocks. Main mandarin varieties are 'Clemenules', 'Orogrande' and 'W. Murcott', mainly budded on 'Carrizo', 'C-35' and 'Swingle'. Main lemons varieties are 'Eureka', 'Fino 49' and 'Messina', mostly budded on *Citrus macrophylla*. The export industry recently formed the Chilean Citrus Committee to address more decidedly market access, promotion and research efforts. Nonetheless the positive achievements, the industry faces several challenges due to increasing production costs caused by the strengthening of the Chilean peso, higher energy cost and labor shortage and by the impact of freezes and drought in some areas.

S22O04

A critical economic analysis of advanced citrus production systems (ACPS)

Roka F.M.

University of Florida (UF), Southwest Research and Education Center, USA. fmroka@ufl.edu

Advanced citrus production systems (ACPS), as practiced by growers in Spain, South Africa, and Australia, combine higher density tree planting with intensive nutrient and water management. Many Florida citrus growers are looking carefully at these systems as a strategy to effectively deal with Huanglongbing (HLB), rising costs of fertilizer and crop protection materials, as well as increasing land prices arising from urban development pressures. This paper presents an ACPS enterprise budget for sweet oranges planted on the flatwood soils of Florida. Citrus planted on Florida flatwood soils require raised beds and swales to handle water drainage during the rainy season (June – September). The budget outlines irrigation requirements with and without freeze protection, additional equipment to handle computerized irrigation scheduling, costs to reconfigure the planting beds for higher density trees, as well as the increased number of trees planted per hectare. Available yield data from higher density plantings are compared against the economic thresholds of ACPS.

S22O05

Spanish citrus cooperatives: keys to success and challenges for the future

Meliá Martí E., Server Izquierdo R.J., and Lajara-Camilleri N.

Centro de Investigación en Gestión de Empresas (CEGEA), Universitat Politècnica de València, Spain. nalade@cegea.upv.es

Cooperatives are one of the most significant elements in the production and marketing of food products in Spain, as in other European countries. Not surprisingly, according to data from 2008, in Spain there are currently 3,989 agricultural cooperatives that bring together 972,380 members and provide 94,156 direct jobs with total revenue of €18,889 million (CCAE, 2.010). Their role in the food value chain is indisputable and they have come to control most of the market share in many products such as tobacco industry (100%), grape must (80%), olive oil (75%), wine (70%), rice production (60%) and a 45% in citrus, milk and nuts. To a lesser extent they are present in the production of table olives, fruits, sheep and goat meat, cereals, oilseeds and cotton in which they account for 35% (CCAE, 2.010). Additionally cooperatives have emerged as the driving forces of the territory, allowing the adoption of technology, leading the internationalization and developing important management tasks. In recent decades, its role has been intensified by globalization, ongoing regulatory requirements and the need for diversification that has emerged in rural settings in order to keep the population. This paper develops a study of agrifood cooperatives in Spain and more specifically of citrus ones. Structural problems of holdings (small holdings in certain crops) have marked the need to group farmers in order to concentrate supply, internationalize, concentrate financial resources to promote joint R&D and ultimately to improve profitability. Through it, strengths of this kind of company are identified, as main responsible characteristics for the preponderant place that these entities have achieved in the sector. Moreover, the challenges that cooperatives are facing to ensure continuity in the current economic context are also discussed.

S22O06

Evolution of development models for Italian organic citrus growing: economic and environmental aspects

Scuderi A., and D'Amico M.

Department of Agri-food and environmental systems and management (DIGESA), Faculty of Agriculture, University of Catania, Italy. alessandro.scuderi@unict.it

Italian organic farming has been experiencing an intense development phase in the last ten years, arising the interest at economic, political and social level for its general effects on the population. In order to design the development models and understand the current differences between organic and traditional growing, the present study chooses the comparative analysis of homogeneous models. Indeed, the studies concerning the technical-economic analysis have revealed the existence of different methodologies. Method references, in such a context, are those to the studies of Spugnoli (1985), Biondi (1987) and Pimentel (1980), who analyzed the traditional farming and outlined a detailed picture of the energetic analysis by defining the conversion values of the main factors involved in the production process. The complexity of such analysis grew directly proportional with that of the production process of each single company, and the variety of indicators of the energy demand of the two production methods according to a structure based on the main inputs introduced in the production process, by adopting Gigajoule (GJ)-measured data for surface units and Megajoule (MJ)-measured data for product units. The analysis carried out in 2010 considered 80 organic farms above all Italian orange and lemon growing out of a sample of 20 organic farms and 20 traditional ones. Results showed interesting differences between organic and traditional methods. Such differences should become the starting point to exalt the *plus* of organic farming for consumers, politicians, business men, along the sector and in general within society, to offer a different role than today's organic farming.

S22O07

The importance of Research and Technical services in the recent growth of the southern African citrus export industry

Hattingh V.

Citrus Research International (CRI), South Africa. vh@cri.co.za

The southern African citrus industry's production and export statistics for the past 10 years were reviewed as an indicator of the longer term strategic direction it has pursued. It was evident that the climatically diverse range of production regions within the industry, spanning South Africa, Swaziland and Zimbabwe, was advantageous in enabling production of a wide range of cultivars across a broad production window of time. The long distance from lucrative world markets was clearly a disadvantage in that the prolonged shipping made the supply chain expensive, requiring high quality standards and sales values to remain profitable. A long history of industry commitment to supporting Research and Technical support services was linked to the specific challenges that this posed to realising the value of the industry's production advantages. This link was further evident in the industry's active pursuit of access to new markets and the retention of access to existing markets, especially in light of an increase in attention to international Sanitary and Phytosanitary trade regulations. The success of the industry's focus on Research and Technical support was reflected in the 120% increase in export volumes over the past 15 years, with a 55% growth over the past 10 years and an increase in the gross export Rand returns of approximately 160% over the past 10 years.

S22P01

Trends in varietal composition in Spanish citrus-growing and underlying variables

Caballero P.[1], Fernandez-Zamudio M.A.[1], Carmona B.[1], Alcon F.[2], and de Miguel Gomez M.D.[2]

[1]Instituto Valenciano de Investigaciones Agrarias (Ivia), Economia y Sociologia Agrarias, Spain; and [2]Universidad Politecnica De Cartagena (UPCT), Economia De La Empresa, Spain. md.miguel@upct.es

Citrus varieties as a whole must meet two basic conditions for fresh consumption: i) they should cover the widest possible commercial calendar, and ii) allow continuous adaptation to consumer preferences. For citrus

growers, this requires permanent technology up-dates and changes in variety grown, driven by income-risk criteria, as well as technical and commercial experience. The duration of an investment depends on the agronomic performance of the variety or its commercial life, which can sometimes be shortened due to lack of consumer acceptance. The survival of a variety depends on many variables: technical, quality, and competitiveness of the variety. Spanish citrus production falls into five main groups of varieties: lemons (13%), clementines (25%), mandarin hybrids (9.5%), navels (32%) and common sweet oranges (12%). The most productive varieties are: 'Fino', 'Navelina', 'Clemenules', 'Lane Late' and 'Valencia'. Regarding changes in varietal composition of interest, the introduction of new varieties or clones of the 'Clemenules' groups ('Orogrande', 'Nulessin'), Navel ('Powell', 'Rohde', 'Barnfield') and 'Valencia' ('Barberina', 'Midknight', 'V. Late Frost' and 'Delta Seedless') is noteworthy. There is constant pressure to supply new varieties of early clementines, whose survival is usually short. Currently, mandarin hybrids represent one group with potential to expand.

S22P02

Evolution of the international citrus trade in the Valencian region.

Aznar Puente J.L., García Martínez G., Silvestre Esteve E., and Orea Vega G.
Universitat Politécnica De València (UPV), Economics and Social Sciences, Spain. ensiles@esp.upv.es

The citrus production in the Valencian Region, is clearly oriented to the final consumer and presents a consolidated export tradition. Spain has become for years the leader in the worldwide citrus market, and the Valencian Region represents the 75% of the total Spanish exports. Consumers are increasingly more concerned about environment issues. They constitute along with prices the two most important variables in their decision process. Citrus growing and especially its transportation produce relevant energy consumption and greenhouse gas emissions. Transportation is a factor that directly affects the price of the goods and could become critical for, in certain cases, penalizing the product. For citrus companies, the energy consumption in transport and the generation of greenhouse gas emissions will vary depending on the mode of transport used. Therefore, in order to preserve their competitiveness, not only it is necessary that companies know which transportation mode is more efficient, but also which one is less aggressive with the environment. According to the above, this paper develops a model that calculates the absorption / emission of CO_2 derived from the citrus cultivation and the citrus export by land transport modes. The objectives pursued are as the following: 1) To identify the main export trade flows of Spain and the Valencian Region, and the transportation needs that they generate; 2) To determine the current transport mode framework in the citrus export, by defining the use of each type of transport mode towards the most significant country destinations; 3) To calculate the CO2 balance emission / absortion associated to the citrus crop growing procedures and the balance caused by the international transportation of the Valencian citrus products; and 4) To compare and calculate the economic costs linked to the land transportation modes to the main foreign markets, that the Valencian citrus export companies currently face.

S22P03

The citrus chain in Entre Rios: characterization and economic impact of some technologies to increase competitiveness and equity

Vera L.M.V., Díaz Vélez R.D.V., Banfi G.B., Garrán S.M., and Gómez C.G.
Instituto Nacional de Tecnología Agropecuaria (INTA), Experimental Concordia. Entre Ríos, Argentina. luismariavera@gmail.com

Citrus chain in Entre Rios, include 42,000 ha of citrus, principally with orange and mandarin, and nearly 1,800 growers. The average area per grower is nearly to 24 ha, but the incidence of the small fields, the modal area is between 15 and 20 ha. Then the technology may be able to resolve the trouble of this kind of productive structure. The high density plantation and the precision citriculture technology make an interesting contribution on this way. This work study the economic aspect of this technology proposal, related with the costs, income distribution, resumed in economics and financial indices, in comparation with the traditional density and management. The experience in the Experimental Concordia, indicate that the cost can be nearly to the traditional working form, but the productivity and quality can increase significantly.

S22P04

The experience of the Entre Ríos Citrus Board, and its contribution to the citrus industry.

Vera L.M.V.

Instituto Nacional de Tecnología Agropecuaria (I.N.T.A.), Experimental Concordia. Concordia City, Entre Ríos, Province, Argentina. luismariavera@gmail.com

Concordia, in Entre Ríos province, became in '60' decade, the National Capital of Citrus in Argentina. That is because the important growth of citrus area and production in the north east of the province since 1945 to this time, and became the principal citrus region of the country. The production was oriented to supply fresh fruit to the local market. But citrus industries for making juice, needed to regulate the quality and commerce of fresh fruit. In 1962 a juice factory was build, called Las Tejas in 1962. It was mixed (public – private) company thought for the small and medium growers. In 1965 and 1967, the hardest frosts, until then, took place. Las Tejas preserved the incomes of the small growers, but the sustainability of the system was not insured. That's why, in 1967, the Entre Ríos Citrus Board (ECB) was created to complete the system. The board had two principal objectives, one was to improve all regional citrus industry, and the other to regulate and improve the commerce and the industrialization. The contributions in both directions were very important. But ECB was dissolved by the State in 1982, basically because of the implementation of a neoliberal vision. Similar process occurred in other citrus region. Now in 2012, the other hardest frozen has taken place, and other similar troubles are also associated, and organizations like ECB seem to be necessary. This work rescues the experience of ECB, in this sense.

S22P05

Overview of organic citrus production in the Algarve (Portugal)

Reis D.[1], Fernandes M.M.[2], Marreiros A.[2], Mourão I.[3], and Duarte A.[4]

[1]Instituto Politécnico de Viana do Castelo (IPVC), Escola Superior Agrária de Ponte de Lima, Portugal; [2]Direcção Regional de Agricultura e Pescas do Algarve (DRAPALG), Portugal; [3]Centro de Investigação de Montanha/Instituto Politécnico de Viana do Castelo (CIMO/IPVC), Escola Superior Agrária de Ponte de Lima, Portugal; and [4]Institute of Mediterranean Agricultural and Environmental Sciences - University of Algarve (ICAAM/UAlg), Faculty of Sciences and Technology, Portugal. dhcreis@gmail.com

The citrus industry in the Algarve holds a leading position in the overall national fruit production. However, only a few citrus producers certify their production as organic farming, which should be increased if we consider the advantages of organic production compared to the conventional systems. From a technical point of view, the regulations of organic citrus fruit production can be applied straightforwardly. Although the control of some pests could be difficult, particularly *Ceratitis capitata* and scale insects, usually they do not cause major problems when organic pest control methods are used. Moreover, yield can be close to that obtained in conventional agriculture. Nowadays, only few farms produce organic citrus fruits in the Algarve and the mean area of citrus orchards is very small (2.3 ha per farm). In addition, some organic farmers have neglected crop management in their citrus orchards. The basis of their low incentive is the relative low prices paid to citrus fruits, as well as the payment schedule, since payments often are performed six months after crop trade, namely through supermarket chains. This has led to a replacement of the citrus production in favour of other crops or to other more rewarding farm activities. If prices go up and / or payment terms become more favourable, Portuguese organic citrus production could increase significantly in a short period of time.

S22P06

Citrus and pomegranate cultivation: concurrence *vs* competition in eastern Spain

Melian Navarro A.[1], de Miguel Gomez M.D.[2], and Fernandez-Zamudio M.A.[3]

[1]Universidad Miguel Hernandez (UMH), Economia Agroambiental, Spain; [2]Universidad Politecnica de Cartagena (UPCT), Economia de la Empresa, Spain; and [3]Instituto Valenciano de Investigaciones Agrarias (IVIA), Horticultura, Spain. md.miguel@upct.es

Citrus growing, traditionally associated with Spanish Mediterranean agriculture, is particularly sensitive to water allocation fluctuations. In this area the structural water resource deficits, as well as the gradual decline in citrus prices received by farmers, have promoted the co-plantation of other crops that are more resistant to water shortage, such as pomegranates, which have been grown in eastern Spain for decades. At present,

demand is growing due to knowledge of pomegranates' nutritional benefits and functional food qualities. In this paper, we analyze the different techno-economic aspects of citrus and pomegranate growing, as they pose ever increasing competition to each other. Comparison of these two crops begins with an assessment, from the farm economy perspective, of the main results published in the scientific literature on regulated deficit irrigation, this being one of the most up-to-date techniques used to address water shortage not clear. Also, the study analyzes the trend in prices received by farmers for the 'Lane Late' orange, 'Clemenules' mandarin, and for the two main Spanish varieties of pomegranate. The economic outlook expected from these two crops is rounded off by an analysis of investments, which shows the profitability of individual farms.

S22P07

PDO and PGI citrus productions: state of the art and perspectives

Scuderi A., and Pecorino B.

Faculty of Agriculture, University of Catania, (DIGESA), Department of agri-food and environmental systems and management, Italy. alessandro.scuderi@unict.it

Within the field of agrifood and certified hortofruit productions, citrus fruits play a very important role, especially for Italy. In fact, 12 appellations out of the 19 ones worldwide recognized have Italian origin. In fact, they have become a significant and strategic sector of the national economy, in particular for the South, which, nevertheless, in the last times is experiencing a severe crisis, with growing loss of market shares and profitability for producers. Therefore, the need of an intervention is pressing now, at structural, economic and financial level, to overcome it. Besides, improving production efficacy and farmers' profits, special attention is to be paid to the economic development of the entire production system, focusing on the concept of global quality. The new strategy of the modernization process of Italian citrus growing is to be based on two priority targets: food safety for consumer protection and enhancement of agrifood production quality. To this end this report analyzed, in a system perspective, the state of the art of quality productions by adopting SWOT analysis mode. Preferring the system approach implied an articulated group of specific targets aiming at obtaining the improvement of the conditions of the production phase and the market efficiency for citrus productions.

S22P08

Comparisons of fruits load estimation methods using geostatistical techniques

Gimenez L.I., Cabrera Brunetti S.C., Avanza M.M., and Mazza S.M.

Universidad Nacional del Nordeste (UNNE), Facultad de Ciencias Agrarias, Argentina. smmazza@gmail.com

Anticipated knowledge of fruits harvest is useful to define management and marketing strategies. Prediction methods use objective field data by counting the fruit in the entire canopy of trees or in sections of them, with known volume, and then apply mathematic expressions for geometric bodies, to calculate the total volume. Variability in load of fruit trees occurs, in time, with alternation between years of high and low production, and in space, between areas of higher and lower productivity. Geostatistical techniques allow using spatial information for estimating variables distributed in space. This study aimed to compare methods for estimating fruit load in 'Valencia late' sweet orange quantifying the spatial dependence. Field data were obtained on a commercial orchard of 'Valencia late' sweet orange, located in Entre Rios, Argentina, during the seasons 2009/10 and 2010/11. Load was estimated 60 days before harvest on sample of the 6.5% of trees, by two methods: counting fruits in the entire canopy (TC) or into a frame of 0.5 m of side (0.125 m3) and applying the cone volume formula (FC); at harvest all fruit in the canopy was counted (RL). Using the residual sums of square as a selection criterion, mathematical models that quantify spatial dependence of harvested (RL) and estimated load (TC and FC) were determined. Real load adjusted to a spherical model, in both seasons, with ranges of spatial dependence of 20.58 m and 16.52 m and estimates of the total variance of 140.574 and 129.043 in seasons 2009/10 and 2010/11 respectively. In season 2009/10 predicted values by both methods fitted to an exponential model, with ranges of 20.82 m and 25.38 m and sills of 104.263 and 86.022 respectively. In season 2010/11 predicted values by both methods fitted to a spherical model with ranges of 16.58 m and 14.62 m and sills of 69.887 and 100.426 respectively. Both methods characterize adequately the structure of spatial dependence but underestimate total variance.

S22P09

Price evolution of 'Clemenules' and 'Navelina' in Spain in the period 2007-2012

Server Izquierdo R.J., García-Martínez G., Lajara-Camilleri N., and Orea-Vega G.

Centro de Investigación en Gestión de Empresas (CEGEA), Universitat Politècnica de València, Spain. nalade@cegea.upv.es

Citrus crop is strongly affected by cyclical swings in their productions. This fact, among others, is reflected in the evolution of prices that are received by farmers. This instability results in an uncertain income for farmers, being even not enough –in some cases- to cover the expenses incurred by the activity. This paper analyzes the evolution in the past five campaigns of the average price received by the farmer ("price in tree") and by wholesalers in some of the most important citrus varieties cultivated in Spain: the clementine 'Clemenules' and the navel orange 'Navelina'. Additionally, volumes are analyzed based on the available information on statistics agencies. The aim of the paper is to characterize the evolution of prices and highlight the differences between the agents considered, in order to be a first step in the study of supply and demand of fresh citrus fruit in Spain.

S22P10

São Francisco Valley as a new belt for the Brazilian citrus industry

Passos O.S.[1], Bastos D.C.[2], Soares Filho W.S.[1], Girardi E.A.[1], and Leão H.C.[3]

[1]Embrapa Cassava and Fruit Crops Center (Embrapa-CNPMF), NUGENE-Citros, Brazil; [2]Embrapa Semiarid Center (Embrapa-CPATSA), Fruit Crops, Brazil; and [3]Fischer S/A Com Ind. Agricultura (Citrosuco Fischer), Technical Department, Brazil. orlando@cnpmf.embrapa.br

The citrus industry is one of the most relevant segments of the Brazilian agribusiness, being responsible for 40% of the sweet orange production and 60% of the juice processing in the World. Some factors for this hegemony are the adequate ecological conditions, which allow cultivation without irrigation, and huge land availability. Threats, however, exist especially at the core of national production, in the State of São Paulo. The most important bacterial and virus diseases appear with increasingly incidence and destructive power. The Northeast, the second national producing region seems to have the best conditions for the citrus industry expansion. Among potential areas, the São Francisco Valley appears as one the best options due to the semiarid climate, government support and tradition in the irrigated fruit production and export. The region, with 360,000 ha potentially irrigable, is located between 8º and 9º S and 40º to 42º W and altitude of 300 m. The annual rainfall is less than 600 mm and the average temperature is 26.2º C, relative humidity of 67% and solar radiation of 3,000 h/year, constituting a comparative advantage to species such as acid lime, lemon and grapefruit. Citrus scions and rootstocks trials have been carried out since the 90´s by Embrapa in cooperation with the private sector in the States of Bahia and Pernambuco, which data are presented in this paper, showing the potentiality of the São Francisco Valley as a new belt for the Brazilian citrus industry.

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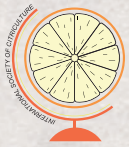

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