

22nd “International Conference on Virus and Other Transmissible Diseases of Fruit Crops” (ICVF)

Rome, June 3-8, 2012

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Welcome to the

*22nd “International Conference on Virus and Other Transmissible Diseases of Fruit Crops”
(ICVF)*

Dear Colleagues,

We are very happy and honored to welcome you for the second time in Rome in 18 years to attend and participate in the 22nd International Conference on Virus and Other Graft Transmissible Diseases of Fruit Crops. Some of you (the “youngest” ones!) remember that in 1994 the Plant Pathology Research Institute hosted the 16th Symposium on Fruit Tree Virus Diseases and the 7th Symposium on Small Fruit Virus Diseases. The Meeting was a great success and we hope to offer to you the same warm hospitality as we did 18 years ago!

More than 150 participants from different countries where fruit crops are economically important are here to present their most relevant scientific results obtained during the last three years.

In addition to the paper and poster presentations, we have organized a round table discussion on “Diagnosis of relevant pathogens: role of National and International organizations in the frame of harmonization”. The round table, chaired by Ms Françoise Petter, EPPO assistant Director, will have the participation of scientists actively involved in this specific scientific field.

Moreover, a workshop is planned to update all the Conference participants on the European Certification of fruit tree and small fruit crops. It will have the active participation of invited experts from different countries who are deeply involved in the establishment of legal, scientific and operative aspects aimed at improving the phytosanitary quality of fruit tree and small fruit propagative material and at reducing the risk of pest spreading through the commercialization of infected germplasm.

The last day of the Conference, we will host a COST workshop on “Phytoplasmas in fruit trees: multidisciplinary approaches toward disease management” which will offer to all participants the opportunity to be informed on special aspects of this class of plant pathogens.

During the Conference we will have the pleasure to listen to four invited lectures presented by researchers who spent most of their scientific careers in improving and advancing our knowledge of viruses, viroids, phytoplasmas and detection and identification of plant viruses and viroids. A big thanks goes to Professors Martelli, Flores, Oshima and Hadidi who kindly accepted to share with us their long experience on these specific topics.

We thank all of you for attending the second Meeting held under a new title covering virus and other graft transmissible diseases of fruit tree and small fruit crops.

We hope you will enjoy the Meeting and the beauty of the eternal city, Roma!

Cordially,

Marina Barba

Convener of the Conference

and

Members of the Organizing Committee

Scientific Committee

Wilhelm Jelkmann, Germany (co-chair ICVF)

Gabi Krczal, Germany (co-chair ICVF)

Marina Barba, Italy (co-chair ICVF)

Stuart MacFarlane, UK (secretary ICVF)

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Scientific Program of ICFV 2012 – Rome 3-8 June

Sunday, 3 June

17:00 – 20:00 Registration, poster hanging and Welcome cocktail

Monday, 4 June

09:00 – 10:00 Registration and poster hanging

10:00 – 10:30 Meeting opening

10:30 – 11:15 Invited lecture

G.P. Martelli - Advances in the knowledge of some fruit tree viruses

11:15 - 11:45 Coffee break

11:45 – 13:00 **Oral session I - Fruit trees: viruses, viroids and phytoplasmas.** Chairs: W. Jelkmann and A. Bertaccini

11:45 - 12:00 Molecular analysis of a 'new' *Foveavirus* associated with a fruit deforming disease of apple - *D. James, A. Varga, G.D. Jespersen, M. Navratil, D. Safarova, F. Constable, M. Horner, K. Eastwell* and *W. Jelkmann*

12:00 – 12:15 Genomic conformation of *Apple mosaic virus* Turkish isolates coat protein gene regions – *F. Ertunç, D. Canik, A. Sezer, Ş. Topkaya, Ç. Ulubaş-Serçe*

12:15 – 12:30 Molecular characterization of *Tomato ringspot virus* (ToRSV) strain responsible for “prune brownline” disease – *N. Fiore, L. Rivera* and *A. Zamorano*

12:30 - 12:45 Incidence of viral diseases on pear plants and the molecular characterization of three pear viruses in China – *H. Ni, S. Yansu, M. Xiaofang, H. Guojun, W. Liping, W. Guoping*

12:45 – 13:00 Detection and identification of *Pear blister canker viroid* occurring in pear trees in Canada - *E.M. Torchetti, C. Birch, M. Cooper, C. Masters, Y. Arocha, F. Di Serio* and *R. Michelutti*

13:00 - 14:00 Lunch

14:00 - 14:30 Poster session

14:30 – 16:00 **Oral session I - Fruit trees: viruses, viroids and phytoplasmas.** Chairs: F. Di Serio and S. Paunovic

14:30 – 14:45 A review of viruses infecting actinidia – *A.G. Blouin, R.R. Chavan, M.N. Pearson, R.M. MacDiarmid, C. Ratti, R. Biccheri, B. Lebas, S. Veerakone, D. Cohen*

14:45 – 15:00 *Pelargonium zonate spot virus* infecting kiwifruit plants in Italy – *R. Biccheri, A.R. Babini, A. Blouin, C. Lanzoni, A. Pisi, C. Poggi Pollini, R. Credi, L. Laghi, P. Rocculi, C. Rubies Autonell, M.N. Pearson* and *C. Ratti*

15:00 – 15:15 Association of phytoplasmas and rod shaped bacteria with citrus disease of unknown etiology in the State of Baja California Sur, Mexico – *A. Poghosyan, V. Lebsky, J.H.-Gonzalez*

15:15 – 15:30 Detection of viruses infecting apple in north western Himalayan region – *A. Brakta, P.D. Thakur* and *A. Handa*

15:30 – 15:45 Phytoplasma detection and identification in kiwi plants and possible correlation with *Pseudomonas syringae* pv *actinidiae* presence - *S. Paltrinieri, M. Piergiacomi, S. Ardizzi, N. Contaldo, E. Biondi, C. Lucchese, A. Bertaccini*

15:45 – 16:15 Coffee break

16:15 – 17:45 **Oral session II - Plum pox virus.** Chairs: D. Skoric and M. Cambra

16:15 – 16:30 An atypical Albanian isolate of *Plum pox virus* could be the progenitor of the Marcus strain - *F. Palmisano, D. Boscia, A. Minafra, A. Myrta, T. Candresse*

- 16:30 – 16:45 Epidemiology of *Plum pox virus*-T and -M isolates in stone fruit orchards in Turkey -
K. Çağlayan, Ç.U. Serçe, M. Gazel, K. Kaya, E. Elçi, F.C. Cengiz, E. Vidal and M. Cambra
- 16:45 – 17:00 *Plum pox virus* - W appears to be the most variables strain of the seven recognized strains of the virus - *A. Sheveleva, P. Ivanov, Y. Prihodko, A. Varga, D. James and S. Chirkov*
- 17:00 – 17:15 Preliminary evaluation of the competitiveness of PPV-Rec and PPV-D under field conditions –
I. Zagrai, L. Zagrai, G. Labonne, S. Dallot, A. Festila, I. Baias
- 17:15 – 17:30 Complete and partial genome sequences of the unusual *Plum pox virus* (PPV) isolates from sour cherry in Russia suggest their classification to a new PPV strain - *M. Glasa, Y. Prichodko, T. Zhivaeva, Y. Shneider, L. Predajňa, Z. Šubr, T. Candresse*
- 17:30 – 17:45 A large scale study of *Plum pox virus* genetic diversity and of its geographical distribution -
M. Glasa, T. Candresse and the SharCo consortium

Tuesday, 5 June

09:30 – 10:15 Invited lecture

A. Hadidi – Next-generation sequencing: applications for the detection and identification of plant viruses and viroids

10:15 – 11:00 Oral session III - Diagnosis and next generation sequencing. Chairs: D. Boscia and M. Glasa

10:15 - 10:30 Direct methods of sample preparation for detection of fruit tree viruses and viroids by real-time RT-PCR amplification - *E. Bertolini, M.C. Martínez, E. Vidal, A. Olmos and M. Cambra*

10:30 - 10:45 Validating PCR protocols for the detection of strawberry viruses in Australia – *F. Constable, L. Ko, C. Bottcher, G. Kelly, N. Nancarrow, M. Milinkovic, D. Persley and B. Rodoni*

10:45 – 11:00 Characterization of divergent *Plum bark necrosis stem pitting-associated virus* isolates and development of a polyvalent PBNSPaV-specific detection test – *A. Marais, C. Faure, B. Bergey, P. Gentit and T. Candresse*

11:00 – 11:30 Coffee break

11:30 – 12:15 Oral session III - Diagnosis and next generation sequencing. Chairs: V. Pallas and D. James

11:30 – 11:45 A first evaluation of siRNA next-generation sequencing for detection and characterization of *Prunus* viruses – *A. Olmos, M. Cambra, A. Marais, C. Faure and T. Candresse*

11:45 – 12:00 Use of 454 pyrosequencing for the fast and efficient characterization of known or novel viral agents in *Prunus* materials - *T. Candresse, A. Marais, C. Faure, S. Carrère and P. Gentit*

12:00 – 12:15 The study of plant virus disease etiology using next-generation sequencing technologies -
H.J. Maree, Y. Ne, M. Visser, B. Coetzee, B. Manicom, J.T. Burger and D.J.G. Rees

12:15 – 12:45 Poster session

12:45 - 13:45 Lunch

13:45 - 16:00 Round Table on “Diagnosis of relevant pathogens: role of National and International Organizations in the frame of harmonization” . Chair: F. Petter

16:00 - 16:30 Coffee break

19:30 Gala dinner

Wednesday, 6 June

09:00 – 09:45 Invited lecture

R. Flores - Viroids: biology and molecular biology of the smallest plant pathogens

09:45 – 11:00 Oral session IV – Host-Pathogen interaction. Chairs: T. Candresse and L. Conci

09:45 – 10:00 *Peach latent mosaic viroid*: data supporting the involvement of RNA silencing in peach calico (extreme chlorosis) - *B. Navarro, A. Gisel, M.E. Rodio, S. Delgado, R. Flores, F. Di Serio*

10:00 – 10:15 *Peach latent mosaic viroid*: further insights on a determinant associated with peach mosaic - *S. Delgado, B. Navarro, S. Minoia, P. Gentit, M.F. Cambra, F. Di Serio, R. Flores*

10:15 - 10:30 Disorganized viroid DNA fragment is transcribed in plant – *H. Guojun, H. Ni, W. Guoping, X. Wenxing*

10:30 – 10:45 Generation of *in vitro* RNA transcripts and infectious full-length cDNA clones of ASPV and ASGV – *A. Arntjen and W. Jelkmann*

10:45 – 11:00 Genome organization of *Cherry leaf roll virus* and analyses of function of virus-encoded proteins – *S. von Bargaen, L. Dierker, M. Rott, J. Langer, C. Büttner*

11:00 - 11:30 Coffee break

11:30 – 13:15 Oral session V - Small fruits: viruses, viroids and phytoplasmas. Chairs: R.R. Martin and S. MacFarlane

11:30 – 11:45 Strawberry marginal chlorosis (SMC): a disease of various bacterial and viral etiologies in France - *J.L. Danet, S. Fimbeau, P. Salar, C. Faure, J.J. Pommier, M. Turque, A. Marais and X. Foissac*

11:45 – 12:00 Identification and phylogenetic analysis of viruses infecting stone fruits and strawberry in Egypt - *F. Fattouh, C. Ratti, E. Aleem, M. Rabea, A.R. Babini and C. Rubies Autonell*

12:00 – 12:15 *Raspberry leaf blotch virus*, a new *Emaravirus* infecting raspberry – *S. MacFarlane, W. McGavin, C. Mitchell, P. Cock*

12:15 – 12:30 Emerging disease in blueberry caused by a novel RNA virus – *R.R. Martin, D.F. Quito-Avila, W.O. Cline, P.F. Harmon, P.M. Brannen*

12:30 – 12:45 Occurrence and variability in European isolates of *Blueberry red ringspot virus* – *J. Špak, K. Petrzik, J. Příbylová, D. Pavingerová, V. Špaková, D. Kubelková*

12:45 - 13:00 Dissecting the epidemiology of *Blackberry yellow vein associated virus* and *Blackberry chlorotic ringspot virus*; a study on population structure, transmission and alternative hosts - *B. Poudel, W.M. Wintermantel, S. Sabanadzovic and I. E. Tzanetakis*

13:00 – 13:15 Phytoplasmas of group 16SrI associated with strawberry (*Fragaria x Ananassa*) in Colombia – *L.M. Perilla and L. Franco-Lara*

13:15 - 14:30 Lunch

14:30 - 15:00 Poster session

15:00 – 16:00 Oral session VI – Control strategies. Chairs: W. Jaraush and T. Malinowski

15:00 – 15:15 Transgenic approach for improving resistance of plum cultivars for sharka disease - *S. Dolgov, R. Mikhailov, O. Shulga, A. Firsov*

15:15 – 15:30 Robust and wide spectrum RNA silencing mediated resistance to *Plum pox virus* - *E. Di Nicola-Negri, M. Tavazza and V. Ilardi*

15:30 – 15:45 Production and *in vitro* assessment of transgenic plums for the resistance to *Plum pox virus* – *S. Monticelli, E. Di Nicola-Negri, A. Gentile, C. Damiano and V. Ilardi*

15:45 – 16:00 Use of horticultural mineral oil treatments for the control of different *Plum pox virus* isolates in nursery blocks - *E. Vidal, L. Zagrai, S. Milusheva, V. Bozhkova, E. Tasheva-Terzieva, I. Kamenova, I. Zagrai, M. Cambra*

16:00 - 16:30 Coffee break

16:30 - 17:15 Oral session VI – Control strategies. Chairs: K. Çağlayan and S. Dolgov

16:30 – 16:45 The present status of commercialized and developed biotech (GM) crops, results of evaluation of plum 'honeysweet' for resistance to plant viruses in the Czech Republic - *J. Polák, J. Kumar, B. Krška, M. Ravelonandro and R. Scorza*

- 16:45 – 17:00 Role of conifer extract as repellent on *Cacopsylla pruni* and their effect on the spreading of ESFY in apricot - *P. Ermacora, F. Ferrini, M. Martini, N. Loi*
- 17:00 – 17:15 Use of micropropagated *Malus* to study latent apple viruses – *A. Liebenberg, A. Kappis, J. Barth, M. Weiter, M. Herdemertens, T. Wetzel* and *W. Jarausch*

17:30 – 19:30 Scientific Committee meeting

Thursday, 7 June

09:00 – 12:30 Workshop on EU Certification

“TOWARD THE EUROPEAN CERTIFICATION OF FRUIT PLANT: STATE OF THE ART” *Comparison between the research community, representatives of EU countries and stakeholders*

09:00 – 09:30 Welcome by Giandomenico Consalvo – President CIVI-Italia

09:30 – 10:45 Oral session VII – Certification. Chairs: P. Giorgetti and M. Barba

09:30 – 09:45 Clean plants, the national clean plant network and harmonizing certification standards in the United States – *R.R. Martin, K.C. Eastwell, S.W. Scott, I.E. Tzanetakis*

09:45 – 10:00 Establishment of virus-free planting material for pome fruits in Latvia – *A. Kāle, A. Gospodaryk, N. Pūpola, I. Moročko-Bičevska*

10:00 – 10:15 Development of molecular diagnostic tools to detect endemic and exotic pathogens of *Prunus* species for Australia – *F. Constable and B. Rodoni*

10:15 – 10:30 Quality systems for production of nursery stock in apple - *E.T.M. Meekes, D. Bakker, H. Konings*

10:30 – 10:45 Establishment, activities and results of plant biotechnology laboratory in Kabul, Afghanistan – *S. Rehman, J. Ahmad, C. Lanzoni, C. Rubies Autonell* and *C. Ratti*

10:45 - 11:15 Coffee break

11:15 - 11:30 State of the art on the implementing measures of Dir. CE 2008/90 - *B. Foletto - D.G. Sanco, EU Commission, Brussels*

11:30 – 11:45 Current status and evaluation of the pests included in the technical protocols for the coming EU certification rules - *Italian Experts Group consulted by MiPAAF*

11:45 – 12:30 Workshop on EU certification: final discussion

12:30 - 13:30 ICVF General Assembly

13:30 – 14:30 Lunch and Poster session

14:30 – 19:00 Technical and touristic tour (Farfa Abbey and visit to the Certification facilities at the CRA-PAV experimental farm of Tor Mancina)

20:00 Country dinner

Friday, 8 June

COST FA0807 Workshop

Phytoplasmas in fruit trees: multidisciplinary approaches toward disease management

09:00 - 09:15 Introduction by COST FA0807 – Chair A. Bertaccini

09:15 – 10:00 Invited lecture

K. Oshima - Global gene expression analysis of phytoplasma in the host switching between plant and insect

10:00 - 11:45 WG1 – Chairs: B. Duduk and J. Franova

10:00 – 10:15 Introduction of WG1 by WG leaders

10:15 - 10:25 Detection and identification of a phytoplasma affecting blackcurrant and redcurrant shrubs - *Y. Arocha, V. Agrawal, J. Scott, A. Bertaccini* and *R. Michelutti*

10:25 - 10:35 Molecular polymorphism in phytoplasmas infecting peach trees in Serbia – *B. Duduk, A. Bertaccini, M. Mitrovic, S. Paltrinieri, N. Contaldo*

- 10:35 - 10:40 Serological proofs for relatedness of the mycoplasma-like organisms (phytoplasmas) from apple proliferation group - *P.G. Ploaie, C. Chireceanu*
- 10:40 - 10:50 Multilocus gene analyses of ‘*Candidatus Phytoplasma mali*’ confirms the genetic diversity of phytoplasma population in the Czech Republic - *J. Fránová, H. Ludvíková, F. Paprštejn, A. Bertaccini*
- 10:50 - 11:00 Early and reliable detection of European stone fruit yellows phytoplasma in peach trees - *J. Polák, J. Salava, P. Komínek and J. Svoboda*
- 11:00 – 11:30 Coffee break
- 11:30 - 11:35 Detectability of phytoplasmas in naturally infected *Picea* and *Pinus* spp. trees by PCR – *M. Kamińska, H. Berniak, A. Kościelak*
- 11:35 – 11:40 First occurrence of pear decline disease in Portugal – *E. Sousa, A. Marques, C. Mimoso, F. Cardoso*
- 11:40 – 11:50 Survey for apple proliferation in orchards close to nurseries in Norway in 2011
D.R. Blystad, B. Toppe, M.A. Holst, M.B. Brurberg
- 11:50 - 13:00 WG4** – Chairs: X. Foissac and S. Hogenhout
- 11:50 – 12:20 Update of Phytoplasma Genome Sequencing Initiative (PGSI): fruit trees phytoplasmas – *S. Hogenhout, M. Kube and X. Foissac*
- 12:20 - 12:30 Evidence for ‘*Ca. P. pyri*’ and ‘*Ca. P. prunorum*’ inter-species recombination – *G. Balakishiyeva, J.L. Danet, A. Mammadov, A. Batlle, A. Laviña, I. Huseynova and X. Foissac*
- 12:30 - 12:40 Enrichment of phytoplasma DNA by selected oligonucleotides and PHI 29 polymerase amplification – *C. Siewert, J. Mitrovic, B. Duduk, J. Hecht, K. Mölling, F. Bröcker, P. Beyerlein, C. Büttner, A. Bertaccini, M. Kube*
- 12:40 - 12:50 Genetic diversity, membrane topology and relationship to virulence of the AAA+ ATPases and HflB proteases of ‘*Candidatus Phytoplasma mali*’ - *E. Seemüller, S. Sule, W. Jelkmann, M. Kube, B. Schneider*
- 12:50 - 13:00 Transcriptome analysis of *Vitis vinifera* cv. Chardonnay infected with GLRaV 3 and Aster yellows phytoplasma in a South African vineyard – *M.C. Solofoharivelo, M.C. Snyman, A. Van der Walt, D. Stephan, M.J. Freeborough, J.T. Burger and S. Murray*
- 13:00 – 14:30 Lunch
- 14:30 – 15:10 WG2** – Chairs: B. Jarausch and N. Sauvion
- 14:30 – 14:40 Introduction of WG2 by B. Jarausch
- 14:40 - 14:50 Summary of questionnaire data for the presence of phytoplasma diseases and their putative vectors through Europe and Middle East – *R. Tedeschi, D. Delić, B. Jarausch, P.G. Weintraub*
- 14:50 - 15:00 Psyllid vectors of the AP group (16SrX) phytoplasmas in Turkey - *Ç.U. Serçe, K. Kaya, M. Gazel, K. Çağlayan, N. Sauvion*
- 15:00 - 15:10 Degree of specificity among the psyllid-phytoplasma interactions and consequences in epidemiology - *N. Sauvion*
- 15:10 – 15:50 WG3** – Chairs: W. Jarausch and E. Torres
- 15:10 - 15:20 Phytoplasmas associated with Apricot chlorotic leafroll disease – *N. Contaldo, J. Mitrovic, S. Paltrinieri, B. Duduk, A. Bertaccini*
- 15:20 - 15:30 Almond witches’ broom phytoplasma, officially declared as a regulated pest in Lebanon – *Y.A. Jawdah, M. Molino-Lova, P.A. Bianco, E. Choueiri, R. Fakhr, S. Hajj-Hassan, L. Haydar and R. Al Achi*
- 15:30 - 15:40 Monitoring distribution of fruit tree phytoplasmas in Bulgaria from 2007 until 2011 – *A. Etropolska and M. Laginova*
- 15:40 - 15:50 Induction of defense genes in apple leaf tissues by an endophytic strain of *Epicoccum nigrum* and its possible use in the control of the apple proliferation disease – *K. Farhan, S. Grisan, R. Polizzotto, F. de Marco, P. Ermacora, R. Musetti*
- 15:50 - 17:00 Joint round table WG2 and WG3**

LIST OF POSTERS

Poster session I – Fruit trees: viruses, viroids and phytoplasmas

- P01 - INCIDENCE OF *FIG LEAF MOTTLE-ASSOCIATED VIRUS* AND *FIG MOSAIC VIRUS* IN EASTERN PROVINCE OF SAUDI ARABIA
K. Alhudaib
- P02 - THE INCIDENCE OF *PRUNUS NECROTIC RINGSPOT VIRUS* IN APRICOT AND PEACH IN SAUDI ARABIA
K. Alhudaib and A. Rezk
- P03 - EVIDENCE FOR ‘*CA. P. PYRI*’ AND ‘*CA. P. PRUNORUM*’ INTER-SPECIES RECOMBINATION
G. Balakishiyeva, J.L. Danet, A. Mammadov, A. Batlle, A. Laviña, I. Huseynova and X. Foissac
- P04 - EPIDEMIOLOGICAL STUDY OF APPLE PROLIFERATION IN SPAIN
A. Batlle, J. Sabaté, A. Laviña
- P05 - DETECTION AND MOLECULAR CHARACTERIZATION OF A NOVEL *CRYPTOVIRUS* FROM PERSIMMON (*DIOSPYROS KAKI*)
M. Morelli, A. De Stradis, P. La Notte, J. Merkuri, D. Boscia and A. Minafra
- P06 - EFFICACY OF APPLE PROLIFERATION PHYTOPLASMA TRANSMISSION BY VEGETATIVE PROPAGATION TECHNIQUES AND IMPACT ON ITS EPIDEMIOLOGY
D. Cornaggia, N. Grasseau and P. Gentit
- P07 - SUSCEPTIBILITY OF SOME APRICOT AND PEAR CULTIVARS ON VARIOUS ROOTSTOCKS TO ‘*CANDIDATUS PHYTOPLASMA PRUNI*’ AND ‘*CA. P. PYRI*’
M. Gazel, Ç. U. Serçe, Ş. Yavuz, H. Gultekin, K. Çağlayan
- P08 - GENOME CHARACTERIZATION OF A DIVERGENT *LITTLE CHERRY VIRUS 1* ISOLATE ASSOCIATED WITH THE SHIROFUGEN STUNT DISEASE
T. Candresse, A. Marais, C. Faure and P. Gentit
- P09 - DETECTION AND CHARACTERIZATION OF PHYTOPLASMAS INFECTING APPLE TREES IN POLAND AND IDENTIFICATION OF THEIR POSSIBLE VECTORS
M. Cieślińska, D. Kruczyńska, K. Jaworska
- P10 - TRANSMISSION TRIALS OF THE FIG MOSAIC DISEASE AGENT
R. Credi, F. Terlizzi, R. Beber, C. Poggi Pollini, C. Ratti
- P11 - FIRST SURVEY ON POME FRUIT VIRUSES IN LEBANON
E. Nassar, E. Choueiri, S. Minoia, F. Di Serio, K. Djelouah
- P12 - PARTIAL CHARACTERIZATION OF *FIG MOSAIC VIRUS* IN INOCULATED FIG AND PERIWINKLE PLANTS BY DOP-PCR
E. Elçi, K. Çağlayan, A. Minafra
- P13 - IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF ‘*CANDIDATUS PHYTOPLASMA PYRI*’ IN PEAR TREES FROM CENTRAL ITALY.
L. Ferretti, E. Costantini, A. Gentili, F. Punelli, G. Pasquini

- P14 - DETECTION AND MOLECULAR CHARACTERIZATION OF *CHERRY GREEN RING MOTTLE VIRUS* (CGRMV) AND *CHERRY NECROTIC RUSTY MOTTLE VIRUS* (CNRMV) IN SWEET CHERRY IN CHILE
N. Fiore and A. Zamorano
- P15 - MOLECULAR VARIABILITY IN THE COAT PROTEIN GENE OF THE DIFFERENT ISOLATES OF *APPLE MOSAIC VIRUS*
L. Grimová, L. Winkowska, P. Ryšánek, P. Svoboda
- P16 - NOVEL GENOMIC RNA SEGMENTS OF *FIG MOSAIC VIRUS*, A NEWLY IDENTIFIED *EMARAVIRUS* INFECTING THE COMMON FIG (*FICUS CARICA*)
K. Ishikawa, K. Maejima, K. Komatsu, Y. Kitazawa, M. Hashimoto, D. Takata, Y. Yamaji and S. Namba
- P17 - INVESTIGATIONS ON THE OCCURRENCE OF THREE LATENT APPLE VIRUSES THROUGHOUT THE YEAR AND SEQUENCE VARIABILITY OF *APPLE STEM PITTING VIRUS*
A. Arntjen and W. Jelkmann
- P18 - DETECTION OF *CHERRY VIRUS A* AND *CHERRY GREEN RING MOTTLE VIRUS* IN GREEK SWEET CHERRY ORCHARDS
A. T. Katsiani, E. V. Drougkas, E. Deligiannis, C. Ktori, N. I. Katis and V. I. Maliogka
- P19 - PARTIAL CHARACTERIZATION OF A DISTINCT *LITTLE CHERRY VIRUS 1* ISOLATE REVEALS HIGH INTRASPECIES GENETIC VARIABILITY
A. T. Katsiani, V. I. Maliogka, K. E. Efthimiou and N. I. Katis
- P20 - THE MONITORING OF OCCURRENCE OF FOUR COMMON VIRUSES IN PLUM CULTIVARS IN LATVIA
A. Gospodaryk, N. Pūpola, A. Kāle, I. Moročko-Bičevska
- P21 - A NEW *TRICOVIRUS* ISOLATED FROM PEACH TREES IN MEXICO
R. De la Torre, V. Pallas and J. A. Sánchez-Navarro
- P22 - DETECTION OF PHYTOPLASMAS IN CIRUELO TREE (*CYRTOCARPA EDULIS*) AND IN SHARPSHOOTER *HOMALODISCA LITURATA* IN THE STATE OF BAJA CALIFORNIA SUR, MEXICO
V. Lebsky, J. H. Gonzalez, R. Servin-Villegas, A. Poghosyan
- P23 - A SIMPLE METHOD FOR PHYTOPLASMAS TRANSMISSION BY GRAFTING
P. Kawicha, J. Hodgetts, and M. Dickinson
- P24 - GENETIC DIVERSITY OF *APPLE CHLOROTIC LEAF SPOT VIRUS* (ACLSV) IN FRUIT CROPS
N. Pūpola, A. Gospodaryk, A. Kāle
- P25 - A SURVEY FOR VIRUSES AND VIROIDS IN USA CHERRY GENETIC RESOURCES
R. Li, G. Kinard, R. Mock, P. Forsline, M. Pooler and E. Stover
- P26 - MONITORING OF PSYLLID SPECIES (*HEMIPTERA, PSYLLOIDES*) IN CONIFEROUS PLANTS IN POLAND
G. Soika, H. Berniak, M. Kamińska, A. Kościelak
- P27 - FIRST REPORT OF MOLECULAR IDENTIFICATION OF 'CANDIDATUS PHYTOPLASMA PYRI' IN PEAR TREES IN BELGIUM
T. Olivier, E. Demonty, G. Peusens, T. Beliën and S. Steyer

P28 - IDENTIFICATION OF *PEACH LATENT MOSAIC VIROID* (PLMVd) IN MONTENEGRO
J. Zindović, I. Mavrić-Pleško, M. Viršček-Marn, Z. Miladinović

Poster session II – *Plum pox virus*

P29 - *PLUM POX VIRUS* SITUATION IN EMILIA-ROMAGNA REGION (ITALY)
P. Fini, P. Grillini, A. D'Anniballe, A.R. Babini, V. Vicchi

P30 - EVALUATION OF *PLUM POX VIRUS* SENSIBILITY ON DIFFERENT STONE - FRUIT VARIETIES IN EMILIA ROMAGNA REGION (ITALY)
A. R. Babini, F. Fontana, P. Fini, C. Poggi Pollini, C. Ratti

P31 - SUSCEPTIBILITY OF DIFFERENT PRUNUS ROOTSTOCKS TO NATURAL INFECTION BY *PLUM POX VIRUS*-T
K. Çağlayan, K. Kaya, Ç. U. Serçe, M. Gazel, F.C. Cengiz, E. Vidal, M. Cambra

P32 - MOLECULAR INVESTIGATION ON RECOMBINANT *PLUM POX VIRUS* ISOLATES IN CENTRAL ITALY
E. Costantini, P. Grillini, A.R. Babini, A. D'Anniballe, P. Fini, V. Vicchi, G. Pasquini

P33 - TEMPORAL ANALYSIS OF *PLUM POX VIRUS* IN CHILE
N. Fiore, A. Zamorano, F. González, I. M. Rosales, C. Hamilton-West

P34 - DYNAMICS OF SPREAD OF PPV-REC AND PPV-D IN AN EXPERIMENTAL PLUM ORCHARD
D. Jevremović, S. Paunović, G. Labonne, S. Dallot

P35 - DETECTION OF *PLUM POX VIRUS* IN REGIONS OF BELARUS
A. Salavei, M. Kastriskaya, N. Valasevich, N. Kukharchyk

P36 - EFFECT OF CO-INFECTION OF *PLUM POX VIRUS* AND *PRUNE DWARF VIRUS* IN *PRUNUS PERSICA*; A QUANTITATIVE ANALYSIS BY REAL-TIME RT-QPCR
E. Svobodová, J. Jarošová, J. K. Kundu

P37 - SEROLOGICAL AND MOLECULAR CHARACTERIZATION OF THE ISOLATES OF *PLUM POX VIRUS* FOUND IN LATVIA.
T. Malinowski, L. Michalczuk, N. Pūpola, Dz. Dēķena

P38 - PARTIAL CHARACTERISATION OF BIOLOGICAL PROPERTIES OF PPV-C ISOLATES FOUND IN BELAROUS AND ESTABLISHMENT OF *IN VITRO* CULTURES OF INFECTED L2 AND OWP-6 ROOTSTOCKS.
T. Malinowski, I. Sowik, A.V. Salavei, N.V. Kukharchyk

P39 - MAPPING THE PATHOGENICITY DETERMINANTS IN THE GENOME OF *PLUM POX VIRUS*
A. Nagyová, M. Kamencayová, Z.W. Šubr

P40 - OCCURRENCE OF *PLUM POX VIRUS* STRAINS IN MORAVIA (CZECH REPUBLIC)
D. Šafářová, S. Gadiou, M. Navrátil

P41- SURVEY OF SHARKA DISEASE (*PLUM POX VIRUS*) ON STONE FRUIT TREES IN NORTHERN HUNGARY
L. Palkovics, A. Almási, J. Ádám, B. Balotai and I. Tóbiás

P42 - DIVERSITY OF *PLUM POX VIRUS* IN PLUM ORCHARDS IN SERBIA
S. Paunović, D. Jevremović, S. Dallot

P43 - *PLUM POX VIRUS* ON SOUR CHERRY IN CROATIA

V. Kajić, S. Černi, D. Škorić

P44 - LARGE SCALE *PLUM POX VIRUS* SURVEY OF CULTIVATED AND WILD CHERRY IN ROMANIA

L. A. Zagrai, I. Zagrai, L. Levy, V. Mavrodieva, A. Festila, I. Baias

P45 - CHARACTERIZATION OF *PLUM POX VIRUS* ISOLATES FROM DIFFERENT PEACH VARIETIES IN MONTENEGRO

J. Zindović, V. Božović, Z. Miladinović, C. Rubies Autonell, C. Ratti

Poster session III – Diagnosis and next generation sequencing

P46 - VIRUS SANITATION AND DEEP SEQUENCE ANALYSIS OF FIG

M. Chiumenti, R. Roberto, G. Bottalico, A. Campanale, A. De Stradis, A. Minafra, D. Boscia, V. Savino, G. P. Martelli

P47 - DEVELOPMENT OF QUANTITATIVE REAL-TIME RT-PCR FOR THE DETECTION OF *HOP STUNT VIROID*

M. Luigi and F. Faggioli

P48 - APPLICATION OF HIGH RESOLUTION MELT (HRM) ANALYSIS FOR SIMULTANEOUS DETECTION OF *CHERRY GREEN RING MOTTLE VIRUS* AND *CHERRY NECROTIC RUSTY MOTTLE VIRUS*

B. Komorowska

P49 - DETECTION OF *PLUM POX VIRUS* WITH IMMUNOSENSOR UTILIZING ANTIBODIES IMMOBILIZED ON GOLD NANOPARTICLES.

U. Jarocka, M. Wąsowicz, H. Radecka, T. Malinowski, L. Michalczuk, J. Radecki

P50 - DETECTION OF TWO STRAINS OF ‘*CANDIDATUS PHYTOPLASMA ASTERIS*’ FROM PEACH AND APRICOT USING LOOP-MEDIATED ISOTHERMAL AMPLIFICATION

C. Miura, T. Keima, T. Watanabe, T. Nijo, K. Maejima, K. Oshima and S. Namba

P51 - DEVELOPMENT OF A MINI-OLIGO ARRAY FOR THE ANALYSIS OF *PLUM POX VIRUS* VARIABILITY

A. Olmos, G. Ancillo, L. Svanella-Dumas, E. Vidal, J. Petit, M. Cambra, T. Candresse

P52 - VALIDATION OF DIAGNOSTIC PROTOCOLS FOR THE DETECTION OF ‘*CANDIDATUS PHYTOPLASMA MALI*’ AND ‘*CANDIDATUS PHYTOPLASMA PRUNORUM*’

G. Pasquini, A. Bertaccini, P.A. Bianco, P. Casati, E. Costantini, L. Ferretti, M. Martini, C. Marzachi, S. Palmano, S. Paltrinieri, M. Barba

P53 - DETECTION OF *PLUM POX VIRUS* IN VARIOUS TISSUES USING FIELD TESTS, SEROLOGICAL AND MOLECULAR TECHNIQUES

M. Viršček Marn, I. Mavrič Pleško, D. Altenbach, W. Bitterlin

P54 - DEEP SEQUENCE ANALYSIS OF VIRAL SMALL RNAs FROM A GREEN CRINKLE-DISEASED APPLE TREE

N. Yoshikawa, N. Yamagishi, H. Yaegashi and T. Ito

Poster session IV – Host-pathogen interaction

- P55 - GENOME SEQUENCE VARIABILITY OF A *PLUM POX VIRUS* ISOLATE RELATED TO ITS LONG-TIME MULTIPLICATION IN DIFFERENT HOST SPECIES
Z.W. Šubr, L. Predajňa, M. Glasa
- P56 - SCREENING OF TURKISH LOCAL APRICOT VARIETIES AND CROSSINGS BY MOLECULAR MARKERS LINKED TO *PLUM POX VIRUS* RESISTANCE
Ç. U. Serçe, B. M. Asma, M. Gazel, K. Çağlayan, M.L. Badenes
- P57 - *APPLE LATENT SPHERICAL VIRUS* VECTORS FOR CROSS PROTECTION AGAINST *ZUCCHINI YELLOW MOSAIC VIRUS*
T. Kato, A. Tamura, T. Natsuaki, and N. Yoshikawa

Poster session V – Small fruits: viruses, viroids and phytoplasmas

- P58 - DETECTION OF VIRUSES AFFECTING *SAMBUCUS* SPP. PLANTS IN POLAND
H. Berniak, M. Kamińska
- P59 - EPIDEMIOLOGICAL STUDIES ON LETHAL REDNESS DISEASE OCCURRING IN STRAWBERRY CROPS IN ARGENTINA
F. Fernandez, N. Meneguzzi, D. Kirschbaum, E. Galdeano, V. Conci and L. Conci
- P60 - A VIRUS COMPLEX RESPONSIBLE FOR INCREASED EXPRESSION OF CRUMBLY FRUIT SYMPTOMS IN RED RASPBERRY ‘MEEKER’
D. F. Quito-Avila, R.R. Martin
- P61 - PLANT RNA ISOLATION AID™ HELPS TO EXTRACT HIGHER QUANTITIES OF TOTAL RNA FROM RASPBERRIES
I. Mavrič Pleško, M. Viršček Marn, M. Kovač, N. Toplak
- P62 - STUDIES OF RUBUS VIRUSES IN SLOVENIA
I. Mavrič Pleško, M. Viršček Marn, D. Koron
- P63 - SURVEY OF SLRSV, RPRSV, SMYEV AND SCRV IN STRAWBERRY FIELDS IN BELGIUM
T. Olivier, E. Demonty, K. De Jonghe, S. Morio and S. Steyer
- P64 - SPREAD OF *HOP LATENT VIROID* (HLVd) IN HOP GARDEN
P. Svoboda, J. Matoušek, J. Patzak,
- P65 - A NEW *FLEXIVIRUS* IDENTIFIED IN BLACKBERRY
S. Sabanadzovic, N. A. Ghanem-Sabanadzovic and I.E. Tzanetakis
- P66 - A NEW *EMARAVIRUS* DETECTED IN BLACKBERRIES AFFECTED BY YELLOW VEIN DISEASE
M. Hassan, K.E Keller, R.R. Martin, S. Sabanadzovic and I. E. Tzanetakis
- P67 - SENSITIVE DETECTION OF THREE BERRY FRUIT VIRUSES BY TAQMAN® qPCR
T. Ho and I. E. Tzanetakis
- P68 - GENOME ORGANIZATION AND SEQUENCE DIVERSITY OF A NOVEL BLACKBERRY *AMPELOVIRUS*
T. Thekke-Veetil, S. Sabanadzovic Keller, K.E., Martin R.R. and I. E. Tzanetakis

Poster session VI – Control strategies

P69 - PRELIMINARY RESULTS ON STUDIES OF RESISTANCE TO *PLUM POX VIRUS-D* IN *PRUNUS* IN ARGENTINA

D. B. Marini, R. J. Farrando, M. E. Ojeda, A. Dal Zotto

P70 - MONITORING OF FRUIT TREE PHYTOPLASMA VECTORS IN AN OVERWINTERING SITE

M. Navrátil, M. Horníková, P. Lauterer, V. Čermák, M. Starý, P. Válová, D. Šafářová

P71 - RECOVERY PHENOMENA IN APRICOT TREES CV. BERGERON INFECTED BY EUROPEAN STONE FRUIT YELLOWS IN THE PROVINCE OF TRENTO (ITALY)

C. Poggi Pollini, S. Franchini, M. Gobber, C. Lanzoni, C. Ratti

Poster session VII – Certification

P73 - CAV'S LABORATORY ACTIVITY WITHIN THE ITALIAN CERTIFICATION SCHEME OF PLANT PROPAGATION MATERIAL

R. Zisa, S. Botti and M. Cardoni

P74 - CIVI-ITALIA, THE ITALIAN NURSERY ASSOCIATION AND ITS ROLE WITHIN THE FRAMEWORK OF THE ITALIAN CERTIFICATION SCHEME

L. Catalano, R. Savini and D. Bologna

P75 - THE VOLUNTARY CERTIFICATION SCHEME FOR FRUIT PLANT PROPAGATING MATERIAL IN ITALY

P. Giorgetti, A. Sgueglia and S. Baralla

ORAL SESSION I
FRUIT TREES:
VIRUSES, VIROIDS AND PHYTOPLASMAS

Invited lecture

ADVANCES IN THE KNOWLEDGE OF SOME FUIT TREE VIRUSES

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Two enquiries for the identification of plant viruses ranking among the “top ten” in the world for their economical and scientific importance, carried out following the initiative of the late Dr. R.G. Milne (1988) and of the journal “*Molecular Plant Pathology*” (2011), included *Plum pox virus* (PPV) and *Apple chlorotic leaf spot virus* (ACLSV) in the list. The properties of both viruses are reviewed taking into account the recent developments in their knowledge. The agent of fig mosaic is a virus (FMV) with enveloped particles and a multipartite single-stranded negative-sense RNA genome. FMV is transmitted by eriophyid mites, the same as six similar viruses which are approved (FMV and *European mountain ash ringspot virus*) species or potentially putative members in the unassigned genus *Emaravirus*. Contrary to previous reports, the FMV genome was recently found to consist of six (-)RNA fragments, only three of which encode proteins with recognized functions. The family *Closteroviridae* comprises two fruit tree viruses [*Little cherry virus 2* (LChV-2), *Plum bark necrosis stem pitting-associated virus* (PBNSPaV)], classified as definitive species in the genus *Ampelovirus*, and one [*Little cherry virus 1* (LChV-1)], currently retained as an unassigned species to the family. Recently secured molecular information has prompted: (i) the revision of genus *Ampelovirus* with the identification of two subgroups, one of which contains LChV-2 and PBNSPaV; (ii) the establishment of a putative novel genus comprising LChV-1, for which the name *Velarivirus* has been proposed.

MOLECULAR ANALYSIS OF A ‘NEW’ *FOVEAVIRUS* ASSOCIATED WITH A FRUIT DEFORMING DISEASE OF APPLE

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There are several fruit deforming diseases of apple that appear to be virus related, but the specific causal agent(s) is unknown. Some of these include apple green crinkle, apple rough skin, and apple star crack disease. Susceptible apple cultivars are often severely affected by these diseases producing fruit that are deformed and unmarketable. A putative ‘new’ foveavirus was identified that may be associated with apple green crinkle disease (AGCaV). The genome of AGCaV consists of 9266 nucleotides, with an organization similar to *Apple stem pitting virus* (ASPV) the type species of the genus *Foveavirus*, family *Flexiviridae*. ORF1 of AGCaV encodes a replicase-complex polyprotein with a molecular mass of 247 kDa; ORFs 2, 3, and 4 (TGB proteins) are estimated at 25.1, 12.8, and 7.4 kDa, respectively; and ORF5 encodes the CP with an estimated molecular mass of 43.3 kDa. The virus has four non-coding regions (NCRs) that include a 5'-NCR (60 nts), a 3'-NCR (134 nts), and two intergenic (IG) NCRs - IG-NCR1 (69 nts) and IG-NCR2 (91 nts). AGCaV is closely related to ASPV, but appears to be a distinct foveavirus.

GENOMIC CONFORMATION OF *APPLE MOSAIC VIRUS* TURKISH ISOLATES COAT PROTEIN GENE REGIONS

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Apple mosaic virus (ApMV) is the most important virus infection of apple and hazelnut orchards in Turkey. Disease is destructive and epidemic on apple and hazelnut plantations, causes great growth reduction and yield loss. Major symptoms are yellowish white evident systemic mosaic on apple leaves and severe systemic mosaic, ring spots and oak leaf pattern on hazelnut. Hazelnut isolates were collected from the Black Sea region and the apple isolates from the major and important apple growing provinces and all were identified serologically and molecularly by using the primers of genes encoding the coat protein region of ApMV. Coat protein gene targets of Turkish isolates were sequenced and recently, 15 data (7 apple and 8 hazelnut) were present in NCBI databank. It was detected that single nucleotide polymorphisms were present in Turkish apple mosaic isolates. In phylogenetic analysis performed with isolates selected from different locations and from different hosts in the world, it was detected that Turkish apple and hazelnut isolates were located in different regions and hazelnut targets were different at the rate of 50% comparing the other isolates selected from NCBI Genbank. Unsignificant differences were present in amino acid sequences.

MOLECULAR CHARACTERIZATION OF *TOMATO RINGSPOT VIRUS* (ToRSV) STRAIN RESPONSIBLE FOR “PRUNE BROWNLIN” DISEASE

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Tomato ringspot virus (ToRSV) mainly infects different species of ornamental, fruit trees, and shrubs. ToRSV cause serious diseases in stone fruit, as that called „prune brownline“, whose main symptoms are a dark brown line at the graft union with pits and grooves in the woody tissue. In Chile this disease has been observed in plum cv. D‘Agen grafted on Myrobalan rootstock. ToRSV was detected by RT-PCR using specific primer pair designed by Griesbach (D1r: 5‘-TCCGTCCAATCACGCGAATA-3‘; U1f: 5‘-GACGAAGTTATCAATGGCAGC-3‘; 449bp). The viral isolate was transmitted and maintained in *Gomphrena globosa* L. Identification was confirmed by RT-PCR using a primer pair (ToRSV2r: 5‘-CTCACGTAAATGTATGGTTCC-3‘; ToRSV2f: 5‘-GACTGGAGGTTTGAGTGGC-3‘) that amplify a fragment of 330bp in the gene encoding for the coat protein (CP). Primers were designed for amplification of the entire gene encoding the CP of the virus (1687bp). The amplicons obtained were purified and cloned. Putative recombinant clones were analysed by colony-PCR using primers to vector sequences flanking the polylinker. Amplicons obtained from three colonies per cloned fragment were sequenced in both directions. Sequence analysis has been carried out using the partial (330bp) and complete (1687bp) CP sequences of the Chilean isolate (ToRSV Ch Gg). In both phenetic trees Ch Gg ToRSV is close correlated to one isolate from raspberry whose origin is USA, and is distant from those that induce “mosaic yellow peach buds disease“ (PYBM) and others isolates more commonly found in raspberries. This is the first molecular characterization of the entire gene encoding the CP of a ToRSV isolate causing “prune brownline“ disease.

INCIDENCE OF VIRAL DISEASES ON PEAR PLANTS AND THE MOLECULAR CHARACTERIZATION OF THREE PEAR VIRUSES IN CHINA

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During 2001-2011, field surveys were carried out to evaluate the incidence of viral diseases on pear plants grown in China. The most frequently found viral disease-like symptoms were chlorotic leaf spot, leaf ring spot, leaf vein yellowing and mosaic. At a few orchards, some trees re-grafted for variety replacement showed uneven leaf reddish, leaf curling and even decline of the tree. A few cultivars at some orchards showed serious fruit malformation with stone-pitting like symptoms. In order to understand the sanitary status of pear trees grown in China, a total of 410 pear trees from nine provinces and 18 orchards were tested by biological indexing and/or RT-PCR for the presence of *Apple chlorotic leaf spot virus* (ACLSV), *Apple stem grooving virus* (ASGV) and *Apple stem pitting virus* (ASPV). The infection rates of ASGV, ACLSV and ASPV were 70%, 57.4% and 43.2%, and over 95% tested pear varieties were infected by one or more those viruses. It was noticed that ACLSV and ASPV were usually associated with leaf ring spot and fruit stone-pitting, respectively. The CP and MP genes of 25 ASPV, partial CP genes (482bp, covering 62.7 % of its CP gene) of 45 ASGV, and CP genes of 22 ACLSV from pear plants were cloned and their sequences were analyzed. Results showed that ASPV isolates from pear plants were highly divergent and their CP and MP genes shared identities of 73.9%~99.5% and 76.5%-99.9% at nt level, respectively. Some ASPV isolates had continuous nucleotide acid inserts or deletion in their CP genes. The CP gene of ASGV isolates showed 86.7-99.4 % nt and 94.3-100 % aa identities. The CP sequences of ACLSV showed 87.3-100% identities at the nt level and 92.7-100% identities at the aa level. The phylogenetic relations of different isolates within each of those three viruses were analyzed, and it was found that the most ASPV isolates and ACLSV isolates from pear grown in China clustered in a group, which were separated from ASPV and ACLSV isolates from apple and other hosts. These results may contribute to a further understanding of molecular diversity and its implication in pathogenicity of pear viruses.

DETECTION AND IDENTIFICATION OF *PEAR BLISTER CANKER VIROID* OCCURRING IN PEAR TREES IN CANADA

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A survey was conducted in Canada for the presence of *Pear blister canker viroid* (PBCVd) in pear trees of different origin. Initial screening was performed in 2010 at the Agriculture and Agri-Food Canada Research Centre by tissue printing hybridization (TPH) using a digoxigenin-labeled riboprobe specific for detecting PBCVd. A total of 118 pear accessions were screened and two of them (Anjou Euwer and Clapp's Favorite Starkrimson) tested clearly positive. In 2011, 25 accessions, including the two accessions tested positive and 23 additional ones generating slight and doubtful hybridization signals in the first TPH analysis, were re-screened. The possible infection by PBCVd, or a close related viroid, of the accessions Anjou Euwer and Clapp's Favorite Starkrimson was confirmed. In contrast no hybridization signal was obtained from the other accessions. The conclusive identification of the infecting viroid was obtained at the CNR Istituto di Virologia Vegetale (Italy) and the Canadian Food Inspection Agency (Canada) by testing total nucleic acid (TNA) preparations from both TPH-positive accessions by RT-PCR, using PBCVd specific primers (Malfitano *et al.*, 2004, *Acta Horticulturae* 657: 367-371), and by direct sequencing the amplification products. Amplified cDNAs of the expected size obtained from the accessions Anjou Euwer and Clapp's Favorite Starkrimson accessions shared high sequence identity (94-97%) with PBCVd reference variant (NC_001830), conclusively showing that they are infected by this viroid. In contrast, TNAs from additional five accessions, selected among those testing negative to the TPH analyses, did not generate any amplification products when assayed by RT-PCR, confirming the absence of PBCVd in these samples. This is the first documented report of PBCVd in Canada and call for prompt extension of similar analyses to other pear cultivations in the country.

A REVIEW OF VIRUSES INFECTING *ACTINIDIA*

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Kiwifruit (*Actinidia deliciosa*) was introduced in New Zealand in 1904 from seeds imported from China. Since then seeds and scionwood have been imported to New Zealand from China, to increase the gene pool for breeding purposes. The first virus naturally infecting kiwifruit, *Apple stem grooving virus*, was identified following symptoms observed in imported plants growing in quarantine (2003). Three novel viruses, two vitiviruses, and a citrivirus closely related to *Citrus leaf blotch virus*, have since been identified from plants originating from the same importation. All belong to the family *Betaflexiviridae*. Members of the family *Bromoviridae*, *Alfalfa mosaic virus* and *Cucumber mosaic virus* (CMV), have been detected in the field. In New Zealand, these were mostly limited to *Actinidia guilinensis* and *A. glaucophylla* seedlings, while CMV was detected in *A. deliciosa* in Italy. These viruses are cosmopolitan, and weeds provide a reservoir for infection. From the same family, *Pelargonium zonate spot virus* (PZSV) has been detected in Italy associated with severe symptoms on leaves and fruit. Four viruses appear to have limited effect on kiwifruit. Two Tobamoviruses, *Ribgrass mosaic virus* and *Turnip vein clearing virus*, are probably present worldwide in kiwifruit and are also present in *Plantago* spp., a common weed in kiwifruit orchards. *Cucumber necrosis virus* family *Tombusviridae* has been detected in kiwifruit at very low titre without apparent symptoms. Additionally, a novel *Potexvirus* has been transmitted to herbaceous indicators from three kiwifruit plants, but direct detection from the original plants was unsuccessful. Finally, *Cherry leaf roll virus* (CLRV), family *Secoviridae*, has been detected in New Zealand on kiwifruit and also in *Rumex* spp. growing below the infected vines. Major symptoms were observed on the kiwifruit, including leaf spots, fruit malformation, reduced yield, bark cracking and cane wilting. The symptoms are similar to those observed in Italy from PZSV infection.

PELARGONIUM ZONATE SPOT VIRUS INFECTING KIWIFRUIT PLANTS IN ITALY

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Pelargonium zonate spot virus (PZSV) is the single member of the *Anulavirus* genus (Fam. *Bromoviridae*) first isolated from tomato in Italy and later reported also from Spain, France, USA and Israel. Up to now PZSV has known to naturally infect only herbaceous hosts as tomato, pepper, artichoke and common weeds often symptomless. Symptoms on leaves and fruits of infected tomato plants are characterized by line patterns, chlorotic and necrotic rings, together with plant stunting, leaf malformation and poor fruit set, which often result in plant death as infected cells show severe cytopathological alterations. The virus is transmitted by mechanical inoculation, grafting, and through seed by means of infected pollen carried on the bodies of thrips. In May 2011 plants of kiwifruit (*Actinidia chinensis*) cv. Hort16A, exhibiting viral symptoms, were observed in two orchards in Faenza province, Emilia-Romagna region. Symptoms include chlorotic and necrotic rings on leaves and depressed areas on the fruits with consequently deformation of the berries. The causal agent has been successfully transmitted to indicator plants, as *Chenopodium quinoa*, *Nicotiana benthamiana*, *N. glutinosa* and *N. tabacum*, by mechanical inoculation. Viral particles with diameter of about 30 nm were observed on viral purification obtained from *C. quinoa* infected leaves. Random amplification and sequencing of nucleic acids isolated from purified virus, allowed identification of a short segment showing high nucleotide identity with 5' end of PZSV RNA2. RT-PCR analyses, performed using PZSV primer pair, specifically identified the virus in all indicator hosts and in leaves and fruits collected from all symptomatic kiwifruit plants. The new PZSV isolate has been characterized by sequencing and by ultrastructural and immuno-transmission electron microscopy investigations. Moreover, in order to determine the effect of the virus on cellular water compartmentation, tissue metabolic activity and overall quality, fruits and leaves from infected and healthy plants have been compared by NMR measurements (Proton transverse relaxation time), isothermal calo-respirometry (metabolic heat production) and visual appearance by computer vision system.

**ASSOCIATION OF PHYTOPLASMAS AND ROD SHAPED BACTERIA WITH A
CITRUS DISEASE OF UNKNOWN ETIOLOGY IN THE STATE OF BAJA
CALIFORNIA SUR, MEXICO**

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Multiple symptoms of yellow-type diseases were observed in citrus trees from various citrus growing areas in the state of BCS. Leaf malformations, chlorosis, blotching and necrotic spots, yellowing of leaf veins and shoots, necrotic leafstalks and shoots, leaf dropping and dieback, these symptoms were reported for distinct citrus diseases worldwide and related with phytoplasmas and other vascular pathogen presence. To determine the possible causal agent/agents of these citrus maladies, samples from symptomatic lemon, sweet orange, mandarin and grapefruit trees were collected and processed for scanning electron microscope (SEM) analysis. Specimens from leaf midribs, leafstalks, young leaflets and stems from symptomatic plants were prepared and observed in Hitachi S-300N SEM. Both phytoplasmas and rod shaped bacteria were observed in phloem tissue of symptomatic plants. The most abundant phytoplasma presence was detected in the samples of mandarin (south of BCS) where bacteria were not detected. On the contrary, in samples of lemon and orange trees collected from northern BCS along with phytoplasmas, some rod-shaped bacteria were observed with specific smooth outer surface. In some sieve tubes their concentration was very high. Phytoplasma size ranged from 500 to 1,000 nm, and bacterial sizes reached 3,000 x 500 nm, similar to former prokaryote sizes reported in the case of citrus greening. A very high level of phytoplasma (but not of rod shaped bacteria) presence was registered also in acacia species, as well as in some herbaceous plants growing in the same plots, which suggests that these species could be wild hosts of this pathogen. In some lemon and orange samples phytoplasma detection was achieved also by nested PCR technique using phytoplasma specific primers. The work is in progress to confirm all the cases of phytoplasma infection detected by SEM, to identify both pathogens and prove that the mixed infection of the two prokaryotes could be at the basis of the disease etiology.

DETECTION OF VIRUSES INFECTING APPLE IN NORTH WESTERN HIMALAYAN REGION

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Apple is cultivated on a commercial scale in the hill state of Himachal Pradesh situated in the North Western Himalayan region of India. Economy of Himachal Pradesh is largely dependent on apple cultivation. Of late, viruses have been observed to be a major constraint in declining apple productivity in the state. Infection of Apple chlorotic leaf spot *trichovirus* (ACLSV), Apple stem pitting *foveavirus* (ASPV), Apple stem grooving *capillovirus* (ASGV) and Apple mosaic *ilarvirus* (ApMV) has been invariably associated with commercial apple plantations of Himachal Pradesh with an incidence as high as 95 percent. Chlorotic spots turning into necrotic lesions on apple leaves were found to be the predominant symptoms. An orchard with a very high incidence of viral diseases (95 percent) was selected for conducting biological and serological detection of apple viruses. Serological detection through DAC and DAS-ELISA resulted in the detection of ACLSV, ASPV, ApMV and ASGV in the form of mixed infection in all the samples of 10 cultivars randomly marked for assays. Biological detection of one of the isolate containing mixed infection of ASGV, ACLSV, ASPV and ApMV on herbaceous hosts resulted in the production of symptoms on *Chenopodium quinoa*, *Phaseolus vulgaris*, *Nicotiana glutinosa* and a few other hosts. Detection on woody indicators (Virginia crab, *M. platycarpa*, Spy 227, Jay Darling and Russian Clone) under field conditions through double grafting, grafting cum budding and double budding of inoculators and indicator budwood resulted in the production of typical viral symptoms on leaves in *M. platycarpa*, Spy 227, Jay Darling and Russian Clone whereas swelling and necrotic symptoms were produced at the graft union in case of Virginia crab and Spy 227 indicators, respectively. Periodic detection of these viruses revealed that leaf samples drawn during March to May months were found suitable for the ELISA of ASGV, ACLSV and ASPV whereas petals were the best source for ApMV detection. Association of ASGV infection in an apple tree was also confirmed by RT-PCR assay. Phylogenetic analysis of the coat protein gene of the Shimla ASGV isolate revealed its 100 per cent sequence similarity with Serbian isolate. Serological indexing for virus free clones resulted in the selection of 20 trees of 11 cultivars free from infection of ASGV, ACLSV, ASPV, ApMV and tomato ringspot virus (ToRSV) in ELISA test of 60 symptomless trees of 20 cultivars. These virus tested trees have been selected to serve as important mother trees for use in raising healthy nursery plants.

PHYTOPLASMA DETECTION AND IDENTIFICATION IN KIWI PLANTS AND POSSIBLE CORRELATION WITH *PSEUDOMONAS SYRINGAE* PV *ACTINIDIAE* PRESENCE

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Recent findings indicated that in kiwi plants showing severe symptoms of canker disease phytoplasmas and *Pseudomonas syringae* pv *actinidiae* (Psa) were identified. Surveys were then carried out during 2011 in kiwi plantations located in North and Central Italy to verify the presence of phytoplasmas and Psa. Analyses carried out on samples collected in May from plants showing severe symptoms of twig dieback and cankers Psa and phytoplasmas were identified. Psa isolation was carried out in NSA medium followed by HR tests in tobacco and by PCR with specific primers while phytoplasma detection was carried out by nested-PCR assays on phloem tissues. Among the samples collected from 12 plants five resulted positive for Psa presence while in 11 samples phytoplasmas belonging to 16SrXII (stolbur), 16SrI (aster yellows) or 16SrX (apple proliferation) groups in some cases in mixed infection with 16SrV (elm yellows) group were identified. Further 50 samples were collected in October only in North Italy from 2 plantations of cv. Jintao and one of cv. Hayward. Plants showed in some of the branches premature reddening of leaves downward curled and crinkling; the green variety (Hayward) show also leaf reddening while in cv. Jintao (yellow) there was a general plant decline and yellowing of the leaves. Scattered plants also showed other symptoms such as irregular shape of non lignified branches and reddening shoot proliferation from the rootstock. These samples showed preliminary negative results for Psa presence while phytoplasma presence was confirmed mainly in the cv. Jintao where groups 16SrI, 16SrXII and 16SrX were identified in single or in mixed infection. These latter results also confirm previous reports (1999) indicating presence of stolbur phytoplasmas in Liguria (Italy). Further work is in progress to evaluate possible interaction of the two prokaryotes in infected plants and phytoplasma influence in the epidemic of canker disease.

ORAL SESSION II
PLUM POX VIRUS

AN ATYPICAL ALBANIAN ISOLATE OF *PLUM POX VIRUS* COULD BE THE PROGENITOR OF THE MARCUS STRAIN

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In the frame of the efforts supported by the European FP7 SharCo project, PPV isolate AL-11pl, found in a naturally infected plum (*Prunus domestica*) in Çërravë, Eastern Albania, was submitted to molecular analysis. RNA was extracted from infected leaves by silica capture and used for cDNA synthesis by random primers and M-MLV RT (Invitrogen). cDNA was amplified by Taq DNA polymerase (Fermentas) in several fragments ranging about 1200bp in average. Purified PCR products were directly sequenced on both strands using the respective PCR primers. Alternatively, some PCR products were ligated into the pSC-A vector (Stratagene) and recombinant plasmids were sequenced. The complete genomic sequence of the AL-11pl was assembled from a set of overlapping PCR fragments. AL-11pl genome consists of 9786nt, excluding the 3' terminal poly (A) tail. The organization is identical to previously sequenced PPV isolates. A start codon (AUG) was identified at positions 147–149, and an amber stop codon at positions 9567–9569, resulting in a single ORF consisting of 9420 nt. Sequence comparisons indicate that the closest isolate is Turkey (Ab-Tk), with a divergence of 6.5%. Divergence with PPV-M is 7.7% (PPV-PS) to 7.9% (PPV-SK68) while other strains are more distantly related: 13.8% (Rec, PPV-BOR3), 15.5% (PPV-D isolates), 20.3% (PPV-EA), 21.2% (PPV-W) and 21.6% (PPV-C). Molecular characterization of AL-11pl shows a high divergence with PPV-M in 1-2688 region, high divergence with PPV-Tk in 1-1584 region, but high homology with PPV-Tk in 1584-2688 region. This result demonstrates that AL-11pl has the expected features for an PPV-M ancestor in an evolutionary scenarios described by Glasa & Candresse (2005). AL-11pl can thus be considered as a putative further strain of PPV, for which PPV-An (Ancestor) name is proposed. *The research leading to these results has received funding from the European Community's Seven Framework Programme (FP7/2007-2013) under Grant Agreement n°204429, SharCo project*

EPIDEMIOLOGY OF *PLUM POX VIRUS-T* AND *-M* ISOLATES IN STONE FRUIT ORCHARDS IN TURKEY

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Although *Plum pox virus* (PPV) was introduced in Turkey 44 years ago, the virus is present in a rather limited number of trees. Our recent results on the PPV epidemiology show that PPV was introduced rapidly in new PPV-free regions. The epidemiology of PPV was studied in a previously (Aegean-Izmir) and the more recently infested (Mediterranean-Antakya) regions from 2009 to 2011. Aphid populations were monitored from the first week of April to the end of June by sticky-plant method. Aphids collected from plum and apricot trees were tested individually by squash real-time RT-PCR. The highest aphid populations were observed at the end of May in both regions but the aphid species landing on the plants were different. The most abundant aphid species caught were *Myzus persicae* (20.15 %), *Hyalopterus pruni* (18.64 %) and *Aphis craccivora* (9.04 %) in Izmir and *Aphis gossypii* (20.55 %), *Aphis spiraecola* (19.38 %) and *Hyalopterus pruni* (13.36 %) in Antakya. Among the aphid species caught in Izmir, the percentage of PPV-viruliferous aphids resulted 40, 34, 25 and 9 in *M. persicae*, *H. pruni*, *A. gossypii* and *A. craccivora* aphid species, respectively. However, in Antakya infection rates were 41, 25, 21, 18 and 16 %, in *M. persicae*, *A. spiraecola*, *A. gossypii*, *H. pruni* and *A. craccivora*, respectively. This is the first report about epidemiological aspect of PPV in two different ecological regions of Turkey.

PLUM POX VIRUS W APPEARS TO BE THE MOST VARIABLE STRAIN OF THE SEVEN RECOGNIZED STRAINS OF THE VIRUS

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To date there are seven recognized strains of *Plum pox virus* (D, M, Rec, EA, C, W, and T). These strains vary in their distribution, and in their biological properties such as transmissibility and symptomatology. They vary also in their serological properties and in their genetic profile which allows the development of strain specific protein-based assays (such as ELISA), and nucleic acid-based assays (such as RT-PCR), respectively. Sequence analyses indicate unusually high genetic variability among isolates of PPV strain W. Recently 14 new isolates of PPV W were identified in Russia. Natural hosts included *Prunus domestica* (plum), *P. spinosa* (blackthorn), *P. nigra* (Canadian plum), and *P. tomentosa* (downy cherry). Symptoms were observed in some infected hosts, while in other cases infected hosts were symptomless. Phylogenetic analysis of the (C-ter)NIb-(N-ter)CP region (nt and deduced aa) of these 14 isolates, plus the published sequences of PPV W isolates from Canada, Latvia, and the Ukraine, revealed 7 distinct clades supported by high bootstrap values. Interestingly, though genetically diverse, all 14 new Russian PPV W isolates were detected by the strain specific primers 3174-SP-F3/3174-SP-R1.

PRELIMINARY EVALUATION OF THE COMPETITIVENESS OF PPV-REC AND PPV-D UNDER FIELD CONDITIONS

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PPV-D, PPV-M and PPV-Rec are the three main strains of *Plum pox virus* (PPV), the most detrimental viral pathogen of stone fruits (*Prunus* sp.). While different experimental results reported that PPV-M is more aggressive than PPV-D, limited information are available about the competitiveness of PPV-Rec relatively to PPV-D or PPV-M. To provide this information, we monitored the PPV-Rec and PPV-D spreading in an experimental plum orchard (577 trees) located in Bistrita, Romania. The dynamic of spread of the two strains was monitored during four vegetative periods (2008-2011). The sanitary status of the trees was assessed visually and by ELISA testing each year. In 2008, the strain status of each PPV isolate was determined by IC-RT-PCR using strain specific primers located in the (Cter) CP, (Cter) NIB – (Nter) CP and the 6K1-CIP coding regions. From 2009 to 2011, strain typing was performed for every new disease case and for all single strain infections detected in the previous years, in order to check for possible over infection with the other strain. Disease incidence increased from 57 % (328/577) in 2008 to 72 % (414/577) in 2011. From a total of 328 infected trees in 2008, 58% of the trees were infected by PPV-Rec, 13 % by PPV-D and 29% were co-infected by PPV-D and PPV-Rec. The results on the rate of progression of PPV-D and PPV-Rec as well as the frequency of co-infections will be presented and a preliminary conclusion upon the competitiveness of PPV-Rec and PPV-D in our conditions will be discussed.

The research leading to these results has received funding mainly from the European Community's Seven Framework Programme (FP7/2007-2013) under Grant Agreement n°204429, SharCo project, and also by the Romanian Research Ministry, contract 34/2009.

COMPLETE AND PARTIAL GENOME SEQUENCES OF THE UNUSUAL *PLUM POX VIRUS* (PPV) ISOLATES FROM SOUR CHERRY IN RUSSIA SUGGEST THEIR CLASSIFICATION TO A NEW PPV STRAIN.

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Plum pox virus (PPV), the agent responsible of the sharka disease, is characterized by a wide genetic diversity and currently known PPV isolates are assigned to 7 molecularly different PPV strains. Despite substantial progress in the ability to assess and describe PPV variability, our understanding of PPV variability and of the geographical distribution of this variability is still incomplete, in particular from the geographical regions which have so far been little surveyed. In the frame of the efforts supported by the European FP7 SharCo project, a set of unusual PPV isolates (RU-17sc, RU-18sc, RU-19sc, RU-20sc) originated from old sour cherry plantation grown in the cultivar collection of the Horticultural research institute (Kuibyshev region) and from sour cherry trees in a private gardens (Krasnoyarsk region) in the Samara oblast, Russia were analyzed. Besides being recovered from an atypical *Prunus* host, these isolates have the additional originality of not being detected in ELISA by the universal 5B monoclonal antibody (Mab). Partial sequence analysis targeting the N1b-CP region of the genome of 4 isolates (RU-17sc, RU-18sc, RU-19sc, RU-20sc) revealed about 30% nucleotide sequence divergence with conventional PPV-M, D and Rec isolates. Phylogenetic analysis clearly assigned these isolates to a distinct cluster, most closely related to PPV-C and PPV-W. The deduced aminoacid sequence of the N-terminus of the capsid protein revealed a D94>E aminoacid substitution probably responsible for the failure to react with the 5B Mab. Complete genome sequence comparisons showed that the RU-17sc and RU-18sc isolates has only 73-80.6% identity with other sequenced PPV strains. These results suggest that these unusual isolates belong to a new PPV strain able to infect cherry, for which the name PPV-CR (Cherry Russian) is proposed.

A LARGE SCALE STUDY OF *PLUM POX VIRUS* GENETIC DIVERSITY AND OF ITS GEOGRAPHICAL DISTRIBUTION

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The understanding of the geographic distribution and dynamics and of the genetic variability of *Plum pox virus* (PPV) populations on a worldwide scale is a prerequisite for an efficient management and control of the Sharka disease. An international effort supported by the European FP7 SharCo project has been ongoing with the aim to provide a realistic view on the current diversity of PPV worldwide, by the analysis of a large number of natural field isolates, collected following a standardized sampling protocol. For about 800 PPV isolates from 27 countries and the corresponding epidemiologically relevant data (information on locality, host, symptomatology...) were systematically collected. Isolates were preserved in lyophilized form in a centralized collection and partial sequence data were generated for two highly informative genome portions i.e. P3-6K1 and N1b-CP, together with the complete genome sequences of 35 epidemiologically or molecularly interesting PPV isolates. Besides providing information on the presence of particular strains in countries from which they were previously unreported, the results obtained provide a detailed cartography of the prevalence of PPV strains in the numerous sampled countries or host plants. Analysis of the partial sequence data indicates a higher than previously expected genetic diversity within the PPV-D strain and confirms the splitting of the PPV-M strain into two subclusters. Moreover, divergent isolates have been identified in the PPV-D, M, Rec, T and W strains and are currently undergoing full-length genomic sequencing. The detailed synthesis of the results obtained on these various aspects will be presented and discussed.

ORAL SESSION III
DIAGNOSIS AND
NEXT GENERATION SEQUENCING

Invited lecture

NEXT-GENERATION SEQUENCING: APPLICATIONS FOR THE DETECTION AND IDENTIFICATION OF PLANT VIRUSES AND VIROIDS

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Current molecular detection methods of plant viruses and viroids allow the detection of characterized pathogen species or specific strains but are limited in detection of uncharacterized ones although methods can be modified through the use of degenerate primers for PCR or RT-PCR or the use of pathogen species or genus universal probes for microarrays to enhance utility. The recent advent of the high throughput next-generation sequencing and sophisticated bioinformatics have overcome the above limitation to support a commonly accepted notion that many viruses and viroids still await discovery. Next-generation sequencing describes platforms that produce large amounts (typically millions to billions) of short DNA sequence reads, length typically between 25 and 400 bp. These reads are shorter than the traditional Sanger sequence reads (750 bp). The Roche model 454 GS 20 was the first commercial platform to be released in 2006; it produced 20 Mbp per run. This was replaced by the GS FLX model in 2007 which is capable of producing over 100 Mbp of sequence in just over 4 hours, which increased in 2008 to 400Mbp. The current Roche 454 GS-FLX+ Titanium sequencing platform is capable of producing over 600Mbp of sequence data in a single run with sequence read lengths of up to 1,000 bp. Two major companies compete now with Roche: Illumina and SOLiD. In 2006 Illumina introduced the GAIIx sequencing platform which generated up to 50 Bbp of data per run. It was replaced in subsequent years by the HiSeq platforms of which the 2012 HiSeq 2500 model has an output of 800 Bbp per run with a maximum read length of 200 bp. SOLiD platforms have been commercially available since 2008. The SOLiD 4 system yields 7 Bbp data per day with read length of 50 bp, the 5500 System generates upto 10-15 Bbp/per day with read length of 75 bp, the 5500xl platform yields 20-30 Bbp per day with read length of 75 bp, and the 3 plus platform yields 60 Bbp data per run. Currently a limited number of plant viruses and viroids have been identified from infected tissues using next-generation sequencing. Either total nucleic acid or total dsRNA from virus infected tissue was isolated and sequenced; in some cases host nucleic acid was partially eliminated by hybridization to material from healthy plants to enrich for virus sequence from infected plant material prior to sequencing. Plant viruses or viroids can also be detected indirectly by their short (21-24 nt) interfering RNAs (siRNA) synthesized in the host in response to pathogen infection. Next-generation sequencing can provide thousands to millions of siRNA sequences from infected plant material. A number of novel viruses have been discovered in cultivated as well as wild plants using next-generation sequencing. All known and unknown viruses detected using this technology have been termed "virome". A novel viroid has been recently discovered in grapevines by this technology. Next-generation sequencing in combination with bioinformatics offers new opportunities to detect and identify known and unknown plant viruses and viroids, even at extremely low titers and/or symptomless infection.

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DIRECT METHODS OF SAMPLE PREPARATION FOR DETECTION OF FRUIT TREE VIRUSES AND VIROIDS BY REAL-TIME RT-PCR AMPLIFICATION

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Biological indexing and serological methods are widely used in fruit tree viruses and viroids detection. Nevertheless, molecular techniques have revolutionized plant pathogen detection and identification and in particular real-time RT-PCR which is widely known as the most sensitive and reliable one. However, conventional real-time RT-PCR requires a RNA purification step that makes this procedure tedious, costly, time-consuming and not suitable for large-scale analyses. Fast and simple sample preparation procedures have been developed and patented for real-time amplification of fruit tree viruses and viroids. Three direct methods of sample preparation prior to molecular analyses were compared and validated using *Plum pox virus* (PPV) and *Peach latent mosaic viroid* (PLMVd) as models. Two of these methods are based on the use of crude plant extracts: i) spotting of crude extracts on a nylon positively charged or Whatman membrane, and ii) dilution of crude extracts in extraction buffer. The third method avoids preparation of crude extracts: iii) tissue-print or squash that consists in pressing plant tissues on a piece of membrane. These three sample preparation methods can be used to store immobilized targets, either frozen or at room temperature, for long time periods without decreasing the accuracy of detection. This new methodology was compared with conventional real-time RT-PCR and hybridization for PPV and PLMVd detection, respectively. Statistical parameters such as kappa Cohen index, likelihood ratios and predictive positive and negative values were used as diagnostic parameters for validation purposes. Direct sample preparation methods, combined with real-time RT-PCR proved to be very sensitive, specific and accurate. Two complete commercially available kits for PPV or PLMVd detection by real-time RT-PCR, based on immobilized targets, were successfully evaluated.

VALIDATING PCR PROTOCOLS FOR THE DETECTION OF STRAWBERRY VIRUSES IN AUSTRALIA

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To assist the integration and implementation of the PCR assays into pathogen testing protocols for production of certified strawberry runners in Australia, a virus inoculated “dummy nucleus” (“DN”) of two strawberry varieties was created in December 2008. The “DN” was maintained in a temperate climate in a similar manner to the Victorian nucleus collection and used to validate PCR assays for the detection of *Strawberry mottle sadwavirus* (SMoV), *Strawberry crinkle cytorhabdovirus* (SCV), *Strawberry mild yellow edge potexvirus* (SMYEV), *Strawberry vein banding caulimovirus* (SVBV), *Beet pseudos yellows crinivirus* (BPYV), and *Strawberry pallidosis associated crinivirus* (SPaV). In 2010/11 the “DN” was also replicated in a subtropical climate in Queensland. The “DN” plants were tested once each year by biological indexing and were tested monthly PCR. PCR testing of the 2008/09 Victorian “DN” showed that viruses were not reliably detected in strawberry plants during the first six months post-inoculation. Reliable detection was only achieved during the following seasons and the rate of virus transmission from mother to daughter plants can reach 100%. Virus testing during spring (October-December) of the Victorian “DN” plants during the 2009/10 and 2010/11 was the most reliable time for virus detection. In some years autumn (March-May) may also be a reliable time for virus detection by PCR. The results for the Queensland “DN” collection during the 2010/11 season indicated that there may not be a trend for the timing of detection of all virus species and no single month or season appears was more reliable than another for virus detection. The results from Victoria and Queensland indicated that biological indexing is less reliable for virus detection than PCR techniques as many of the inoculated indicators that were expected to show symptoms were symptomless even though viruses could be detected by PCR. However, the PCR tests were also not 100% reliable because there were only a few months of the year in which viruses were detected in all known infected plants. This highlights the importance of timing for testing and using a combination of molecular and biological tests for certification.

CHARACTERIZATION OF DIVERGENT *PLUM BARK NECROSIS STEM PITTING-ASSOCIATED VIRUS* ISOLATES AND DEVELOPMENT OF A POLYVALENT PBNPaV-SPECIFIC DETECTION TEST

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The plum bark necrosis/stem pitting disease was first reported in California in 1986 on *Prunus salicina* cv. Black Beaut. The putative causal agent was later characterized and identified as *Plum bark necrosis and stem pitting-associated virus* (PBNPaV), belonging to the *Ampelovirus* genus of the *Closteroviridae* family. This virus is now reported to infect many stone fruit species in European countries but also, more recently in China. To better understand PBNPaV diversity, we have pyrosequenced the complete genomes of divergent PBNPaV isolates from 3 sources: a Japanese plum (*Prunus salicina*) collected in France but from uncertain origin; a Japanese plum (*P. salicina*) affected by the Peach red marbling disease (PRM) and a peach (*P. persica*) from the Nanjing region in China. The genomic RNAs of these divergent isolates show the same genomic organization than the PBNPaV reference isolate. The less divergent isolates, found in the Japanese plum and in the PRM source, are closely related to each other but only about 82.8% identical to the PBNPaV reference isolate. On the other hand, they are highly homologous with two recently described PBNPaV divergent isolates from China (98.9-100% aa identity in the partial HSP70 region). The most divergent isolate, from the Chinese peach source, shows only about 71.2% identity to other PBNPaV isolates and is not detected by currently available PBNPaV detection techniques. The complete sequencing of these divergent isolates allowed the identification of a conserved genomic region in the P61 gene and the development of a new primer pair with improved polyvalence. Taken together these results indicate a much broader diversity of PBNPaV than previously thought and provide for a more robust detection of this still poorly characterized pathogen.

A FIRST EVALUATION OF siRNA NEXT-GENERATION SEQUENCING FOR DETECTION AND CHARACTERIZATION OF *PRUNUS VIRUSES*

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The plant post-transcriptional gene silencing antiviral defense mechanism results in the generation by plant Dicer-like enzymes and in the accumulation of small, 21-24 nucleotides long RNAs derived from viral genomes. These so called small interfering RNAs (siRNAs) can be sequenced with an extremely high throughput using the Illumina next generation sequencing technology. Analysis of such siRNAs populations has been reported to allow the efficient detection of plant viruses in samples of unknown sanitary status. We have evaluated this strategy on a few multiply infected *Prunus* samples. While high numbers of siRNAs could be readily sequenced, only a low proportion of siRNAs of viral origin could be identified. On the contrary, high amounts of siRNAs derived from the genome of Peach latent mosaic viroid were detected, allowing the reconstruction of the whole genome of the isolate present. Various strategies were used to analyse the virus-derived siRNAs, allowing an effective assessment of the sanitary status of the *Prunus* samples analysed but showing only a partial ability to reconstruct viral genomes without a priori information.

USE OF 454 PYROSEQUENCING FOR THE FAST AND EFFICIENT CHARACTERIZATION OF KNOWN OR NOVEL VIRAL AGENTS IN *PRUNUS* MATERIALS

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While detection of a well-known virus in *Prunus* material is now relatively straightforward, the task of performing a full indexing for the presence of any known or novel viral agent remains a challenge, due to the wide variability of plant viruses, the large number of viruses infecting *Prunus* crops and the fact that many of them remain uncharacterized. Besides biological indexing, which has broad polyvalence but is lengthy and labor/cost intensive, there is therefore a need for novel approaches for the detection, without any prior information, of all viruses present in a given *Prunus* host. Novel sequencing technologies, which generate massive amount of data at a fraction of the cost and effort of previous ones, offer great opportunities for virus identification and characterization. We have developed an indexing strategy based on the pyrosequencing of purified double stranded RNAs. The procedure, from dsRNA purification to random amplification prior to sequencing, has been streamlined and can be performed in a few days using only basic laboratory equipment. A strategy for the analysis of the large amounts of sequence data produced has also been developed. An automated data processing pipeline is being developed, to allow data analysis in laboratories with limited bioinformatics expertise. Thanks to a multiplexing approach, the analysis currently costs 300-400 euros per sample, including salaries, which compares favourably with the full cost of a biological indexing. Using this strategy, we have evidenced multiple infections in *Prunus* materials, implicating both well known viruses (PPV, PNRSV ...) and novel viral agents. The procedure takes a few days and appears robust, although further testing is clearly needed. It should find applications both in the research laboratory for the fast characterization of novel viruses and in plant quarantine, germplasm collections and certification schemes for the validation of the health status of precious *Prunus* material.

THE STUDY OF PLANT VIRUS DISEASE ETIOLOGY USING NEXT-GENERATION SEQUENCING TECHNOLOGIES

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The use of next-generation sequencing (NGS) has been established as a reliable approach to study the genetic composition of an array of environmental samples. This technology has been applied in numerous studies ranging from microbe populations in the oceans, to the virus complexity of grapevine diseases. In plant pathology NGS technologies have not been limited to metagenomic sequencing for pathogen discovery, but have also been used for other applications such as the study of plant-pathogen interactions. In this study, we used a metagenomic sequencing approach on dsRNA to elucidate the virus etiology of a variety of diseased fruit crops with NGS. Samples with known disease profiles were selected for this proof of principle experiment. Double-stranded RNA was extracted from the cambium tissue of virus diseased apple, citrus and grapevine plants with a cellulose extraction protocol and sequenced in an unbiased manner using the Illumina sequencing-by-synthesis technology on a HiScanSQ. The apple sample contained multiple latent viruses and was routinely used as a positive control in ELISA diagnostics, the citrus sample displayed typical apple stem grooving virus symptoms and the grapevine samples, typical leafroll disease symptoms. Approximately 3 million reads for each sample was generated and used in the *de novo* assemblies. Assembled contigs were subjected to BLAST searches against the NCBI non-redundant DNA and protein databases to identify and classify contigs according to the sequence they aligned to with the highest bit score. For each of the samples the virus profile could be confirmed as determined through other techniques. In the apple sample the expected viruses (ASPV and ACLSV) could be identified as well as an unknown mycovirus whereas in the citrus sample two genetic variants of CTV could be identified as well as the expected ASGV. For the two grapevine samples multiple virus infections from different genetic variants (GLRaV-3, GVA) could be identified including unknown mycoviruses. The results of this proof of principle experiment clearly indicate that the metagenomic sequencing approach of dsRNA can be successfully implemented to study plants with unknown virus etiology.

ORAL SESSION IV
HOST-PATHOGEN INTERACTION

Invited lecture

VIROIDS: BIOLOGY AND MOLECULAR BIOLOGY OF THE SMALLEST PLANT PATHOGENS

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Viroids are the causal agents of diseases in plants relevant for food consumption (potato, tomato, cucumber, hop, coconut, grapevine, subtropical and temperate fruit trees like citrus, avocado, peach, plum apple and pear) and for ornamental purposes (chrysanthemum and coleus). Some viroids are essentially restricted to their natural hosts, but others have expanded host ranges. While certain viroids cause alterations in leaves, stems, bark, flowers, fruits, seeds, and reserve organs—as well as delays in foliation, flowering and ripening—others have devastating consequences. Some viroids only induce symptoms in a particular organ (bark or fruit), whereas others have more general effects. Infections caused by quite a few viroids result in very mild or no discernible symptoms, being the lack of symptoms common in naturally-infected wild plants that can serve as reservoirs. Symptom expression is favored by high temperature and light intensity, explaining why viroids mainly affect plants grown in warm climates (and in greenhouses), and why curing some viroid infections is refractory to thermotherapy. Vegetative propagation of infected material is the most efficient dissemination route for viroids, and has probably caused that certain grapevine and citrus cultivars contain mixtures of different viroids. Most viroids are also transmitted mechanically and some through seed or pollen. Despite many of the symptoms described above being similar to those incited by viruses, viroids have unique structural, functional and evolutionary features: they are single-stranded, circular RNAs of just 250-400 nucleotides, without protein-coding ability but endowed with autonomous replication. Thus, viroids depend almost entirely on host factors to complete their infectious cycle, and can be essentially regarded as parasites of their host transcriptional apparatus while viruses mostly parasitize the translation machinery of their hosts. Viroids are grouped within the families *Pospiviroidae* and *Avsunviroidae*, whose members replicate in the nucleus and chloroplast, respectively. Viroids replication occurs through an RNA-based rolling-circle mechanism involving synthesis of longer-than-unit strands catalyzed by host DNA-dependent RNA polymerases (redirected to transcribe RNA templates), processing to unit-length (mediated by hammerhead ribozymes in the family *Avsunviroidae*), and circularization. Following infection, the viroid RNA moves to its replication organelle, with the resulting progeny then invading adjacent cells through plasmodesmata and reaching distal parts via the vasculature. Viroids are targets of the RNA silencing machinery of their hosts, and some might trigger their pathogenic effect through this route. Finally, viroids most likely have a very ancient evolutionary origin (fulfilling the paradigm that the simplest is the oldest), with the mutation rate estimated for a chloroplast-replicating viroid being the highest reported for any biological entity.

PEACH LATENT MOSAIC VIROID: DATA SUPPORTING THE INVOLVEMENT OF RNA SILENCING IN PEACH CALICO (EXTREME CHLOROSIS)

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Peach calico (PC), an extreme chlorosis (albinism) of peach leaves, stems and fruits, is induced by sequence variants of the chloroplast-replicating *Peach latent mosaic viroid* (PLMVd, genus *Pelamoviroid*, family *Avsunviroidae*) harboring a characteristic insertion of 12-14 nt that assumes a hairpin conformation. Here, we show that the primary and not the secondary structure of the inserted hairpin plays a major role in PC, a finding consistent with the hypothesis that PC could be elicited by an RNA silencing mechanism. More specifically, viroid-derived small RNAs (sRNAs) of 21-24 nt generated by the host response against invading nucleic acids, may target for cleavage cell mRNAs thereby triggering a signal cascade eventually resulting in PC. To test this hypothesis, we have performed deep sequencing (Illumina) of sRNA libraries from healthy, PC-expressing and latently-infected peach leaves. Then, by semi-quantitative RT-PCR and RNA 3' ligase-mediated rapid amplification of cDNA ends (RACE) we have determined that two PLMVd-derived sRNAs, containing the PC-associated insertion and exclusively accumulating in albino tissues, target for cleavage the mRNA encoding the chloroplastic heat-shock protein 90 (cHSP90). These data are the first direct evidence that a viroid may modify host gene expression of its natural host via RNA silencing. Moreover, since cHSP90 participates in chloroplast biogenesis and plastid-to-nucleus signal transduction in *Arabidopsis*, down-regulation of this protein is consistent with the chloroplast malformations previously reported in PC-expressing tissues, and strongly supports the involvement of RNA silencing in PC. Whether symptom elicitation driven by RNA silencing is a particular case or reflects a general situation in viroid pathogenesis remains to be addressed. Since our study has also identified many other peach mRNAs potentially targeted by PLMVd-sRNAs, RNA silencing might have additional roles in the plant-viroid interplay, including viroid evolution and adaptation.

PEACH LATENT MOSAIC VIROID: FURTHER INSIGHTS ON A DETERMINANT ASSOCIATED WITH PEACH MOSAIC

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Peach latent mosaic viroid (PLMVd) displays an unusual pathogenic behavior. While consistent with its name most isolates do not incite foliar symptoms, some incite peach mosaic (PM) or an extreme albinism (peach calico, PC). PLMVd variants inducing PC have a characteristic insertion of 12-13 nt and accumulate in symptomatic leaf sectors but not in the adjacent green sectors. Based on these two findings and on additional experimental data (including deep sequencing of viroid-derived small RNAs) we have recently proposed that PC could be mediated by RNA silencing. Extending similar analyses to PM first entails identification of the molecular determinant of PM, a question posing serious difficulties because it is not associated with a specific insertion. Moreover, contrasting with PC that is a well-defined syndrome, PM varies very much in intensity and distribution. After examining different PLMVd-PM isolates over the years, we have focused on a few of them. This communication reports results obtained with one of such isolates, recovered from the peach cultivar O'Henry grown in Aragón (Spain), expressing an intense mosaic in leaf sectors intermingled with asymptomatic sectors. Previously, RNAs extracted from both sectors of the same leaves were used to generate by RT-PCR full-length PLMVd-cDNAs that were cloned and sequenced. Their multiple alignment revealed specific changes correlating with symptoms. Two clones (Ab4 and Ab7), differing in a single substitution, served for producing dimeric head-to-tail inserts and then *in vitro* transcripts that were mechanically inoculated onto GF-305 peach seedlings. Intriguingly, while Ab4 incited strong chlorotic symptoms in most leaves, Ab7 only incited mild lesions, providing a first insight of a PLMVd nucleotide directly involved in PM. Moreover, RT-PCR amplification, cloning and sequencing showed that the differential nucleotide substitution was preserved in most of the clones from the symptomatic sectors, but no in those from the surrounding green sectors.

DISORGANIZED VIROID DNA FRAGMENT IS TRANSCRIBED IN PLANT

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Viroids have been adopted as a good model for discovering new biological principles and offering perspectives to continue advancing some frontiers of life science. Their disorganized DNA fragment being infectious have been reported in the viroids of *Potato spindle tuber viroid* (PSTVd), *Hop stunt viroid* (HSVd) and *Peach latent mosaic viroid* (PLMVd), but the counterintuitive property gets poor further supports in recent years which promote raising a suspicion about its reliability and generality, and also whether the property can extend to similar RNA fragment remain unknown. To identify the property will provide basic information to document the transcription mechanism for a kind of novel DNA, a disorganized non-coding DNA without integration in plant genomic DNA and lacking protein encoding function. Here we constructed monomeric DNA with blunt ends, monomeric DNA with cohesive ends and dimeric DNA of *Citrus excortis viroid* (CEVd) to infect tomato seedlings, then identify if they are transcribed using RT-PCR and confirmed by analyzing their descendant sequences, results showed that the monomeric DNA with cohesive ends and dimeric DNA of CEVd can be recovered their transcribed RNA version in the host tissue after they are inoculated in 20 days. The transcribed RNA was also assessed about its stability, DNA template amount impact on infectivity, cyclization, and biological function. The cohesive ends of monomeric DNA can act as infection helpful factor was also confirmed in PLMVd. We confirmed here the novel life phenomenon that a disorganized viroid DNA fragment can be transcribed in plant, it will helpful to understand the transcription mechanism adopted by plant cell and develop some protocol such to facilitate and simplify genetic transform manipulation by freeing of vector.

GENERATION OF *IN VITRO* RNA TRANSCRIPTS AND INFECTIOUS FULL-LENGTH cDNA CLONES OF ASPV AND ASGV

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In commercial apple cultivars infection with viruses causes up to 60% losses in yield. *Apple stem pitting virus* (ASPV), *Apple stem grooving virus* (ASGV), and *Apple chlorotic leaf spot virus* (ACLSV) are the three most common latent viruses in apple worldwide. These viruses are highly variable and infect several woody host plants with different symptoms. Infectious cDNA clones provide the opportunity to study pathogenicity and symptomatology of a determined variant of a virus. Two strategies for the generation of an infectious full-length cDNA clone of ASPV were attempted. Initially a ligation strategy was attempted by subdividing the genome of ASPV isolate PB 66 into three fragments. These were ligated into the plasmid p1657 containing the 35S promoter. Due to an incomplete 5'-end of the sequence the resulting cDNA clones showed no infectivity on different host plants. The second strategy was based on PCR of the full length of ASPV and ASGV. Comparison of the 5' – and 3' end of different ASPV isolates showed highly conserved domains which were used as primers for PCR. Generation of infectious *in vitro* RNA transcripts of ASPV and ASGV were obtained by the addition of the T7 promoter sequence to the forward primers of full-length PCR fragments. *In vitro* RNA transcripts of ASGV infected 5 out of 6 mechanically inoculated *Nicotiana occidentalis* 37B plants, whereas transcripts of ASPV infected only 4 out of 42 tobacco plants. The Circular Polymerase Extension Cloning method (CPEC) was used to generate an infectious full – length cDNA clone of ASPV and ASGV in pBin V297. *N. occidentalis* 37B plants were infected by inoculation with *Agrobacterium tumefaciens* containing the pBin vector with the full- length cDNA clone for both viruses. The infection rates were 3% for ASPV and 22% for ASGV.

GENOME ORGANIZATION OF *CHERRY LEAF ROLL VIRUS* AND ANALYSES OF FUNCTION OF VIRUS-ENCODED PROTEINS

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Cherry leaf roll virus (CLRV) infects many stone and some small fruits. Recently, the complete genome was determined revealing the genome organization of the bipartite positive stranded plant RNA virus (*Secoviridae* family, genus *Nepovirus*). Now we aim to characterize the functions of CLRV-encoded proteins. As a prerequisite, it is necessary to understand the processing of mature proteins from polyproteins encoded by vRNA1 (P1) and vRNA2 (P2). The viral proteinase and putative cleavage sites will be analyzed *in vitro* in order to determine the specific processing sites utilized during proteolytic processing of P1 and P2. CLRV is transmitted by pollen and seed. Systemic infection of a host plant is achieved by cell to cell movement via plasmodesmata and long-distance transport through the vascular system. Members of the family *Secoviridae* are transported as virions, thus requiring the coat protein (CP). Further, the viral movement protein (MP) inducing tubular structures by multimerization within plasmodesmata is necessary for passage of virus particles to adjacent cells. Virus-like particles (VLPs) have been observed within tubules in anther cells and pollen grains of CLRV-infected birch and walnut. However, the underlying interactions of CLRV-CP and MP involved in cell to cell movement have never been investigated. We applied the yeast two-hybrid system (YTHS) to address this question. Dimerization of the MP (385 aa, 42 kDa) and the CP (512 aa, 54 kDa) could be shown as well as the specific protein interaction of both viral proteins. Additionally, binding of the viral MP to a plant protein (At-4/1) which is localized at plasmodesmata was demonstrated. At-4/ has been shown to interact with the tubuli-forming MP of *Tomato spotted wilt virus* (TSWV) and facilitates intra- and intercellular trafficking. This suggests that CLRV and TSWV are utilizing the same cell to cell transport mechanism in their host plants.

ORAL SESSION V
SMALL FRUITS:
VIRUSES, VIROIDS AND PHYTOPLASMAS

STRAWBERRY MARGINAL CHLOROSIS (SMC): A DISEASE OF VARIOUS BACTERIAL AND VIRAL ETIOLOGIES IN FRANCE

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Marginal chlorosis has been observed on strawberry plants in France since 1988. Previous studies have demonstrated the association of two phloem restricted bacteria with the disease: *Candidatus* Phlomobacter fragariae and stolbur phytoplasma. The improvements of detection techniques as well as the discovery of two new phloem-restricted bacteria associated with SMC; *Candidatus* Phytoplasma fragariae in Lithuania and the gamma 3 proteobacterium previously known as the agent of “basses richesses” syndrome of sugar beet (SBRp) in Italy lead us to reassess SMC etiology in France. Two specific triplex real time PCRs were engineered to detect the strawberry mitochondrial *cox* gene as internal control in addition to stolbur phytoplasma and *Ca. Phlomobacter fragariae* in the first test, and the detection of *Ca. Phytoplasma fragariae* and SBRp in the second test. SMC surveys were carried out in different locations in France in production fields as well as in nurseries. Phloem-restricted bacteria associated with typical SMC were detected using the two triplex real time PCR and additional tests were carried out to also detect the *Strawberry mild yellow edge virus* (SMYEV), a potexvirus also associated with strawberry yellows. Stolbur phytoplasma was again more frequently detected in nurseries than in production fields, whereas *Ca. Phlomobacter fragariae* was prevalently detected in production fields. SBRp and *Ca. Phytoplasma fragariae* were associated with 1-5 % of the disease cases. SMYEV was detected in nurseries and in production fields with incidences between 13 to 30%. SMYEV coat protein sequences revealed a significant genetic diversity of SMYEV in the French strawberry production.

IDENTIFICATION AND PHYLOGENETIC ANALYSIS OF VIRUSES INFECTING STONE FRUITS AND STRAWBERRY IN EGYPT

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A total of 102 samples of *Prunus persica* (peaches), *P. armeniaca* (apricot) and *P. domestica* (plums) plus 50 samples of *Fragaria X ananassa* (strawberry) were collected from commercial fields and nurseries in Alexandria, El Beheira and El Menofia governorates in Egypt during 2009 and 2010. Samples collection was based on virus diagnostic symptoms observation on different identified cultivars. DAS-ELISA and RT-PCR analyses were used to identify infection by *Plum pox virus* (PPV), *Prune dwarf virus* (PDV), *Prunus necrotic ringspot virus* (PNRSV), *Apple chlorotic leaf spot virus* (ACLSV), *Apple mosaic virus* (ApMV) and *Strawberry latent ringspot virus* (SLRSV) in stone fruits. However, strawberry's samples were tested for *Strawberry vein banding virus* (SVBV), *Strawberry crinkle virus* (SCV), *Strawberry mild yellow edge virus* (SMYEV), *Strawberry mottle virus* (SMoV) and SLRSV. In stone fruits only PPV, PDV and SLRSV were detected. As for strawberry, SMYEV was the only virus identified in all tested samples. RT-PCR identified more infected samples than ELISA due to its higher sensitivity. PCR amplification of different amplicons from the detected isolates allowed partial sequence and phylogenetic analysis of different isolates of PPV (13), PDV (7), SLRSV (4) and SMYEV (2). This study allowed to point out cultivars infected by one or more viruses and illustrates the evolutionary relationship between the Egyptian viral isolates. Moreover each isolate from viruses identified in the present work has been compared to others worldwide reported.

***RASPBERRY LEAF BLOTCH VIRUS, A NEW EMARAVIRUS
INFECTING RASPBERRY***

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In the last 3 or 4 years raspberry leaf and bud mite (*Phyllocoptes gracilis*; RLBM) has emerged as a serious cause of disease in some raspberry plantations in England and Scotland. Chemical treatments to control the mite are either being removed from use by EU directive or are ineffectual, and the incidence of the mite is increasing. We have recently identified a virus, Raspberry leaf blotch virus (RLBV), which is transmitted by the mite and could be a significant contributing factor to the disease severity. Sequencing of RLBV revealed that it has a genome comprised of five RNAs of negative strand polarity, with the complementary strand of each RNA molecule potentially expressing a single protein. This genome structure and viral protein sequences suggest that RLBV is a new member of the very recently described *Emaravirus* genus of plant viruses. Other members of this genus include, European mountain ash ringspot associated virus, Fig mosaic virus and Rose rosette virus, all of which are transmitted by mites.

AN EMERGING DISEASE IN BLUEBERRY CAUSED BY A NOVEL RNA VIRUS

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A new disorder was observed on southern highbush blueberry (*Vaccinium corymbosum* interspecific hybrids) in several southeastern states. Symptoms included irregularly shaped circular spots or blotches with green centers on the top and bottom of leaves. The disease was reported initially in the state of Georgia in 2006 and 2007; but in 2008, it was found in production areas in the states of Florida, Mississippi, South Carolina, and North Carolina. Diagnostic tests failed to isolate any fungal or bacterial pathogens typically associated with such symptoms. Double-stranded RNA (dsRNA) extracted from symptomatic leaves suggested the presence of virus(es) possibly involved in the disease. Sequencing revealed the presence of a novel virus with a genome of ~14 kb divided into 4 positive-sense RNA segments. Primers developed from conserved areas of the RdRp detected the virus in more than 50 individual plants that exhibited necrotic ring blotch symptoms in North Carolina, Georgia and Florida. The perfect correlation between the virus and symptoms in plants from across several states suggests that the virus, for which we propose the name Blueberry necrotic ring blotch virus (BNRBV), is the causal agent of the disease. Sequence analysis showed that BNRBV possesses protein domains conserved across ssRNA viruses from the Virgaviridae, Bromoviridae and the newly created genus Cilevirus. Furthermore, BNRBV has two Helicase (HEL) domains located on RNA 1 and 2. Both HEL domains possess conserved motifs and, more importantly, represent two distinct clades indicating virus recombination events as a result of co-infections.

OCCURRENCE AND VARIABILITY IN EUROPEAN ISOLATES OF *BLUEBERRY RED RINGSPOT VIRUS*

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The red ringspot disease of highbush blueberry (*Vaccinium corymbosum* L.) identified in the USA by Hutchinson and Varney (1954) is caused by *Blueberry red ringspot virus* (BRRV) genus *Soymovirus*, family *Caulimoviridae*, with circular dsDNA genome and particles of 42 – 46 nm in diameter. Complete genome sequence of the New Jersey (NJ, USA) isolate was described. Recently, BRRV was detected in Japan, Slovenia and Poland. In the Czech Republic BRRV was found after extensive survey in 2 plants of cv. Darrow with reddish ring spots and blotches on the upper surface of leaves in a field germplasm collection in South Bohemia in 2009. The electronmicroscopical examination of ultrathin sections prepared from leaves showed spherical virus particles present in inclusion bodies formed of electron-dense matrix with electron-lucent area enclosed in an endoplasmic reticulum. Complete genomes of Czech and Slovenian isolates of BRRV were sequenced. Their comparisons showed 95.9% identity of both isolates but only 92.2% identity with the US NJ isolate. Phylogenetic analysis performed on a 186-aa-long region from the reverse transcriptase protein of 17 known isolates defined four clusters: one containing Japanese sequences from cvs. Weymouth and Blueray, one branch of a single American isolate from cranberry (Cran I), cluster containing cvs. Sierra and Duke isolates from Japan and less defined cluster containing the other US isolates together with the two European ones. We conclude that the phylogenetic tree more probably reflects natural variability of American BRRV isolates present in imported rootstocks rather than variability generated in the field. Experiments on BRRV elimination from cv. Darrow are in progress. The work was supported by grant AV0Z50510513 of the Academy of Sciences of the Czech Republic.

DISSECTING THE EPIDEMIOLOGY OF *BLACKBERRY YELLOW VEIN ASSOCIATED VIRUS* AND *BLACKBERRY CHLOROTIC RINGSPOT VIRUS*; A STUDY ON POPULATION STRUCTURE, TRANSMISSION AND ALTERNATIVE HOSTS

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Blackberry yellow vein disease (BYVD), caused by virus complexes, is the most important blackberry disease in the southern United States. *Blackberry yellow vein associated virus* (BYVaV) is the most prevalent virus in BYVD plants, and was detected in more than 50% of the samples exhibiting BYVD symptoms. *Blackberry chlorotic ringspot virus* (BCRV) is another virus frequently found in BYVD plants, and in addition to blackberry, it can also infect rose and raspberry. Despite the importance of the viruses as major components of BYVD, their epidemiology is grossly understudied. Population diversity, transmission modes and potential alternate hosts of both viruses were examined. The population structure of BYVaV was determined after sequencing four genomic regions of 34 isolates. For BCRV, the complete RNA 3 of 30 isolates from three different hosts was evaluated. Sequence variability among five coding regions of BYVaV and the coat protein and movement protein of BCRV was evaluated. Two whitefly species were tested for their ability to transmit BYVaV, and seed transmission assays for BCRV were performed using three hosts. Over 25 species of plants were tested as potential alternative hosts of these viruses. BYVaV populations are fairly diverse; whereas US isolates of BCRV are more uniform. The greenhouse and banded-winged whiteflies were identified as efficient vectors of BYVaV. BYVaV was graft-transmissible to rose; and if natural infection of rose is confirmed this can be of great concern to the ornamental industry with respect to movement of plant material. BCRV was found to naturally infect apple and was seed transmissible to the three hosts tested. The information generated in this study has provided reliable detection protocols capable of detecting a wide range of virus isolates.

PHYTOPLASMAS OF GROUP 16SrI ASSOCIATED WITH STRAWBERRY (*FRAGARIA X ANANASSA*) IN COLOMBIA

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Cajicá (15 Km north of Bogotá-Colombia) is a small town located at 2,600 m of altitude on the Andes cordillera. In June 2010, an experimental semi-organic strawberry (*Fragaria X Ananassa*) crop was planted under a plastic greenhouse to test fruit yield of the “Ventana” variety. In 2011, the crop was visited in order to determine the cause of a dramatic drop in production. Symptoms such as fruit phyllody, flower viviparity, leaf reddening, abnormal shoot production and changes in sepal size were observed. Leaves from 4 symptomatic plants were sampled and DNA extracted by a CTAB extraction method, followed by an additional cleaning step (Power Clean DNA MoBio). Nested PCR tests were performed with P1A/P7A-R16F2n/R16R2. Amplicons were purified and later sequenced using R16F2n/R16R2 primers, but *Bacillus* spp. was detected. However, when fU3/rU5 primers were used, a band of 845 pb was obtained and phytoplasma sequences were found. The sequence was compared with NCBI database using BLAST and homology of more than 99% with phytoplasmas of group 16SrI was found. A dendrogram obtained using previously reported sequences confirmed the relationship of phytoplasmas with the aster yellows group (*Candidatus Phytoplasma asteris*’-related strains). Although symptoms indicating the presence of phytoplasmas were observed in all tested samples, the presence of this pathogen was confirmed only in one of the evaluated strawberries plants. This is the first report of phytoplasma presence in strawberry in Colombia.

ORAL SESSION VI
CONTROL STRATEGIES

TRANSGENIC APPROACH FOR IMPROVING RESISTANCE OF PLUM CULTIVARS FOR SHARKA DISEASE

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Since the first record of Sharka disease in Bulgaria, the disease has progressively spread via infected plant materials throughout Europe where it has destroyed well over 100 million stone fruit trees. The disease has serious agronomic and political consequences due to the enormous economic losses. Because only few PPV resistance genes have been found to naturally occur in *Prunus*, scientists have utilized genetic engineering techniques to develop resistant plums by inserting specific genes from the PPV genome into the DNA of *Prunus* host plants. For improving the plants resistance to plum pox virus (PPV) we used two technologies based on co-suppression gene and RNA-silencing. Binary vector pCamPPVcp which contained *ppv-cp* gene in sense-orientation and self-complementary fragments of gene *ppv-cp* were used for realization post-transcriptional gene silencing. Seven independent transgenic lines with *ppv-cp* gene and five transgenic lines with a two inverted repeats of *ppv-cp* gene fragment were produced in our laboratory. Stable integration of genes into genome of plants was confirmed by PCR analyses. The accumulation of coat protein was evaluated by Western blot assay in five from six lines. Plum clones were infected by bud-grafting. PPV detection was analyzed RT-PCR by using primers targeting the 3' untranslated region and HC-Pro gene of PPV. Western blot analysis was performed using rabbit polyclonal antibodies to PPV coat protein (Loewe). We observed absence of PPV in one line of St-pCamPPVcp and all four tested lines of St-pCam PPVRNAi. Field testing of transgenic plants is underway in the field plots.

ROBUST AND WIDE SPECTRUM RNA SILENCING MEDIATED RESISTANCE TO *PLUM POX VIRUS*

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Plum pox virus (PPV) is a quarantine virus that causes sharka, one of the most important diseases of stone fruits worldwide. Seven PPV strains have been characterized, of which PPV-D, -M and Rec are the most important from an agro-economical point of view. The best agricultural sustainable approach to prevent sharka disease consists in developing PPV-resistant plants. In this context we have shown that *Nicotiana benthamiana* plants transformed with PPV-M sequences (PPV-IsPaVe44), covering the 5'UTR region, P1 and HC-Pro genes and arranged to express self-complementary "hairpin" RNAs, are immune to the homologous PPV-ISPave44 (Di Nicola-Negri et al., 2005, *Transgenic Res.* 14:489-94). However, as the RNA silencing-mediated resistance operates in a sequence-specific fashion, transgenic plants harboring the four hairpin constructs were also challenged with viral isolates belonging to different PPV strains. All the transgenic plant lines were resistant to PPV-D, -M and Rec strains. Moreover, the transgenic plant line harboring the 5' UTR/P1 sequence was also resistant to isolates of PPV-EA and PPV-C strains that are distantly related to ISPave44 (Di Nicola-Negri et al., 2010 *Plant Cell Rep* 29:1435-1444). Since it's known that some abiotic - low temperature - and biotic stresses - mixed viral infection - could have a detrimental impact on RNA silencing-mediated viral resistances, 5' UTR/P1 plants were challenged with PPV under different conditions. Transgenic plants were resistant to PPV infection both at high (30°C) and low temperature (15°C). Furthermore, no susceptibility to PPV was observed in 5'UTR/P1 plants previously inoculated with *Potato virus Y* (PVY), *Cucumber mosaic virus* (CMV) or *Artichoke mottled crinkle virus* (AMCV) suggesting that prior virus-mediated expression of HCPro (PVY), 2b (CMV) and P19 (AMCV) RNA silencing suppressors was not able to defeat PPV resistance. The overall data suggest that the 5' UTR/P1 hairpin construct can be profitably used to confer resistance to the sharka disease in *Prunus* species.

PRODUCTION AND *IN VITRO* ASSESSMENT OF TRANSGENIC PLUMS FOR THE RESISTANCE TO *PLUM POX VIRUS*

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Plum pox virus (PPV) is the quarantine pathogen causing sharka, the most devastating disease of stone fruits. Genetic engineering represents an interesting tool to obtain elite cultivars resistant to PPV. However, the containment of transgenic plants and PPV is an important aspect to be considered during the plant evaluation process. Here, we report the production of transgenic plum clones together with their *in vitro* evaluation for PPV resistance. Hypocotyl slices of mature plum seeds, cv Stanley, were transformed by *Agrobacterium tumefaciens* with a PPV-M derived hairpin construct (h-UTR/) that showed high performance in model plant. Two transgenic clones, St24 and St28, were obtained. A protocol to establish *in vitro* culture, proliferation and maintenance of PPV-D infected GF305 peach [*Prunus persica* L.(Batsch)] and virus-free microcuttings was developed. Micropropagated shoots of the transgenic clones were *in vitro* micro-grafted onto PPV-D infected GF305 rootstock. After the permanent union between the grafted tissues the transgenic scions were analysed by ImmunoCapture-RT-PCR (IC-RT-PCR) for PPV detection. More than 97% of ST24 and 73% of ST28 tested plants were resistant to the heterologous strain of PPV. Transgene-specific siRNAs were detected in both resistant clones, indicating that post-transcriptional gene silencing underlies the mechanism of resistance. These results confirm the role of the genetic engineering for the development of plum clones highly resistant to PPV and offer a quick and contained infection test procedure of practical interest.

USE OF HORTICULTURAL MINERAL OIL TREATMENTS FOR THE CONTROL OF DIFFERENT *PLUM POX VIRUS* ISOLATES IN NURSERY BLOCKS

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The effect of horticultural mineral oil (HMO) treatments (Sunspray Ultrafine at 1%) on the natural spread of *Plum pox virus* (PPV), was evaluated in three experimental nursery plots of Nemaguard and Mariana GF8-1 *Prunus* rootstocks. The experimental plots were established under high natural PPV-inoculum pressure of different types in Plovdiv (Bulgaria)/PPV-M and -Rec, Bistrita (Romania)/PPV-D and -Rec and Llíria (Spain)/PPV-D. Treatments were weekly applied during the vegetative period from springtime to fall (in summertime the treatments were interrupted). Nursery plants were yearly analysed by MagicDAS-ELISA (Plant Print Diagnostics) based on the monoclonal antibody 5B-IVIA. The aphid population of each experimental nursery plot was monitored by Moericke yellow traps and by the sticky-shoot method. Springtime was the period when the maximum peak of aphid population was registered in the three experimental nursery plots. PPV incidence was significantly reduced ($p < 0.05$) in all the experimental plots and in both assayed rootstocks, although the HMO treatments did not avoid PPV infection, probably due to the high PPV prevalence present in the neighbouring area of the experimental nursery blocks. This result shows that HMO treatments could be used as a possible environmental friendly control strategy, in different ecological areas, to reduce PPV incidence in *Prunus* nurseries regardless the PPV isolate involved, due to the physical action of the film of oil that covered the surface of the treated plants.

THE PRESENT STATUS OF COMMERCIALIZED AND DEVELOPED BIOTECH (GM) CROPS, RESULTS OF EVALUATION OF PLUM 'HONEY SWEET' FOR RESISTANCE TO PLANT VIRUSES IN THE CZECH REPUBLIC

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Commercialization of biotechnological (GM) crops started in 1995. A significant increase of 9 million hectares planted biotechnological (Biotech) crops per year was realized in 1996-2009. In the years 2010-2011 it was already 12 million hectares (8%). 16.7 million farmers in 29 countries planted 160 million hectares of biotech crops in 2011. Developing countries grew close to 50% of global Biotech crops at present. The U.S.A. is the lead producer of Biotech crops with 69 million hectares, 43% of global. GM soybean remains dominant crop, followed by Bt maize, cotton, and canola. Golden Rice is advancing towards the completion of its regulatory requirements. GM crops are contributing to the reduction of CO₂ emissions. Not only field crops, but also horticultural transgenic crops are developed and commercialized. The impressive progress is in Biotech vegetable projects which include tomato, potato, cabbage, brassica, cauliflower, bean, sweet pepper, chili, zucchini, squash, eggplant, cucumber, carrot, and sweet corn. Genetic engineering has the potential to revolutionize fruit tree breeding.. The development of transgenic fruit cultivars is in progress. Papaya resistant to *Papaya mosaic virus* is grown in U.S.A. and China. Biotech grapevine resistant to viral, bacterial, fungal disease with abiotic stress tolerance and health benefits is grown in South Africa. Biotech banana, apple, pear, and strawberry cultivars are under the development. A result of over the past 20 years an international research is development of 'HoneySweet' plum highly resistant to PPV. GM plum 'HoneySweet' resistant to *Plum pox virus* (PPV) was deregulated in U.S.A. in 2010. Plum clone C-5 (cv. 'HoneySweet' at present) was ten years evaluated in the Czech Republic for resistance to PPV, PDV, and ACLSV under the high and permanent infection pressure of PPV and combinations with PDV, and ACLSV. Plum 'HoneySweet' was proved to be highly resistant in leaves and fruits to PPV. The mixed infection of PPV with PDV and/or ACLSV has practically no influence on the quantity and quality of HoneySweet fruits. Fruits from 'HoneySweet' trees graft-inoculated with PPV and combinations with PDV, and ACLSV are even better quality than fruits from healthy control trees of plum 'Stanley'.

ROLE OF CONIFER EXTRACT AS REPELLENT ON *CACOPSYLLA PRUNI* AND THEIR EFFECT ON THE SPREADING OF ESFY IN APRICOT

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Conifers act as shelter plants for *Cacopsylla pruni*, vector of European stone fruit yellows (ESFY), during autumn-winter time. The abandonment of the conifers in the Spring is reported as a critical point in the life cycle of *C. pruni*; a mechanism induced by conifers on the insect repellent has been suggested to describe this behavior. An experimental trial was established under semi-field conditions in an insect-proof tunnel. A total of 90, one year old, apricots cv. Reale d'Imola were planted at distance of 1 meter in two rows, spaced of 4 meters. Plants were divided into three theses: untreated, treated with systemic insecticide, treated with *Picea abies* extract. After two days from the treatments, a total of 500 insects, collected in areas with high ESFY disease pressure, were released in the tunnel. The colonization of the test plants was visually recorded for three times during the 20 days following the placing of the insects. The results show a greater presence of *C. pruni* on untreated apricots and a reduced colonization of both the plants treated with insecticide and the plants treated with conifer extract. The above mentioned differences were confirmed and emphasized by the results obtained by quantifying the presence of the new generation insects on the three theses. In order to estimate ESFY diffusion in the case of study, real-time PCR analyses were carried out on apricot plants samples at the end of the vegetative season. The results show a reduced diffusion of ESFY both in the insecticide and conifer extract treated theses. The obtained results suggest a repulsive mechanism by *P. abies* extract on *C. pruni*. In fact in the semi-field adopted conditions, apricot colonization by *C. pruni* was reduced and ESFY diffusion was comparable between insecticide-treated plants and conifers extract-treated plants. Characterization of *P. abies* extracts is undergoing in order to identify compound involved in the repulsive mechanisms.

USE OF MICROPROPAGATED *MALUS* TO STUDY LATENT APPLE VIRUSES

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Latent apple viruses like apple stem grooving virus (ASGV, Capillovirus) and apple stem pitting virus (ASPV, Capillovirus) could be maintained in *Malus x domestica* cultivars micropropagated *in vitro*. As both viruses exhibited high genetic variability among different isolates analysed, maintenance of defined isolates in homogenous culture lines proved to be a valuable tool for further studies. *In vitro* grafting was successfully applied to transmit both viruses to healthy plants or to establish defined co-infections of different viruses and isolates. Specific quantitative RT PCR assays for ASGV and ASPV were developed to determine the virus concentration in the different culture lines. Furthermore, the interaction of both viruses with ‘*Candidatus* Phytoplasma mali’, the causal agent of apple proliferation disease, was analysed by *in vitro* graft transmission and qRT-PCR analysis of the inoculated plants. The influence of the different viruses and virus-phytoplasma combinations on the growth of micropropagated apple was studied. Whereas virus-infected *M. x domestica* showed no affected growth compared to healthy controls, phytoplasmas induced typical symptoms. In contrast, *Malus sieboldii* is known to react with necrosis upon graft-inoculation with latent apple viruses *in vivo*. This reaction could be seen also in the *in vitro* system when virus or virus-phytoplasma inocula were grafted *in vitro* on micropropagated *M. sieboldii*. The analysis of this necrotic reaction of *M. sieboldii* has become of major interest since *M. sieboldii* and its hybrids confer resistance to apple proliferation (AP) disease and have been used in a recent breeding program aiming to develop AP-resistant rootstocks of agronomic.

ORAL SESSION VII
CERTIFICATION

CLEAN PLANTS, THE NATIONAL CLEAN PLANT NETWORK AND HARMONIZING CERTIFICATION STANDARDS IN THE UNITED STATES

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The National Clean Plant Network (NCPN) was established in the 2008 Farm Bill with three major objectives: a) develop and maintain G1 (Foundation) blocks that are to serve as sources of clean plant material for certification programs; b) carry out pathogen elimination in vegetatively propagated crops; and c) develop improved diagnostic tests for systemic pathogens. Limited funding was provided for four years to support and enhance existing facilities (clean plant centers), and development and validation of diagnostic assays. Currently the program supports a total of 19 programs at 16 centers working on five groups of perennial specialty crops: berries, citrus, grapes, hops, and tree fruits. A governing board was formed for each crop to develop lists of pathogens (viruses, phytoplasmas and systemic bacteria) that need to be tested, review proposals for funding, and coordinate activities between the centers. The program supports tree fruit service functions at Prosser, WA; Davis, CA; and Clemson, SC; and berry research and service functions in Corvallis, OR and Fayetteville, AR; and service functions in Raleigh, NC. In addition to efforts to develop these G1 blocks, the Farm Bill also provided funding to support certification programs, which in the U.S. are regulated at the state rather than the federal level. This funding is being used to harmonize certification programs across states for each of the crops. Ideally, the validation of diagnostic assays will be expanded to include international collaborators and many aspects of the certification standards being developed will be harmonized with programs in other countries.

ESTABLISHMENT OF VIRUS-FREE PLANTING MATERIAL FOR POME FRUITS IN LATVIA

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During the past twenty years, many commercial apple orchards have been established by use of introduced cultivars and clonal rootstocks. Due to specific requirements of adaptation to local climatic conditions, mainly domestic cultivars and cultivars originating in Russia, Belarus and Kazakhstan are grown. In Latvia certification programme for virus-free planting material is not established yet and planting material of pome fruits correspond to *Conformitas Agraria Communitatis* standard. In order to establish nuclear stock collection for several in the country widely grown pome fruit cultivars candidate graft material from four apple cultivars 'Auksis', 'Antei', 'Antonovka', 'Sinap Orlovskii' and six pear genotypes 'Belorusskaya Pozdnaya', 'Conference', 'Concorde', 'Condo', 'Vasarine Sviestine', P-67-21 were budded on seedling rootstocks grown in sterilized substrate and insect proof greenhouse. Next year before thermotherapy the presence of common viruses were detected by multiplex RT-PCR. All apple cultivars were infected with *Apple chlorotic leaf spot virus* (ACLSV). Cultivars 'Auksis' and 'Sinap Orlovskii' were also infected with *Apple stem pitting virus* (ASPV) and *Apple stem grooving virus* (ASGV), but cultivar 'Antei' was infected with ASPV. All pear cultivars were infected with ApMV and cultivar 'Conference' was also infected with ASPV and ASGV. For elimination of viruses the plants were kept in a growth chamber for 40 days at temperature of $38 \pm 0.5^\circ\text{C}$. The shoot tips (5 mm) were excised from heat treated plants and grafted onto a seedling rootstocks. Apple cultivars 'Auksis' and 'Antonovka' were negative on all tested viruses during two vegetation periods. Cultivars 'Antei' and 'Sinap Orlovskii' remained infected with ASGV, which was detectable only in the second year after thermotherapy. Pear cultivars 'Belorusskaya Pozdnaya' and 'P 67-21' remained infected with ApMV. The work is in progress to test candidate mother plants by woody indicators for viruses and other graft-transmittable organisms included in EPPO guidelines for pome fruits.

DEVELOPMENT OF MOLECULAR DIAGNOSTIC TOOLS TO DETECT ENDEMIC AND EXOTIC PATHOGENS OF *PRUNUS* SPECIES FOR AUSTRALIA

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The biosecurity of the Australian almond, summer fruit and cherry industries is maintained at the border by the Australian Quarantine Inspection Service (AQIS) in Post Entry Quarantine (PEQ) facilities and internally through schemes that supply high-health planting material throughout Australia. Currently the AQIS recognises two bacteria, six phytoplasmas or phytoplasma-like diseases, 13 viruses, one viroid and 70 fungi, to be of quarantine significance for *Prunus* species. AQIS also recognises that there are many diseases of *Prunus* of unknown aetiology. A recent review of the bacteria, phytoplasmas, viruses, viroids and fungi that are currently known to infect *Prunus* species identified the pathogens that are significant to Australian PEQ: five bacteria are significant at the quarantine level and another four are significant at the plant health certification level within Australia. 11 phytoplasmas or phytoplasma groups are known to infect *Prunus* species and no phytoplasmas have been reported to infect *Prunus* species within Australia. 34/47 viruses that are known to infect *Prunus* species are significant at the PEQ level. Only one of three viroids that infect *Prunus* species are reported as significant at the PEQ level. Based on these findings a new research project aimed at improving Australia's stone fruit biosecurity is underway to: Update the PEQ list for *Prunus* species with recently reported pathogens and updated information of known pathogens. Adopt, develop and validate molecular diagnostic tools under Australian conditions for the important quarantine pathogens of *Prunus* species. Develop a PEQ diagnostic manual for a number of *Prunus* species. The development of these molecular protocols will support industry biosecurity plans that have been developed for Australian almonds, summer fruit and cherry industries.

QUALITY SYSTEMS FOR PRODUCTION OF NURSERY STOCK IN APPLE

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Growers of nursery stock aim to produce a high quality product. Plants are selected and propagated, stocks are indexed and possibly certified through quality-plus systems. Detection tests for plant pathogens e.g. viruses are carried out in order to remove infected plant material. Within quality plus systems several techniques are combined to detect a range of virus(like) diseases, but more and more the focus shifts to molecular testing. Although molecular detection tests are rapid, they are generally aimed at a specific target and might therefore miss (new) variants of a virus. In recent years laboratories like inspection services started to obtain accreditation for the detection tests. Validation is the key to describe the reliability of a detection test. As an example we will focus on screening for presence of *Apple stem pitting virus* (ASPV), *apple stem grooving virus* (ASGV), *Apple chlorotic leaf spot virus* (ACSLV) and *Apple mosaic virus* (ApMV). Comparison of RT-PCR's – published and modified primer sets –, testing by grafting on indicators, and adjustments needed for routine testing of samples will be discussed in relation to quality plus systems and availability of virus collections.

ESTABLISHMENT, ACTIVITIES AND RESULTS OF PLANT BIOTECHNOLOGY LABORATORY IN KABUL, AFGHANISTAN

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Horticultural industry is a vital component of Agriculture sector of Afghanistan and a primary engine of Afghanistan's recovering economy is its agriculture industry, which engages approximately 80% of the working population. This sector was thriving in the 1970s, but is today incapable of competing in the international market. To recover and develop the horticulture sector of the country, EC supports PHDP (Perennial Horticulture Development Project), to provide true to type/ecotype and healthy planting materials, and the Plant Biotechnology Laboratory to ensure the health status of local germplasm. This laboratory started screening the health status of the Afghan Germplasm National Collection in order to ensure the multiplication of not only the best selected varieties or ecotype, but also to avoid production and distribution of virus-infected fruit trees. Symptoms inspection and sample collection for viral diseases was carried out in all the National Collection fields, including peach, plum, apricot, almond, apple, grape and citrus plants, located in different areas of the country (Kabul, Kunduz, Mazar, Herat, Kandahar and Jalalabad). During the routinely activity of the Laboratory, citrus plants from the National Collection experimental farm in Jalalabad (Nangarhar province) showing vein flecking, yellowing and plant decline symptoms were found to be infected by *Citrus tristeza virus* (CTV). Identified isolates have been characterized molecularly amplifying a fragment corresponding to the major coat protein gene from all positive samples. Moreover *Prunus necrotic ringspot virus* (PNRSV) was detected in samples collected from three almond accessions from the PHDP research station of Mazar Sharif (Northern Afghanistan). To our knowledge, our results identified for the first time CTV and PNRSV in Afghanistan. The presence of those viruses in different accessions of the national collection is of concern for Afghan horticulture. Implementation of the certification schemes is therefore necessary to quarantine the production and the employment of virus-free propagating material.



COST FA0807 WORKSHOP

**PHYTOPLASMA IN FRUIT TREES:
MULTIDISCIPLINARY APPROACHES TOWARD**



Invited lecture

GLOBAL GENE EXPRESSION ANALYSIS OF PHYTOPLASMA IN THE HOST SWITCHING BETWEEN PLANT AND INSECT

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Phytoplasmas are biologically unique since they can parasitise a diverse range of hosts, including plants and insects. Phytoplasmas reside endocellularly within the plant phloem and feeding insects (mainly leafhoppers), and are spread among plants also by insects. It is of interest how phytoplasmas can adapt to two diverse intracellular environments (i.e., plant and insect cells). We have previously demonstrated that a complex between Amp, a surface membrane protein of phytoplasma, and insect microfilament may play a major role in the insect transmissibility of phytoplasma. However, the mechanisms enabling the switch between plant and insect hosts are poorly understood. To investigate the molecular mechanism underlying the “host switching” between plant and insect, we performed the global gene expression analysis of ‘*Candidatus Phytoplasma asteris*’ OY-M strain. As a result, the phytoplasma alters many gene expressions in response to the plant and insect host, such as genes for transporters, secreted proteins, and metabolic enzymes. These results suggest that the phytoplasma may use transporters, secreted proteins, and metabolic enzymes in a host-specific manner. As phytoplasmas reside within the host cell, the proteins secreted from phytoplasmas are thought to play crucial roles in the interplay between phytoplasmas and host cells. Our microarray analysis revealed that the expression of the gene encoding the secreted protein PAM486 was highly upregulated in the plant host, which is also observed by immunohistochemical analysis, suggesting that this protein functions mainly when the phytoplasma grows in the plant host

DETECTION AND IDENTIFICATION OF A PHYTOPLASMA AFFECTING BLACKCURRANT AND REDCURRANT SHRUBS

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During October-November/2011, blackcurrant (*Ribes nigrum* L.) and redcurrant (*Ribes rubrum* L.) shrubs exhibiting symptoms of leaf redness and downward curling in Ontario, Canada were tested for phytoplasma presence. Total DNA was extracted (Fast DNA Spin kit, MP Biomedicals, USA) and used in a nested PCR assay with universal primers that target the phytoplasma 16S rRNA gene, P1/P7 followed by either R16F2n/R2 or fU5/rU3 for the nested reaction. R16F2n/R2 PCR amplicons were obtained for all the symptomatic plants collected, purified (Cycle Pure kit, Omega, USA) and sequenced bi-directionally (University of Health Network, Toronto, Canada). Sequences were compared to those of reference phytoplasmas in GeneBank. BLAST analysis showed a 100% of 16S rDNA sequence identity between the two phytoplasmas detected in both blackcurrant and redcurrant plants, and a 99% of sequence identity to those of members of group 16SrX, subgroup 16SrX-A. Virtual and actual RFLP of the R16F2n/R2 and fU5/rU3 amplicons with *AluI*, *RsaI*, *SspI* and *MseI* restriction endonucleases yielded RFLP patterns similar to those of phytoplasma members of the group 16SrX 'apple proliferation', subgroup 16SrX-A. Phylogenetic analysis (MEGA version 4.1, USA) based on the 16S rDNA sequences confirmed RFLP and BLAST results. Phytoplasmas of group 16SrX have been previously reported in Ontario, affecting *Prunus* and *Pyrus* species. However, results represent the first record of a 16SrX-A infecting blackcurrant and redcurrant, and provide a valuable tool for further epidemiological studies of a 16SrX phytoplasma in these two plant species.

MOLECULAR POLYMORPHISM IN PHYTOPLASMAS INFECTING PEACH TREES IN SERBIA

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During a survey carried out in 2008 to verify phytoplasma presence in fruit trees in Serbia, samples were collected from two single peach plants (30-Niš and 103-Radmilovac) showing yellows disease. Phytoplasma detection was carried out using PCR assays with primer pair P1/P7 in direct reaction, followed by nested PCR with F1/B6, R16F2n/R2, and R16(I)F1/R1. RFLP analyses carried out on R16F2n/R2 amplicons with *TruII*, *TaqI*, *Tsp509I*, *AluI*, and *BfaI* restriction enzymes showed that in peach 30, a mixed phytoplasma infection was present. Identification of 16SrII and 16SrXII phytoplasmas was confirmed by RFLP analyses carried out on amplicons obtained with primers R16(I)F1/R1 with *TruII* and *Tsp509I* restriction enzymes. RFLP analyses on F1/B6 and R16F2n/R2 amplicons with *TruII* showed that 16SrII group phytoplasmas were present also in peach 103. F1/B6 and R16F2n/R2 amplified products (about 1,700 bp and 1,200 bp respectively) of both peach samples were purified using Qiagen PCR Purification Kit, cloned in DH5 alfa and a number of clones was screened by PCR with M13 primers followed in nested PCR by F1/B6 and R6F2n/R2 primers (according with amplicon). RFLP analyses with *TruII* on R16F2n/R2 amplicons of seven M13 clones obtained from peach 30 show the presence of 6 profiles referable to 16SrXII and two referable to 16SrII phytoplasmas. *AluI* restriction enzyme did not show polymorphisms but distinguished between groups 16SrII and 16SrXII while *Tsp509I* showed different profiles between the two 16SrII group phytoplasmas. Similar results were obtained with *TruII* and *AluI* restriction enzymes on 3 clones amplified with R16(I)F1/R1. Peach 103 clones were all identical after RFLP analyses. This is the first report of 16SrII phytoplasmas in peach and the 16Sr DNA variability detected in both 16SrXII and 16SrII phytoplasmas is an indication of phytoplasma population presence in the infected plant.

SEROLOGICAL PROOFS FOR RELATEDNESS OF THE MYCOPLASMALIKE ORGANISMS (PHYTOPLASMAS) FROM APPLE PROLIFERATION GROUP

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Two polyclonal antibodies were produced in rabbits for apricot chlorotic leaf roll (ACLR) MLO purified from apricot, and for European Aster yellows (EAY) cultivated in artificial media. Both antisera were used as primary antibody to detect apple proliferation (AP15), pear decline (PD) and ACLR, in a comparative study with EAY MLO purified from periwinkle. AP15 (from Udine, Italy) was kindly provided by E. Seemüller, Germany, and in our laboratory, it was identical by symptoms (virescence and proliferation) in periwinkle with EAY isolated in Romania and with American severe western yellows. An indirect dot blot ELISA method was performed on nitrocellulose membrane stripes using secondary antibodies Goat anti-rabbit IgG conjugated with Alkaline Phosphatase (Sigma product No. A3937) and Goat anti-rabbit IgG conjugated with colloidal gold (Sigma product No. G3779). Positive reactions were identified for alkaline phosphatase using SIGMAFAST-BCIP/NTB (Sigma product No. B0274 substrate). When EAY and AP15 MLOs purified from periwinkle were tested with antiserum for ACLR, these gave positive reaction. Pear decline MLO extracted from pepper infected by *Cacopsylla pyri* and from pear trees was also detected with anti ACLR MLO serum. MLO purified from *C. pyri* insects used in experimental transmissions was also detected. These results support the hypothesis that EAY, AP15, PD and ACLR MLOs are identical serologic. The EAY antiserum recognized AP15 and EAY MLOs (phytoplasmas) purified from periwinkle when both molecular markers (AP and Colloidal gold) were used. The MLOs from AP15 and AP15 group (PD, ACLR) were also identical with EAY which infect more than 500 species of cultivated and spontaneous plants. Recent investigation in Germany, based on PCR analyses of 16S ribosomal DNA revealed that AP, PD and ACLR are identical pathogens.

MULTILOCUS GENE ANALYSES OF ‘*CANDIDATUS PHYTOPLASMA MALI*’ CONFIRMS THE GENETIC DIVERSITY OF PHYTOPLASMA POPULATION IN THE CZECH REPUBLIC

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Apple trees are affected by the apple proliferation (AP) disease in several European countries. The AP is usually associated with the presence of ‘*Candidatus Phytoplasma mali*’ (‘*Ca. P. mali*’), a quarantine pathogen in EU. Samples from several apple trees showing proliferation disease symptoms were collected during 2010 at 28 different locations all over the Czech Republic. Phytoplasma strains were detected through polymerase chain reaction and restriction fragment length polymorphism analyses of 16S rDNA-spacer region-23rDNA, ribosomal protein gene sequences *rpl22* and *rps3* and non ribosomal DNA fragment (nitroreductase-like gene). All 74 apple trees examined were positive for phytoplasmas, predominantly for ‘*Ca. P. mali*’ (60 trees). ‘*Ca. P. asteris*’ alone and in also mixed infection with ‘*Ca. P. mali*’ was detected sporadically. In the 16S plus spacer region of ‘*Ca. P. mali*’ two profiles P-I (39 trees) and P-II (5 trees) singly or together (8 trees) were identified. The presence of two genetic lineages designated in literature as pattern ‘1’ (7 trees) and ‘2’ (31 trees) singly and also mixed (7 apple trees) was detected while the only pattern named ‘a’ was identified in 45 apple trees. ‘*Ca. P. mali*’ strains belonging to ribosomal protein rpX-A subgroup were identified in the majority of apple samples (51 out of 55 plants positive in direct PCR amplification with rpAP15f/rpAP15r primers), while phytoplasmas belonging to rpX-B subgroup were detected sporadically (four trees). Nearly equal distribution of apple proliferation subtypes AP-15 and AT-2 (in 21 and 22 trees, respectively) was determined, while apple proliferation subtype AT-1 was detected in 7 trees. Nucleotide sequence analyses of the 16S-23S ribosomal operon, ribosomal proteins L22, S3 and nitroreductase-like protein gene of five selected apple proliferation phytoplasma strains confirmed the PCR/RFLP analyses results. This is the first study of molecular diversity among ‘*Ca. P. mali*’ strains in the Czech Republic.

EARLY AND RELIABLE DETECTION OF EUROPEAN STONE FRUIT YELLOWS PHYTOPLASMA IN PEACH TREES

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The presence of European stone fruit yellows (ESFY), syn. '*Candidatus* Phytoplasma pruni' detection in peach trees grown in South Moravia, Czech Republic was studied during five years. ESFY symptoms were evaluated visually and biological indexing was carried out on the peach indicator GF-305. Three different procedures of molecular detection of ESFY were used. The first was PCR with primers ECA1/ECA2 according to Jarausch et al. (1998), the second one with primers fAT/rPRUS according to Smart et al. (1996), and third procedure was nested PCR with primers R16R0/R16F1 in a first step and primers R16F2/R16R2 in a second step according to Lee et al. (1995). Detection of ESFY in peach trees by biological indexing was found to be less suitable, the presence of pathogen was proved in less than 60% of symptomatic trees. Results of the all three applied PCR procedures were the same, but reactions of nested PCR were more strong. The presence of ESFY was detected in ca 75% of symptomatic trees. Results of PCR were negative in symptomless peach trees. The presence of ESFY symptoms is still most reliable criterion for detection of ESFY disease in peach trees grown in the Czech Republic. Further improving of PCR procedures is necessary for early and reliable detection of ESFY in peach trees.

DETECTABILITY OF PHYTOPLASMAS IN NATURALLY INFECTED *PICEA* AND *PINUS* SPP. TREES BY PCR

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Coniferous plants in Europe are natural hosts of three phytoplasmas - 'Candidatus Phytoplasma asteris', phytoplasma belonging to X-disease phytoplasma group and 'Ca. P. pini'. Very recently 'Ca. P. pini' was identified in trees and shrubs of eight species. Unfortunately, the previous studies yielded unsatisfactory results concerning phytoplasma detection in coniferous plants. This work was undertaken to study round-year detectability of phytoplasmas in the needles of naturally infected coniferous plant species and to examine detectability of 'Ca. P. pini' in plant material stored at different conditions (fresh needles, needles dried at room temperature and stored at 20°C or -18°C, lyophilized material stored at 4°C and frozen needles stored at -70°C). Experiments with phytoplasma infected pine and spruce trees indicated that phytoplasma detection measured by PCR assay was different depending on the host plant as well as time of testing. In *Picea tabuliformis* tree, 'Ca. P. pini' was detected in samples of needles collected every month. In contrast, in genotypes such as *Picea pungens* and *P. abies*, phytoplasma detectability was lower. In two *P. abies* trees infected with phytoplasma X-disease, the pathogen was detected in 70% of the tested samples mainly in nested PCR using P1/P7 followed by R16F2n/R16R2 primer pairs. In two *P. pungens* trees affected with 'Ca. P. pini' phytoplasma was detected only in spring and summer months. In affected *Pinus banksiana* and *P. sylvestris* plants 'Ca. P. pini' could be detected in direct PCR in fresh plant material as well in needles stored for 12 months at -70°C or stored at 4°C after lyophilization. Detectability of phytoplasma in needles dried at room temperature and stored at -18°C or at 20°C for 12 months was relatively lower; in these samples phytoplasma could be detected only by nested PCR.

FIRST OCCURRENCE OF PEAR DECLINE DISEASE IN PORTUGAL

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All over the world over than a thousand phytoplasma-associated diseases is known, causing high economic losses. They affect all kind of plant species, such as vegetables, fruit trees and ornamental plants. There is no treatment to this kind of organisms, being most of them included in EPPO quarantine list. Throughout the years, biomolecular techniques were developed to detect and identify phytoplasmas, where the most important is nested-PCR for detection complemented with RFLP for identification and recently the real-time PCR. Fruit trees' phytoplasmas are widespread, mainly in Europe, and cause severe fruit production losses. This study concerned the apple proliferation group phytoplasmas that gathers three major phytoplasmas: '*Candidatus* Phytoplasma mali', '*Ca. P. pyri*' and '*Ca. P. prunorum*', associated with apple proliferation (AP) and pear decline (PD) diseases in pome fruit trees and European stone fruit yellows (ESFY) in stone fruit trees. These phytoplasmas are vectored by psyllids *Cacopsylla picta*, *C. pyri* and *C. pruni*, respectively. Until now, there is no evidence of any of these diseases in Portugal, as well as of their vectors, except for *C. pyri*, the only one reported until now in Portuguese pear orchards. Portuguese pear cv. Rocha is widely cultivated in Portugal, being a very important icon to national agriculture. This cultivar is exported worldwide, representing a major income in Portuguese economy. Due to its importance, it is fundamental to control all diseases infecting pear trees. As *C. pyri* is very abundant in Portuguese pear orchards and cause severe damages by itself, it is important to know if the insect or the trees are infected with PD. As there are no previous records of the disease in Portugal, the main goal of this study was to assess the presence of disease in order to prevent and control future spread. As the other two vectors were never found in Portugal, one of the goals of this study was to look for them in apple orchards (where *C. picta* feeds) and prunus (peach, apricot, and cherry) orchards (where *C. pruni* feeds). The search was not successful so testing for AP and ESFY phytoplasmas was limited to vegetal samples, all negative for Portuguese orchards. Some of the captured insects revealed to be positive for the PD phytoplasma, as well as some pear tree samples. The positive results, obtained by nested-PCR were confirmed by RFLP, nucleotide sequencing and PD specific real-time PCR assays. Work on this disease is now in progress.

SURVEY FOR APPLE PROLIFERATION IN ORCHARDS CLOSE TO NURSERIES IN NORWAY IN 2011

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Apple proliferation (AP) is one of the most serious plant diseases in the main apple production areas all over Europe. The losses are estimated to be very high. Apple production is important in specific areas in both the western and the eastern parts of Norway. AP is listed as a quarantine disease in Norway and until 1996 it was never reported in Norway. A survey in the years 1996 and 1997 revealed 14 diseased trees in orchards throughout the country. It turned out that several of these cases were relatively old trees and it was suspected that some infected material entered the country in the 1970-ties and has been spread to some extent by vegetative propagation. No conclusive evidence for natural spread by vectors was found. The infected trees at these locations were eradicated. From 2000 until 2008 only two or three more infected trees were detected and eradicated. In 2010, however, some new serious cases of AP disease were found in the western parts of Norway. This led to the initiation of a survey program for orchards close to nurseries in the most important fruit districts. During autumn 2011 orchards close to nurseries were visited for visual inspection and sampled by the Norwegian food safety authorities. Small branches from sampled trees were sent to Bioforsk for testing by PCR analysis. In the orchards where no AP-like symptoms were found a random sampling was carried out. DNA was extracted using the DNeasy® Plant Mini Kit (Qiagen), according to the manufacturer's instructions. PCR was performed according to the TaqMan real-time PCR protocol as described by Nicolíć et al. 2009. In 2011, altogether 250 samples from 126 orchards close to nurseries were analyzed for AP. Altogether 139 samples representing 43 orchards were infected. AP was found in both symptomatic and asymptomatic trees. There was not found any infected trees in the nursery production in any of the nurseries in Norway. This is due to a strict use of healthy propagation material and good control of potential vector populations.

EVIDENCE FOR ‘*Ca. P. PYRI*’ AND ‘*Ca. P. PRUNORUM*’ INTER-SPECIES RE-COMBINATION

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The genetic diversity of three temperate fruit tree phytoplasmas; ‘*Candidatus* Phytoplasma prunorum’, ‘*Ca. P. mali*’ and ‘*Ca. P. pyri*’ has been established by multilocus sequence analysis. Genotyping of some Spanish and Azerbaijanese ‘*Ca. P. pyri*’ isolates revealed that they possess both ‘*Ca. P. pyri*’ and ‘*Ca. P. prunorum*’ genetic markers, supporting for the first time the existence of inter-species recombination between these two species. Particularly, isolate Spa3 had aceF and imp genes of ‘*Ca. P. prunorum*’ while its pnp and secY genes corresponded to ‘*Ca. P. pyri*’ genotypes. Isolate Spa2 also had two genes corresponding to ‘*Ca. P. prunorum*’ genotypes and two genes clustering within the ‘*Ca. P. pyri*’ phylogenetic branch, whereas AZ-POI45 had only a ‘*Ca. P. prunorum*’ pnp gene and three other gene markers corresponding to ‘*Ca. P. pyri*’. Altogether, these results indicated that these isolates of ‘*Ca. P. pyri*’ are inter-species recombinants. Surprisingly, for the Spa2 isolate, two different imp sequences could be detected, one corresponding to the one of ‘*Ca. P. prunorum*’ and the other clustering with ‘*Ca. P. pyri*’. This could not be associated with a mixed infection as only ‘*Ca. P. pyri*’ 16S rDNA sequence could be detected. A simple recombination event between ‘*Ca. P. pyri*’ and ‘*Ca. P. prunorum*’ can lead to the exchange of many gene markers at the same time. However, to allow recombination between two phytoplasma species, they have to share a common host. Peach has been described as a common host for these two phytoplasmas. Recombination might also have occurred in a common insect vector, such as *Cacopsylla pyri*, which is more polyphagous in autumn and might acquire both phytoplasmas from their respective host plant. Because none of the three ‘*Ca. P. pyri*’ recombinants had the same MLST genotype, it is likely that the recombination event is quite frequent. Indeed, it represented three cases from two distinct geographical areas over 19 different ‘*Ca. P. pyri*’ isolates tested. One could certainly consider that if recombination occurs between species it could also occur within species. We cannot confirm such a phenomenon because, despite incongruence between the phylogenetic trees of the different markers, the bootstrap validity levels were not high enough to consider it evident.

ENRICHMENT OF PHYTOPLASMA DNA BY SELECTED OLIGONUCLEOTIDES AND PHI29 POLYMERASE AMPLIFICATION

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Phytoplasma have resisted all attempts of cell-free cultivation so far. This problem hampers genome research. Elaborate and material intensive approaches are used to enrich the phytoplasma DNA. We present an amplification-based approach to obtain phytoplasma DNA from a few grams of plant tissue for downstream applications such as genomic draft sequencing. Total DNA was extracted by CTAB extraction from tobacco and parsley infected by stolbur strains 284/09 and 231/09, respectively. For enrichment, genomic DNA was amplified using oligonucleotides deduced from the four published complete phytoplasma genomes, random hexamers and Phi29 polymerase. Twenty-eight oligonucleotides were selected by frequency and distribution in the complete genomes. In additional experiments, the application of phytoplasma-specific primers P1/P7 was evaluated. *De novo* assemblies of short-reads with a length of 36 bases were generated. BLASTX against NCBI's NRPROT database using contigs with a minlength of at least 300 bp was analysed with MEGAN and taxonomical assignment of the contigs performed. Enrichment was measured by illumina's sequencing by synthesis approach. Up to a fifteen-fold increase of obtained phytoplasma draft sequence resulted from the usage of the determined oligonucleotides. Individual assemblies of short single-reads resulted in an average contig length of 1.3 kb for strain 231/09 and 2.5 kb for strain 284/09 and a total contig length of >474 kb and >498 kb, respectively. Preliminary, results indicate only weak phytoplasma enrichment in the amplification experiments supplemented with P1 and P7 oligonucleotides. Combining the reads of the individual experiments resulted in a draft sequence of >516kb for strain 231/09 and >557 kb for strain 284/09. Sequencing and annotation results highlight the potential of this strategy for uncharacterized phytoplasma genomes in particular. It is shown that cost-saving short-read sequencing can be used to generate efficient draft sequences from these templates.

GENETIC DIVERSITY, MEMBRANE TOPOLOGY, AND RELATIONSHIP TO VIRULENCE OF THE AAA+ ATPASES AND HFLB PROTEASES OF '*CANDIDATUS PHYTOPLASMA MALI*'

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One of the economically most important phytoplasma pathogen in Europe is '*Candidatus Phytoplasma mali*', the agent associated with apple proliferation disease. As other phytoplasmas, '*Ca. P. mali*' resides in plants in the sieve elements of the conducting phloem tissue where it may induce severe histopathological aberrations including the deposition of pathological callose and sieve tube necrosis. These symptoms lead to the accumulation of starch in the aerial parts of the trees and depletion of starch in the roots and may severely affect the performance of the trees. In more recent studies, accumulation of hydrogen peroxide and phloem protein and the upregulation of genes involved in synthesis of these compounds were also identified. In addition to this data on possible pathogenicity factors, phylogenetic comparison of sequences of one of the *hflB* genes (AP464) annotated in the '*Ca. P. mali*' chromosome revealed that mild and severe strains cluster separately, according to their virulence. *HflB* (synonym *ftsH*) genes encode membrane-associated ATP- and Zn²⁺-depending proteases that are conserved among bacteria and degrade misassembled and short-lived proteins and are thus contributing to quality maintenance of proteins in the membrane and the cytoplasm. The HflBs are composed of ATPase domains and a protease module. The high copy numbers of *hflB* genes in '*Ca. P. mali*' and other phytoplasmas is unusual in prokaryotes and seems to indicate their importance for these plant pathogens. To further investigate the possible role of HflB proteins in the virulence of '*Ca. P. mali*', we PCR-amplified and sequenced all genes annotated as *hflB* in the chromosome of strain AT from a representative number of mild and severe strains. Analysis of deduced amino acid sequences revealed that seven of the annotated HflBs lack the protease module and have thus to be classified as AAA+ ATPases (ATPases Associated with various cellular Activities). Furthermore, several predictors indicated that the enzymatically relevant C-termini of most of the AAA+ ATPases and about half of the HflB proteases are facing the extracellular space and may thus be involved in the impairment of sieve element function. Other findings are that full-length genes from the only cytoplasmatic ATPase (AP11) can only be amplified from mild and moderately virulent strains whereas severe strains yielded truncated genes only. Degeneration was also observed in one of the cytoplasmatic proteases (AP454) from which only a fragment could be amplified from all strains. In contrast, amplification of all AAA+ proteins facing the extracellular space resulted in full-length genes. In addition, phylogenetic analysis showed that the amino acid sequences of mild and severe strains of most of the proteins examined cluster distantly, mostly associated with the presence of virulence-related amino acid markers. In conclusion, the data reported here support previous indications that the AAA+ ATPases and HflB proteases of '*Ca. P. mali*' appear to be involved in pathogenicity.

TRANSCRIPTOME ANALYSIS OF *VITIS VINIFERA* cv. CHARDONNAY INFECTED WITH GLRAV-3 AND ASTER YELLOWS PHYTOPLASMA IN A SOUTH AFRICAN VINEYARD

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Grapevine is one of the most important fruit crops grown in South Africa. Fruit productivity and quality can be greatly affected by diseases caused by various pathogens. Grapevine leafroll disease is prevalent in the grapevine production areas of South African. More recently, vineyards with aster yellows (AY) phytoplasma infection were reported in parts of the Western Cape winelands of South Africa and AY is becoming a threat to the grapevine industry. Using next-generation sequencing, we investigated the effects of *Grapevine leafroll associated virus-3* (GLRaV-3), the predominant virus associated with leafroll disease in South Africa and aster yellows phytoplasma on the transcriptome of *Vitis vinifera* cv. Chardonnay plants grown in the field. Young shoots were collected from a 6 year-old vineyard from the Western Cape region infected with GLRaV-3 and AY. The samples were tested for the presence of common grapevine viruses in South Africa, GLRaV-3 and aster yellows phytoplasma. Twelve samples were selected for RNA sequencing: three samples negative for common viruses, GLRaV-3 and AY; three positive for GLRaV-3; three for aster yellows and the remaining three were positive for both GLRaV-3 and AY. Total RNA was extracted from these samples and sequenced using the Illumina HiSeq 2000 platform. The infected samples were compared to non-infected samples. Preliminary data showed that more genes are differentially expressed in the mixed infected plants (GLRaV-3 and aster yellow) compared to singly infected plants. The significance of the data will be discussed. To our knowledge, this is the first report investigating the effects of mixed infection between a grapevine virus and phytoplasma from field samples.

SUMMARY OF QUESTIONNAIRE DATA FOR THE PRESENCE OF PHYTOPLASMA DISEASES AND THEIR PUTATIVE VECTORS THROUGH EUROPE AND MIDDLE EAST

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Phytoplasmas occur in hundreds of commercial and wild host plants and are associated with many different diseases. In nature they are mainly spread by insects of the families Cicadellidae (leafhoppers), Cixiidae, Delphacidae and Derbidae (planthoppers), and Psyllidae (psyllids). With the development of diagnostic techniques the number of new phytoplasma diseases described worldwide markedly increased. However, there are still no effective control measures able to reduce the spread and the damage of phytoplasma diseases in many crops, mainly due to the lack of information on their epidemiology. Therefore, one of the main tasks of the WG2, which concerns phytoplasma epidemiology and vector ecology, is the establishment of a phytoplasma and vector monitoring system throughout Europe. For this purpose, a questionnaire about phytoplasma diseases and their putative vectors throughout European regions have been drafted and distributed to all members of the COST action FA0807. The obtained data have been used to create a database on the presence of phytoplasma diseases and their putative insect vectors in different European and Middle East regions. Information on the collection methods as well as on the phytoplasma detection tools is provided. Moreover a series of maps summarizing the five major phytoplasma-associated diseases (AP, ESFY, PD, BN and FD) and their known and putative vectors have been drawn up.

PSYLLID VECTORS OF THE AP GROUP (16SrX) PHYTOPLASMAS IN TURKEY

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A survey was conducted in 2010 and 2011 in order to determine the presence of known vectors of apple proliferation group phytoplasmas (16SrX), namely ‘*Candidatus Phytoplasma prunorum*’, ‘*Ca. P. mali*’ and ‘*Ca. P. pyri*’, in different plants hosts (wild plums, conifers, and/or orchard trees). *C. pruni* individuals were collected from *Prunus spinosa*, some other *Prunus* spp. and *Abies* spp. from different provinces of Turkey. Molecular typing based on COI genes and an ITS region indicated that all individuals were from the species B. ‘*Ca. P. prunorum*’ infection rate in overwintered adults of *C. pruni* was estimated as 4.70%. Phytoplasma infection rate in new generation adults of *C. pruni* collected only from Mersin province was estimated as 4.64%. Psyllids, collected from wild and cultured forms of pome fruit trees, were identified as *C. picta*, *C. melanoneura*, *C. affinis*, *C. crataegi*, *C. pyrisuga*, *C. pyri* and *C. pyricola* according to their morphological characters. The most common psyllid among the collected ones, *C. picta*, was infected with ‘*Ca. P. mali*’ with the ratio of 4.36%, but one sample was infected by ‘*Ca. P. prunorum*’. *C. crataegi* individuals collected from hawthorn were also infected by ‘*Ca. P. mali*’ (2.90%). *C. melanoneura* and *C. affinis* were infected by ‘*Ca. P. pyri*’ with respectively a ratio of 3.63% and 2.27%, and never by ‘*Ca. P. mali*’. The psyllid *C. pyri* were found positive for ‘*Ca. P. pyri*’ (2.75%). The individuals of the species *C. pyrisuga* and *C. pyricola* were negative for phytoplasma presence. These preliminary data indicated that, *C. pruni*, the main vector of ‘*Ca. P. prunorum*’ is prevalent in the country. The main vector of ‘*Ca. P. pyri*’ was *C. pyri*, and *C. picta* might be the main vector of ‘*Ca. P. mali*’ Experimental transmission assays by using these vectors are in progress.

DEGREE OF SPECIFICITY AMONG THE PSYLLID-PHYTOPLASMA INTERACTIONS AND CONSEQUENCES IN EPIDEMIOLOGY

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The psyllid vectors often show close association with the pathogens they transmit. In particular, the eight known vectors of the genus *Cacopsylla* transmit only one phytoplasma species, and on the contrary a single phytoplasma species may be transmitted by only one or no more than two psyllid species. When two species are described they are generally very closely related from a taxonomic point of view, such as for the vectors of ‘*Candidatus Phytoplasma pyri*’. Recent results confirmed this degree of specificity of the vector-psyllid interaction (*C. pruni* species complex) or suggested that this could be even more specific (different populations of the same species would be able to have a distinct transmission efficiency towards ‘*Ca. P. mali*’). On the other hand, other reports are suggesting that several species living on the same host plant may transmit the same phytoplasma (‘*Ca. P. mali*’ and the psyllids on hawthorn). We discuss why these new results have important consequences for the investigation and the risk assessment of the role of a specific psyllid species in the spread of a phytoplasma disease.

PHYTOPLASMAS ASSOCIATED WITH APRICOT CHLOROTIC LEAFROLL DISEASE

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Apricot trees are affected by apricot chlorotic leafroll disease in all major cultivation areas in Europe: the disease is killing the plants few years and it widely associated with European stone fruit yellows (ESFY) ‘*Candidatus Phytoplasma prunorum*’ belonging to 16SrX-B. Samples from apricot trees showing typical chlorotic leafroll symptoms were collected in June/July 2010 in Serbia and Italy in orchards where the disease was present in scattered distribution in 7 and 8 year old orchards. Ten symptomatic and two asymptomatic plants were tested in total. Phytoplasma detection was achieved by polymerase chain reaction and restriction fragment length polymorphism analyses using nested-PCR assays. Primers employed were P1/P7 followed by F1/B6 or R16F2n/R2. RFLP analyses on F1/B6 amplicons indicated the presence of 16SrXII in one of the samples from Serbia and of 16SrX-B in one of the samples from Italy. RFLP analyses with *TruII*, *RsaI*, *HinfI*, *HhaI*, and *HpaII* on R16F2n/R2 amplicons showed the presence of 16SrX-B phytoplasmas in 4 samples, two from the Italian orchard and two from the Serbian one. In the remaining three Serbian apricot samples a mixed infection of 16SrX-B and 16SrXII and a mixed infection of 16SrX-B, 16SrXII and 16SrI phytoplasmas were identified; one of the samples was negative. In two of the remaining samples from the Italian orchard a 16SrX-B and 16SrXII mixed infection was detected, while in the last sample 16SrX-B and 16SrIX-C phytoplasmas were identified. The use of nested PCR with PA2 primers on P1/P7 allow to detect 16rXII phytoplasma in the last sample from Serbia. In all the experiments carried out the two asymptomatic plants were negative. This is the first report of presence in symptomatic apricot of phytoplasmas different from ESFY; work to verify the epidemic relevance of these phytoplasmas in the apricot chlorotic leafroll disease is in progress.

ALMOND WITCHES' BROOM PHYTOPLASMA, OFFICIALLY DECLARED AS A REGULATED PEST IN LEBANON

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Surveys conducted during 2009/10 showed that almond witches' broom phytoplasma (AlmWB) disease, incited by 'Candidatus Phytoplasma phoenicium', was widespread in Lebanon and was detected in 16 out of 26 districts. Almond, peach and nectarine were severely affected. The North of Lebanon was considered as the epidemic center from which the disease had spread to the other regions. Due to the devastating losses already incurred and in order to reduce further future losses, HE the Minister of agriculture held a press conference on January 17, 2011 during which he officially declared AlmWB as a regulated pest in Lebanon and that a National plan will be implemented for the integrated management of the disease. Farmers will be encouraged to eliminate infected trees in all areas and will be compensated by providing free seedlings of replacement crops. The major stone fruit cropping regions in Bekaa (West Bekaa and Rachayya) will be given priority and the extension service will actively help in the eradication process. The field activities started on March 2012 with financial support from the Italian government. Updates on the actions taken will be presented during the meeting.

MONITORING DISTRIBUTION OF FRUIT TREE PHYTOPLASMAS IN BULGARIA FROM 2007 UNTIL 2011

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A survey was conducted from August to November during the years 2007 until 2011 in selected fruit tree orchards in the territory of all 28 districts of Bulgaria, as part of the official fruit trees monitoring program of National Service on Plant Protection (now Bulgarian Food Safety Agency). The plant material was collected from symptomatic and non-symptomatic trees and was sampled in late autumn when the concentration of the phytoplasma in the tree is highest. The priority was to collect samples from plant material originating from fruit tree nurseries and mother stock samples. All samples were analyzed by PCR in the Central Laboratory for Plant Quarantine. During the described period phytoplasma-infected plants were found in 10 of the 28 investigated regions. ‘*Candidatus Phytoplasma mali*’ the agent of apple proliferation (AP) and ‘*Candidatus Phytoplasma prunorum*’, the agent of European stone fruit yellows (ESFY) were found in samples from five different regions. Pear samples from 7 different regions in Bulgaria were found to be infected with ‘*Candidatus Phytoplasma pyri*’, the agent of pear decline (PD). The incidence of apple proliferation in 2009 and 2010 was reduced compared to 2007 and 2008 because of the applied phytosanitary measures. As a result, in 2011 no cases of AP infection were found. ESFY infection was found in 2 samples in 2009, in 5 samples in 2010, and as well in 5 samples in 2011. PD infection was found in 2 samples in 2009, in 6 samples in 2010, and in 4 samples in 2011. The infected plants have been destroyed through burning and the affected areas have been taken under quarantine. Phytosanitary prevention measures should be taken for ESFY and especially for PD to minimize infection spreading.

INDUCTION OF DEFENSE GENES IN APPLE LEAF TISSUES BY AN ENDOPHYTIC STRAIN OF *EPICOCCUM NIGRUM* AND ITS POSSIBLE USE IN THE CONTROL OF THE APPLE PROLIFERATION DISEASE

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Endophytes are micro-organisms living inside host plants without causing disease symptoms or visible injury. The ecological role of these organisms is still not well determined but most of them exhibit positive effect for the host plants by promoting growth, improving resistance to multiple stresses and protecting them against pathogens and insects. Apple proliferation (AP), associated to ‘*Candidatus Phytoplasma mali*’ (‘*Ca. P. mali*’) is one of the most economically important disease affecting apple in Europe. The possibility to use endophytes as biocontrol agents or resistance-inducers against phytoplasmas has been recently reported. Musetti et al., reported that an endophytic strain of *Epicoccum nigrum*, inoculated in *Catharanthus roseus*, experimentally infected with ‘*Ca. P. mali*’, reduced symptoms severity and phytoplasma titre inside the plant tissues, inducing ultrastructural modifications both to the phytoplasma and to the host. Starting from these promising results and aiming to assess the possibility of using *E. nigrum* in the AP control an experiment has been carried out using the natural host of ‘*Ca. P. mali*’, *Malus domestica*. Four groups of young apple trees (10 individuals each group) were set up: only *E. nigrum* inoculated, E⁺ AP⁻; *E. nigrum* inoculated then infected with ‘*Ca. P. mali*’, E⁺ AP⁺; only ‘*Ca. P. mali*’ infected, E⁻ AP⁺; control plants E⁻ AP⁻. AP symptom expression, plant and phytoplasma ultrastructural modifications as well as phytoplasma titre in the different groups of plant are evaluated, together with the expression of some defence-related genes. In this work, we investigated the expression of apple genes coding for three pathogenesis-related (PR) proteins, PR 1, PR 2, PR 5 and for three jasmonate (JA)-pathway marker enzymes, Allene Oxide Synthase 2 (AOS 2); 12-Oxyphytyldienoate reductase 3 (12-OPR 3); JA-inducible Proteinase Inhibitor II (PI II), comparing their relative expression levels in apple plants inoculated or not by *E. nigrum* (E⁺AP⁻ and E⁻AP⁻ plant groups). Expression profiling of the selected genes revealed a different expression pattern according the two different plant groups. In particular, PR1 and PR5 resulted significantly induced (respectively 8 and 4 folds) in E⁺AP⁻ plants 3 day after endophyte inoculation (dai) compared to the uninoculated controls (E⁻AP⁻). The expression level of the induced genes tends to return comparable to the control two weeks and two months after endophyte inoculation. On the other hand variations in the expression level of the genes encoding the three JA-pathway marker enzymes at 3 dai seem to be not considerable in E⁺AP⁻ leaf tissues compared to the control. From these preliminary results, we can hypothesize that *E. nigrum* inoculation can induce in apple defense responses related to salicylic acid pathway and PR protein gene expression, inhibiting, on the other hand, JA-related defense pathway. Further analyses will be carried out aimed to understand the mechanism(s) by which *E. nigrum* interacts with apple plants and to assess the real possibility of its usefulness in AP disease control.

POSTER SESSION I
FRUIT TREES:
VIRUSES, VIROIDS AND PHYTOPLASMAS

INCIDENCE OF *FIG LEAF MOTTLE-ASSOCIATED VIRUS* AND *FIG MOSAIC VIRUS* IN EASTERN PROVINCE OF SAUDI ARABIA

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Fig plant, *Ficus carica* L., is grown in Saudi Arabia and is being affected by fig plant mosaic diseases (Fig leaf mottle-associated virus, Fig mosaic virus). The main symptoms are chlorotic mottling, blotching and various types of leaf deformation. Samples were collected, with consideration of the economically importance and distribution of the cultivars, from different areas of Hofuf Saudi Arabia. Each sample was consisted of 10-15 leaves. Samples were labelled and stored in plastic bags at 4°C; then transferred to the laboratory, for total nucleic acids (TNAs) extraction. One hundred mg of leaf veins and or cortical scrapings were used for extraction. Samples were macerated in 1 ml of grinding buffer. TNAs were recovered with a silica-capture procedure and stored at -20°C till used. 8-10µl of TNA extracts were mixed with 1µl random hexamer primer, (Boehringer Mannheim, GbmH) (0.5 µg/µl). RT-PCR assay of leaves extracts of infected fig accession using specific primers gave positive results and non with FLMaV-2. Mixed infection of FLMaV-1 and FMV were found. To our knowledge this is the first record and identification of FLMaV-1 and FMV in Saudi Arabia. Further studies are needed to investigate the fig mosaic disease throughout the country.

THE INCIDENCE OF *PRUNUS NECROTIC RINGSPOT VIRUS* IN APRICOT AND PEACH IN SAUDI ARABIA

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Cultivated areas with fruit trees in Saudi Arabia are estimated at 233,513 h and produce more than 1.6 million tons of fruits annually. *Prunus necrotic ringspot virus* (PNRSV), *Prune Dwarf Virus* (PDV) and *Plum Pox Virus* (PPV) are most important and common viruses infecting stone fruit trees and present in nearby countries, Jordan, Lebanon, Syria and Egypt. In spring 2009, field surveys were carried out in an area of stone fruit production (Al Juof - North of Saudi Arabia) to assess viruses of stone fruit trees. Apricots and peaches were observed showing chlorotic rings, necrotic spots, and a shothole appearance. A total of 166 leaf samples (65 Apricots and 101 Peach) were collected to be analyzed for the causal virus. Double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) was performed using the commercially available for PNRSV. Result showed that 30 (11 Apricot and 19 Peach) out of 166 leaf samples were infected with PNRSV. Total RNA, from same samples used in ELISA test, was extracted in accordance with its instructions. RT-PCR was performed using for PDV, PNRSV and PPV specific primers. RT-PCR resulted in the amplification of a 346 bp fragment as expected and negative result with PPV and PDV. The obtained result indicating the presence of PNRSV in Apricot and Peach in Saudi Arabia. The amplified fragment was sequenced and deposited in GenBank (Accession No. HM584814). The sequence was compared with PNRSV isolates and had 100% identity with AF170170 from Czech Republic while 97% identity for PNRSV from USA (AF013287). Further investigations needed for other commercial orchards and nurseries. This result demonstrates first detection of PNRSV in Saudi Arabia.

EVIDENCE FOR ‘*CA. P. PYRI*’ AND ‘*CA. P. PRUNORUM*’ INTER-SPECIES RECOMBINATION

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The genetic diversity of three temperate fruit tree phytoplasmas; ‘*Candidatus* Phytoplasma prunorum’, ‘*Ca. P. mali*’ and ‘*Ca. P. pyri*’ has been established by multilocus sequence analysis. Genotyping of some Spanish and Azerbaijanese ‘*Ca. P. pyri*’ isolates revealed that they possess both ‘*Ca. P. pyri*’ and ‘*Ca. P. prunorum*’ genetic markers, supporting for the first time the existence of inter-species recombination between these two species. Particularly, isolate Spa3 had *aceF* and *imp* genes of ‘*Ca. P. prunorum*’ while its *pnp* and *secY* genes corresponded to ‘*Ca. P. pyri*’ genotypes. Isolate Spa2 also had two genes corresponding to ‘*Ca. P. prunorum*’ genotypes and two genes clustering within the ‘*Ca. P. pyri*’ phylogenetic branch, whereas AZ-POI45 had only a ‘*Ca. P. prunorum*’ *pnp* gene and three other gene markers corresponding to ‘*Ca. P. pyri*’. Altogether, these results indicated that these isolates of ‘*Ca. P. pyri*’ are inter-species recombinants. Surprisingly, for the Spa2 isolate, two different *imp* sequences could be detected, one corresponding to ‘*Ca. P. prunorum*’ and the other clustering with ‘*Ca. P. pyri*’ *imp* sequences. This could not be associated with a mixed infection as only ‘*Ca. P. pyri*’ 16S rDNA sequence could be detected. A simple recombination event between ‘*Ca. P. pyri*’ and ‘*Ca. P. prunorum*’ linear chromosomes can lead to the exchange of many gene markers at the same time. However, to allow recombination between two phytoplasma species, they have to share a common host. Peach has been described as a common host for these two phytoplasmas. Recombination might also have occurred in a common insect vector, such as *C. pyri*, which is more polyphagous in autumn and might acquire both phytoplasmas from their respective host plant. Because none of the three ‘*Ca. P. pyri*’ recombinants had the same MLST genotype, it is likely that the recombination event is quite frequent. Indeed, it represented three cases from two distinct geographical areas over 19 different ‘*Ca. P. pyri*’ isolates tested. One could certainly consider that if recombination occurs between species it could also occur within species. We cannot confirm such a phenomenon because, despite incongruence between the phylogenetic trees of the different markers, the bootstrap validity levels were not high enough to consider it evident.

EPIDEMIOLOGICAL STUDY OF APPLE PROLIFERATION IN SPAIN

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Apple proliferation (AP) phytoplasma, classified as '*Candidatus Phytoplasma mali*' was first described in Italy in the 1950s and is associated with severe epidemics and considerable economical losses in apple orchards from all over Europe. In Spain it was identified in apple varieties for production of cider in 2010. In Europe two psyllid species have been reported as vectors of the phytoplasma: *Cacopsylla picta* (syn. *C. costalis*) and *Cacopsylla melanoneura*. However, their distribution, infectivity and transmission capacity vary among different geographical areas. The aim of the present study was to determine the incidence of '*Ca. P. mali*' in host plants and to follow the population evolution and percentage of individuals of *C. melanoneura* and *C. picta* carrying the AP phytoplasma in the affected plots. The incidence of the disease in the affected plots ranged between 20 to 80%. The most affected plots were those planted with the local cider cultivars. The phytoplasma was also identified in the cultivar Reineta gris. The surveys conducted in 2010 and 2011 in several apple growing areas showed that both vectors species of *Cacopsylla* reported as vector of the disease were present in the Basque Country and Asturias (North Spain). The population dynamics of these species showed two peaks, one for adults re-immigrants, which in both years occurred in early April and another one for new generations between June and July. The higher population level of these species, vectors of '*Ca. P. mali*', has been found in the most affected plot (Orberlaún, Basque Country). The high percentage of individuals carriers of the phytoplasma was obtained between the months of June and July (25-33% of positives) for *C. picta* in Orbelaún (Basque country) and for *C. melanoneura* (33-100%) in Siero (Asturias) in 2010 and 2011.

DETECTION AND MOLECULAR CHARACTERIZATION OF A NOVEL CRYPTOVIRUS FROM PERSIMMON (*DIOSPYROS KAKI*)

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Two dsRNA molecules with an estimated length of 1.5 Kbp were identified and characterized from leaves of a Japanese persimmon (*Diospyros kaki*) tree, showing veinlets necrosis on both sides of leaf blades. DOP-PCR recognized two genomic fragments of a bipartite cryptic virus, for which the name of Persimmon cryptic virus (PeCV) is proposed. RLM-RACE led to the sequencing of a 1,510 bp contig identified as dsRNA-1 and a 1,491 bp complete segment identified as dsRNA-2. The two genomic fragments resulted both monocistronic and harbored conserved domains related to RNA-dependent RNA polymerase (RdRP) and capsid protein (CP) of species associated to *Alphacryptovirus* genus. Phylogenetic analysis of RdRP sequence showed highest amino acid identity with Black raspberry cryptic virus (BrCV, 63 %), Pepper cryptic virus 2 (PCV-2, 52 %) and -1 (PCV-1, 46 %). Whereas the CP putatively encoded by dsRNA-2 shared highest identity with Mulberry cryptic virus 1 (MCV-1, 49 %), PCV-2 (39 %) and PCV-1 (32 %). N-J phylogenetic analysis confirmed those relationships and delivered PeCV in a cluster with phyto-cryptoviruses belonging to genera *Alpha-* and *Betacryptovirus*, quite far distinguished from myco-cryptoviruses, gathered in genus *Partitivirus*. Virus-specific primers for RT-PCR were successfully designed inside the CP region to detect PeCV in several symptomless trees found in different orchards of Apulia (Southern Italy), thus proving that infection may be fairly common and presumably latent. An antiserum (kindly provided by Dr. M. Turina, CNR-IVV, Torino, Italy) specific to the CP of family-related *Beet cryptic virus 2* (BCV-2) was profitably used for western blot detection of a 45-50 KDa band, coherent with predicted size of dsRNA-2 product. Furthermore, antibodies were useful for ISEM observation and subsequent decoration of PeCV particles, proved to be isometric, around 30 nm in diameter, with rounded shape lacking in fine structural details, not easily permeable by negative stain.

EFFICACY OF APPLE PROLIFERATION PHYTOPLASMA TRANSMISSION BY VEGETATIVE PROPAGATION TECHNIQUES AND IMPACT ON ITS EPIDEMIOLOGY

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In Europe, the apple proliferation phytoplasma is considered as a quarantine pest (2000/29EC) and it is widespread in central and southern Europe where it is associated with important economic losses. While its natural transmission by insect vectors is well studied, few publications concern its transmission by grafting. We studied the efficiency of transmission of apple proliferation phytoplasma by grafting reproducing three of the main techniques for vegetative propagation of material used in nursery. For each experiment, we collected budwoods of young trees artificially infected by a French strain of apple proliferation phytoplasma. These trees were previously controlled by PCR and displayed characteristic symptoms of the diseases during the previous growing season if material was collected during the winter time, or during the season if material was collected during the springtime and summer season. Those symptoms were foliar reddening, witches broom and enlarged stipules. Collected budwoods were grafted by three different techniques: in 2005 during springtime by chip budding on M7 rootstock, in 2006 during summer time by chip budding on M7 rootstock and in 2007 during winter time by bench grafting on M7 rootstock. The material grafted in 2006 and 2007 were monitored during one growing season by visual inspection and PCR tests then by visual inspection respectively till 2010 and 2011 whereas the material grafted in 2005 was monitored in the same way till 2008. The main result revealed that after one year, only material grafted by bench grafting displayed characteristic symptoms of apple proliferation. Those symptoms were further confirmed by PCR testing. Moreover, only plants showing symptoms were positive by PCR tests. The results of these experiments showed the limited impact of the propagation process in nursery on the spreading of the apple proliferation phytoplasmas in nurseries and in orchards.

SUSCEPTIBILITY OF SOME APRICOT AND PEAR CULTIVARS ON VARIOUS ROOTSTOCKS TO ‘*CANDIDATUS PHYTOPLASMA PRUNI*’ AND ‘*CA. P. PYRI*’

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Four pear and five apricot cultivars were bud-inoculated on different rootstocks and evaluated for their host response to pear decline (PD) and European stone fruit yellows (ESFY) phytoplasmas over a period of 3 years. As a pear cultivars Santa Maria, Williams, Deveci and Ankara were grafted on *Pyrus communis*, OHF333 and BA29 rootstocks whereas apricot cultivars Tyrinte, Sakıt, Tokalı, Şekerpare and Hacıhaliloğlu were grafted on Myrobolan 29 C and wild apricot. In all grafting experiments each combination were replicated ten times. Visual inspection and PCR/RFLP analyses were performed 6 months intervals. Despite not any typical symptoms of PD and ESFY were observed on any combination, phytoplasma infection from the first year after plantation was confirmed by PCR/RFLP analyses. *P. communis* and OHF 333 were the most susceptible rootstocks for local pear cv. Deveci and as well as for Santa Maria and Williams cultivars. None of the cultivars, grafted on BA 29 were found phytoplasma infected in three years. For ESFY wild apricot seems to be more susceptible for all apricot cultivars compared to Myrobolan 29 C. Apricot cultivar, Tyrinte resulted to be the most susceptible to ESFY independently from the rootstock employed.

GENOME CHARACTERIZATION OF A DIVERGENT *LITTLE CHERRY VIRUS 1* ISOLATE ASSOCIATED WITH THE SHIROFUGEN STUNT DISEASE

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Shirofugen stunt (SS, Desvignes, 1999) is a syndrome of *Prunus serrulata* cv. Shirofugen indicators grafted with some sweet (*P. avium*) or sour (*P. cerasus*) cherry sources. It is characterized by strong rosetting with dwarfed and deformed leaves, reduced vigour, gradual necrosis at the union and sometimes die-off of the indicator after a few vegetative cycles. These symptoms are reminiscent of the recently described 'Kwanzan stunting' syndrome (Matic *et al.*, 2009). The causal agent of SS is currently unknown. Double-stranded RNAs purified from the V2356 (cv. Successa) german sour cherry source of SS were sequenced using pyrosequencing technology. The 15646 reads obtained were assembled into 279 contigs. Five of these contigs, totalling almost 16.9 kbp and 5332 reads (34% of sample reads) showed high Blast scores and homology to *Little cherry virus 1* (LChV1). They were further assembled manually into three supercontigs spanning the full LChV1 genome with only two small gaps (17 and 55 bases). Completion of the sequencing of the viral genome was performed using targeted PCR and primers designed from the contigs. No evidence for other viral agents could be identified in the remaining contigs or singletons. The V2356 LChV1 isolate is only about 76% identical with the reference LChV1 sequences and, in particular, with the ITMAR isolate associated with the Kwanzan stunting syndrome (Matic *et al.*, 2009). It is however highly homologous (97-100% identity in the short region sequenced by Bajet *et al.*, 2008) with divergent LChV1 from North America, providing the first complete sequence for such divergent isolates. Although not providing a definite proof, the failure to detect any other viral agent in the V2356 SSD source strongly suggests that the divergent LChV1 isolate identified could be responsible for the SSD syndrome. The association of similar agents in other SS sources is currently under evaluation. Bajet, N.B. *et al.*, 2008. Occurrence of two Little cherry viruses in sweet cherry in Washington State. *Plant Disease*, 92, 234-238. Desvignes, J.C., 1999. *Virus diseases of fruit trees*. CTIFL editions, France. Matic, S. *et al.*, 2009. 'Kwanzan stunting' syndrome: detection and molecular characterization of an Italian isolate of *Little cherry virus 1*. *Virus research*, 143, 61-67.

DETECTION AND CHARACTERIZATION OF PHYTOPLASMAS INFECTING APPLE TREES IN POLAND AND IDENTIFICATION OF THEIR POSSIBLE VECTORS

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During 2009-2011 a survey of the 14 apple orchards and several home gardens in different regions of Poland was conducted for the presence of phytoplasmas. In 2011, to determine putative vectors of phytoplasmas, the insects were captured in ten locations using yellow sticky traps or by the beat tray method. Total DNA extracted from samples of 134 apple trees was subjected to nested PCR with primer pairs P1/P7 followed by R16F2n/R16R2. Primer sets targeting 16S rRNA and ribosomal protein (rp) gene sequences *rpl22* and *rps3* of '*Candidatus Phytoplasma mali*' (apple proliferation phytoplasma group) as well as primer pairs specific to the '*Candidatus Phytoplasma asteris*' (aster yellows phytoplasma group) target genes 16Sr RNA, rp, *secY* and *tuf* genes were also used in this study. Phytoplasmas were detected in 12 trees. 16S rDNA RFLP analysis with *RsaI*, *HhaI*, *MseI*, *SspI* allowed to identify '*Ca. P. mali*' in eight apple trees. Based on RFLP patterns of rp gene sequences, these isolates were identified as belonging to subgroup rpX-A, type AP. PCR/RFLP results were confirmed by sequence analysis of the 16S rDNA and rp fragments. In turn, RFLP analyses on 16S rRNA, rp, *secY* and *tuf* genes showed that four apple trees were infected with phytoplasma belonging to aster yellows group, subgroup B. In ten selected orchards 368 adult of *Cacopsylla melanoneura*, 108 of *C. picta* and 77 of leafhoppers were captured. Phytoplasma presence in the insects (batches of five) was analysed by nested PCR with universal primers as well as primer pairs specific for 16SrI and 16SrX groups. '*Ca. P. mali*' was identified in two out of 73 batches of *C. melanoneura* and in four out of 21 batches of *C. picta*. '*Ca. P. asteris*' was detected in a single leafhoppers' batch out of the 15 tested.

TRANSMISSION TRIALS OF THE FIG MOSAIC DISEASE AGENT

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In recent times, a number of different fig (*Ficus carica*)-infecting viruses have been identified. However, only *Fig mosaic virus* (FMV) appears to be strictly associated with fig mosaic (FM) disease and the intracellular quasi-spherical double-membrane-bound bodies (DMBs), reported by various authors, are now considered the particles of this novel virus (Elbeaino *et al.*, J. Gen. Virol., 90, 1281-1288, 2009). A FM-affected tree, also infested by the eriophyid mite vector *Aceria ficus*, was found in Emilia-Romagna (northern Italy). This FM source was used for first investigations and a disease-inducing agent was unexpectedly mite-transmitted to a periwinkle (*Catharanthus roseus*) plant (Credi, 7th ICPP, abstract 1.13.14, 1998). Based on this finding, transmission studies have been lastly extended. A FM-free *A. ficus* colony was established and reared on healthy fig plants. Small portions of heavily infested leaves were placed onto leaf pieces of the diseased periwinkle (FM-E source) and maintained in a humid environment for a 30-h period. Periwinkle leaf pieces, with potentially infected mites, were then transferred to healthy fig seedlings. Of the 115 test plants, 7 (6%) displayed mosaic symptoms a month postinoculation. Subsequently, one of these was selected and employed as inoculum source (FM-R) for further back-inoculation assays. Of the 135 fig and 6 periwinkle test plants, those successfully inoculated by *A. ficus* resulted to be 133 (98.5%) and 2 (33.3%), respectively. RT-PCR analyses showed that the donor sources of FM used in our transmission experiments likely contained a single infection of FMV. The virus was also detected in *A. ficus* individuals that fed on a diseased fig plant. Moreover, electron microscopy observations revealed the presence of DMBs in leaf parenchyma cells of symptomatic, and FMV-positive, fig and periwinkle plants. From our work convincing evidence was obtained indicating FMV as the real causal agent of FM.

FIRST SURVEY ON POME FRUIT VIRUSES IN LEBANON

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In order to evaluate the incidence of virus diseases in apple, pear and quince in Lebanon, a large-scale survey was carried out in the main pome fruit-producing areas. To this aim, the most important commercial orchards and nurseries in the Country were tested by a combination of biological (woody indexing and mechanical inoculation), serological (ELISA) and molecular (RT-PCR) assays in order to detect the following viruses: *Apple chlorotic leaf spot virus* (ACLSV), *Apple stem pitting virus* (ASPV), *Apple stem grooving virus* (ASGV), and *Apple mosaic virus* (ApMV). A total of 968 samples (713 apples, 175 pears and 80 quinces) were tested by DAS-ELISA, revealing that ACLSV, ASPV and ASGV were prevalent in apple trees, with an average rate of infection with at least one of the viruses corresponding to 27%. The incidence of the same viruses in pear samples was 1 %, whereas no virus was detected in quince. ACLSV was the most predominant virus (16%) followed by ASPV (13 %) and ASGV (2.5%). In contrast, ApMV was not detected in any of the testes samples. RT-PCR assays performed on a fraction of 100 samples, confirmed the DAS-ELISA results, although additional positive samples were identified, showing higher sensitivity of the former with respect to the latter detection method. The implications of our study for improving the phytosanitary status of pome fruit trees in Lebanon will be discussed.

PARTIAL CHARACTERIZATION OF *FIG MOSAIC VIRUS* IN INOCULATED FIG AND PERIWINKLE PLANTS BY DOP-PCR

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Fig mosaic virus (FMV) belongs to recently proposed genus *Emaravirus* of the family *Bunyaviridae* and is one of the causal agent of fig mosaic disease. In this study, partial characterization of FMV in experimentally inoculated fig (DE10) and periwinkle (V19) plants by *Aceria ficus* Cotte. was performed by DOP-PCR analyses. DsRNAs were isolated from leaves after first symptom appearance and cDNA was synthesized by using random primers. DOP-PCR was performed and amplicons were transformed to *Escherichia coli* DH 5 α after ligation of pGEM-T-Easy vector. Plasmids were selected and isolated after growing on media and sequenced. Sequence analyses were done by VectorNTI program and NCBI. The obtained partial sequences showed high similarity with Italian (98 %) and Californian (99 %) FMV isolates. Electron microscope observations confirmed double membrane bodies which are related to FMV, in palisade mesophyll cells of inoculated fig seedling.

IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF ‘*CANDIDATUS PHYTOPLASMA PYRI*’ IN PEAR TREES FROM CENTRAL ITALY.

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During a field survey carried out in early autumn, pear trees showing symptoms resembling those associated with phytoplasma presence were found in an orchard located in Latium region (Central Italy). Samples from four distinct symptomatic plants were collected and submitted to molecular detection. DNA extracts from collected samples were amplified on 16S gene using the universal primer pairs P1/P7 and R16F2n/R2 in direct and nested PCR, respectively. Amplicons of the expected size were obtained from all tested samples and the presence of ‘*Candidatus Phytoplasma pyri*’ (16SrX-C subgroup) was detected for the first time in Latium region by RFLP analysis of the R16F2n/R2 amplicons after digestion with *RsaI* and *SspI* restriction enzymes. In order to further investigate the genetic characteristics of the identified ‘*Ca. P. pyri*’ isolates a preliminary characterization was performed on *tuf* gene using a PCR/RFLP based method. All samples were then submitted to a multilocus sequence analysis on *aceF*, *pnp*, *secY* and *imp* genes. RFLP analysis of the *tuf* genes amplicons showed the presence of two distinct profiles. Out of four tested trees, 3 samples showed the same RFLP profile, identical and not distinguishable from those of pear decline (PD) reference control whereas a different profile was found in the remaining sample. On the basis of multilocus sequence analyses, high identity percentage (99-100%) was observed among the *secY* and *imp* nucleotide sequences of all tested isolates, always corresponding to ‘*Ca. P. pyri*’. High sequence similarity with ‘*Ca. P. pyri*’ was also observed on *aceF* and *pnp* genes in 3 out of 4 analyzed isolates whereas the remaining sample showed the *aceF* and *pnp* genes highly identical (99%) to a ‘*Ca. P. prunorum*’ genotype. This sample not corresponded to those showing a different RFLP profile on *tuf* gene suggesting no correlation on the genomic variability observed on the investigated genes. The obtained results show that molecular variability occurs also among ‘*Ca. P. pyri*’ isolates from the same orchard and confirm the evidences on the existence of inter-species recombination in ‘*Ca. P. pyri*’, already reported by other author. This is the first report of possible recombinant genotype in ‘*Ca. P. pyri*’ Italian isolates.

DETECTION AND MOLECULAR CHARACTERIZATION OF *CHERRY GREEN RING MOTTLE VIRUS* (CGRMV) AND *CHERRY NECROTIC RUSTY MOTTLE VIRUS* (CNRMV) IN SWEET CHERRY IN CHILE

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In Chile, sweet cherry represent one of the most valuable crops with approximately 13,140 ha of plantations in 2010, and the country is the main producer of cherries in the southern hemisphere. In one orchard of the Maule region (South of Santiago), plants of the cv. Bing, with and without brown angular necrotic spots, areas of dead tissue, and shot holes of the leaves, have been sampled during spring and summer time. Samples were analyzed by RT-PCR with specific primers for the detection of *Prunus necrotic rigspot virus* (PNRSV), *Prune dwarf virus* (PDV), *Apple chlorotic leaf spot virus* (ACLSV), *Cherry green ring mottle virus* (CGRMV) and *Cherry necrotic rusty mottle virus* (CNRMV). All plants with symptoms tested positive for CGRMV and CNRMV. In a few plants without symptoms was detected CGRMV, while PNRSV was found in some plants with and without symptoms. Primers were designed for amplification of the gene encoding the coat protein of CNRMV and CGRMV. The amplicons obtained were purified and cloned. Putative recombinant clones were analysed by colony-PCR using primers to vector sequences flanking the polylinker. Amplicons obtained from three colonies per cloned fragment were sequenced in both directions. The sequence analysis allowed us to establish a phenetic relationship between Chilean CNRMV isolates with another detected in India, and Chilean CGRMV isolates with those found in USA, Canada, Poland, Lebanon and Italy. It is known that CGRMV does not cause symptoms in cherry varieties of commercial interest, therefore results suggest that CNRMV is the cause of the observed symptoms, which may be more severe when the plant is simultaneously infected by PNRSV. This is the first molecular characterization of Chilean isolates of CGRMV and CNRMV.

MOLECULAR VARIABILITY IN THE COAT PROTEIN GENE OF THE DIFFERENT ISOLATES OF *APPLE MOSAIC VIRUS*

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Hop (*Humulus lupulus* L.) is without doubt one of the most intensively cultivated perennial crop in the Czech Republic representing a very important agricultural export commodity with a traditional place among the global hop producers. It is a host of several plant viruses with *Apple mosaic virus* (ApMV) identified as the predominant viral pathogen on the cultivated plants. Its infection used to have dramatic influence on yield and quality of producing hop in the past; however, thanks to the certification scheme for the production of healthy plants for planting, the ApMV has been almost eliminated from crop production till nowadays. ApMV isolates from 23 asymptomatic Czech, European and non-European hop plants were obtained from the Hop Research Institute Co., Ltd. Besides, ApMV isolates from European elder, mountain ash, hazelnut, peach and apricot were obtained and included in the study. The complete coat protein (CP) genes were amplified by using the RT-PCR. DNA amplicons were cloned and their nucleotide sequences were determined. The study objectives were to define the molecular variability of genomic information among the Czech ApMV isolates from hop and to compare them to those isolates from different areas or to those from various hosts. The identities of CPs were 95.2-100% and 91.2-100% at the nucleotide and amino acid sequences, respectively. In phylogenetic analysis, no reasonable correlation between geographic origins of hop plants was observed. However, the host origin played a significant role in the CP heterogeneity. Together with several previously characterized ApMV isolates from the GenBank database, ApMV strains could be classified into three subgroups in both nucleotide and amino acid levels: subgroup I with ApMV isolated from almond tree, subgroup II with all pome fruit isolates and subgroup III with hop, European elder, mountain ash, hazelnut, peach, apricot, prune and *Prunus mahaleb*.

NOVEL GENOMIC RNA SEGMENTS OF *FIG MOSAIC VIRUS*, A NEWLY IDENTIFIED *EMARAVIRUS* INFECTING THE COMMON FIG (*FICUS CARICA*)

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Recently, fig mosaic virus (FMV) was identified as the causal agent of fig mosaic disease (FMD). FMV was reported to be a four-segmented negative-strand RNA virus in the newly formed genus *Emaravirus*, whose putative members are thought to consist of four or five RNA segments. In this study, we collected fig (*Ficus carica*) leaves with typical FMD symptoms from Japan and Serbia, and detected 14 FMV isolates. In an attempt to amplify all four of the known viral RNA segments of FMV (RNA1, RNA2, RNA3, and RNA4) simultaneously, we performed RT-PCR using primers based on the conserved 13-nt stretches found at the 3' and 5' termini of FMV genomic segments. The resulting simultaneous amplification of all FMV genomic segments yielded the four previously identified segments of FMV and two novel segments from all 14 FMV isolates, and northern blot analysis was performed to characterize these two novel RNA segments. Consequently, both the sense and antisense strands of these novel RNA segments were detected in FMV-infected fig leaves, confirming that they replicate as FMV genomic segments. Sequence analysis revealed that the novel RNA segments are similar in their organization: each contains a single open reading frame on the viral complementary RNA strand flanked by untranslated regions that include the conserved, complementary terminal sequences. Moreover, the novel RNA segments showed similar molecular evolutionary patterns to those of known FMV genomic RNA segments. Our findings indicate that these newly discovered RNA segments are previously unidentified FMV genomic segments, which we have designated RNA5 and RNA6.

INVESTIGATIONS ON THE OCCURRENCE OF THREE LATENT APPLE VIRUSES THROUGHOUT THE YEAR AND SEQUENCE VARIABILITY OF *APPLE STEM PITTING VIRUS*

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Apple stem pitting virus (ASPV) is a latent virus of apple and belongs to the genus *Foveavirus*, family *Flexiviridae*. Mixed infections with *Apple chlorotic leaf spot virus (ACLSV)* and *Apple stem grooving virus (ASGV)* can cause up to 60% loss of yield. Virus RNA extractions were made from buds or leaves, phloem, and roots from four different trees at monthly intervals throughout the year followed by RT-PCR tests for evaluation of seasonal detection reliability for ASPV. In tests using published ASPV primers phloem was the most reliable tissue for detecting the virus throughout the year in 20 year old trees. Mixed infections of ASPV, ACLSV and ASGV were established by grafting onto young apple trees. These were examined monthly by RT-PCR for the three viruses, beginning one year after infection. The results showed that April was the preferable month for virus detection in leaves while analyses in the summer months were less reliable. To investigate further variability of ASPV, the complete sequences of European ASPV isolates PB 66 and Han were obtained and compared. Isolates PB 66 and Han showed 80% and 74% nucleotide sequence identity, respectively, with PA 66. The ASPV isolates PB 66 and Han showed 75% nucleotide sequence identity. An alignment of the coat protein gene revealed deletion events associated with PB 66 and Han when compared to PA 66. The coat protein of ASPV Han has also an additional insertion event. The complete coat proteins were compared with database entries and a phylogenetic tree was constructed.

DETECTION OF *CHERRY VIRUS A* AND *CHERRY GREEN RING MOTTLE VIRUS* IN GREEK SWEET CHERRY ORCHARDS

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Sweet cherries are susceptible to a number of virus species of the family *Betaflexiviridae*, but so far the presence of such viruses has not been extensively studied in Greek orchards. During surveys conducted in spring 2007 and 2009 for the presence of betaflexiviruses in cherry plantations of Northern Greece, *Cherry virus A* (CVA) and *Cherry green ring mottle virus* (CGRMV) were identified using a generic nested RT-PCR targeting a fragment of the viral RdRp, which has been developed previously in our lab. For the specific detection and study of the incidence of each of these viruses, the nested PCR step of the generic *Betaflexiviridae* assay was modified to include CVA and CGRMV specific primer pairs. Thus, new degenerate primers were designed based on the available in the databases and herein obtained RdRp sequences of both viruses and used in respective nested PCR assays. The developed methods showed high sensitivity and broad detection range. The application of the assays for screening 86 cherry trees from various areas of Northern Greece showed a rather high incidence for both CVA (19/86) and CGRMV (20/86). Sequencing of the amplified products confirmed the specificity of the assays while it further indicated the presence of variability within each of the virus species tested. To our knowledge these findings represent the first report of CVA in sweet cherry trees in Greece and call for large scale surveys for the study of both viruses in more *Prunus* species, for which currently no information exists.

PARTIAL CHARACTERIZATION OF A DISTINCT *LITTLE CHERRY VIRUS 1* ISOLATE REVEALS HIGH INTRASPECIES GENETIC VARIABILITY

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Little cherry virus 1 (LChV1) and *Little cherry virus 2* (LChV2), two members of the family *Closteroviridae*, are associated with Little cherry disease (LChD) which is distributed worldwide and has a great impact on fruit quality of infected trees. Both viruses are characterized by high intraspecies variability with LChV1 being the most variant. During surveys conducted in 2002 for the presence of these viruses in sweet cherry orchards of Northern Greece, one sample in which LChV-1 and -2 could not be detected with specific primer pairs, gave positive reaction in closterovirus infection using a generic nested RT-PCR targeting the N terminus of the viral HSP70. Sequencing and comparative analysis of the product amplified with the generic assay indicated the presence of a virus distantly related to the so far known LChV-1 isolates and therefore it was further characterized. Using combinations of LChV-1 specific and generic primers a 5900 nucleotides long fragment of the virus genome including the helicase (partial), RdRp, the small hydrophobic protein, the HSP70 homolog and the p61 (partial) genes was determined. High sequence divergence was found compared to other known LChV-1 isolates which was ranging from 7-10% in the RdRp up to 21% in amino acids in the p61 protein. Intriguingly, the variability was increasing from the 5' to 3' end of the genome. Phylogenetic analysis using the N terminus of the HSP70h placed this isolate in a separate clade most closely related with a partially characterized Californian isolate. These findings demonstrate that LChV-1 variability may be even higher than previously assumed, thus putatively affecting the reliability of the currently used detection assays and necessitates further investigation.

THE MONITORING OF OCCURRENCE OF FOUR COMMON VIRUSES IN PLUM CULTIVARS IN LATVIA

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Viral diseases of fruit trees can cause considerable losses in commercial orchards. Timely monitoring of virus infections and elimination of infected trees from the orchards is the key point in preventing the spread of viruses. In previous studies using ELISA the presence of *Ilarviruses* in plum orchards were detected. In this study the occurrence of *Apple mosaic virus* (ApMV), *Prunus dwarf virus* (PDV), *Prunus necrotic leaf spot virus* (PNRSV) and *Plum pox virus* (PPV) were investigated and detection rate by ELISA and RT-PCR were compared. In total 654 leaf samples of different cultivars were collected from several commercial plum orchards during 2008. The samples were collected randomly from trees without symptoms. PNRSV and PDV were detected by RT-PCR in 30.7% and 16.4% of the samples examined, respectively. The results indicated that these two viruses are widespread in comparison to ApMV and PPV, which were detected in 1.8% and 1.5% of the samples, respectively. Many plum trees were infected with two viruses. Most often (11 %) detected mixed infection was PDV+PNRSV. ApMV in combination with PDV was detected in 0.5% of the tested samples and ApMV with PNRSV was detected in 1.2% of the samples. Mixed infection of three viruses was detected only in few samples. The PPV-D strain was detected by strain specific primers in all positive samples. In total, 52.4% of the samples tested by RT-PCR were negative. No specific relation between particular virus and host cultivars was observed. As expected, comparative analyses showed that RT-PCR was more sensitive than ELISA. With RT-PCR the number of positive samples for PNRSV and PDV was almost two times higher than obtained by ELISA. Therefore methods based on nucleic acid analysis are now widely used and considered more reliable for diagnosis of fruit tree viruses than ELISA.

A NEW *TRICOVIRUS* ISOLATED FROM PEACH TREES IN MEXICO

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The survey of peach samples from different Mexican regions by multiplex RT-PCR with the capability to detect and identify eight stone fruit tree viruses revealed the presence of an unexpected amplicon of 277 nucleotides which electrophoretic mobility did not match with any of the analyzed viruses. The cloning and further analysis of the nucleotide sequence revealed a percentage of identity of 83% and 77% with the coat protein gene of *Cherry mottle leaf virus* and *Peach mosaic virus*, respectively. The use of specific primers target to conserved regions of tricoviruses permitted the amplification of the 3' end 2,205 nucleotides. Blast analysis of the new nucleotide sequence rendered the higher percentage of identity for Peach mosaic virus (DQ117579) with a 68.2%. The Blast analysis of the amino acids sequences corresponding to the end of the movement protein (409 residues), the coat protein and the nucleic acid binding protein, revealed that the higher percentages of identity/similarity corresponded to *Cherry mottle leaf virus* (66%/82%), *Peach mosaic virus* (78%/90%) and *Cherry mottle leaf virus* (57%/75%), respectively. All together, these results permit to suggest the idea that the new viral sequence characterized in Mexican peach orchards corresponds to a new tricovirus. The complete nucleotide sequence of this potential new tricovirus is under progress.

**DETECTION OF PHYTOPLASMAS IN CIRUELO TREE (*CYRTOCARPA EDULIS*)
AND IN SHARPSHOOTER *HOMALODISCA LITURATA* IN THE STATE OF BAJA
CALIFORNIA SUR, MEXICO**

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Ciruelo (*Cyrtocarpa edulis* var. *edulis*), trivial name “ciruelo del monte”, is a characteristic wild fruit tree in Baja California Peninsula, very popular among local inhabitants for its edible fruits with slightly acid taste. Symptoms of interveinal chlorosis and total yellowing of apical and internodal leaves reduced in size, with deformed leaf lamina and marginal reddening, shortened internodes and leafstalks, proliferation of shoots and dried flowers were observed in some trees throughout the state of Baja California Sur (BCS). A high incidence of sharpshooters *Homalodisca liturata* (Homoptera: Cicadelidae) was noted in ciruelo during insects monitoring with yellow sticky traps. Samples of apical leaflets, midribs, leafstalks, shoots and flowers from symptomatic and asymptomatic plants were collected during field surveys, processed and subjected to scanning electron microscope Hitachi S-3000N (SEM) analysis. The specimens prepared from insects salivary glands were processed with the same technique. Phytoplasma-like bodies ranging from 400 to 1,800 nm were detected in phloem tissue (in sieve tubes and in phloem parenchyma cells) of samples from both symptomatic and asymptomatic plants. The concentration of pathogen in phloem tissue was high, with high affinity to host cell membrane when the concentration was low. The phytoplasma cells were detected also in salivary glands of sharpshooters, with average size of 1,000 nm. The molecular detection and identification of phytoplasmas in plants and insects is in progress. The role of ciruelo as a wild host plant of phytoplasmas and of *H. liturata* as a possible phytoplasma vector and their importance in epidemiology of yellow-type diseases in BCS are discussed.

A SIMPLE METHOD FOR PHYTOPLASMAS TRANSMISSION BY GRAFTING

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Phytoplasmas are insect-transmitted bacterial pathogens infecting a diverse range of plants, and causing economic losses in numerous crops, ornamentals, and fruit trees. They can be transmitted from original diseased host plants and diseased periwinkle to healthy periwinkle by grafting, insect vectors and use of dodder plants. This research details a simple method for transmitting phytoplasmas affecting periwinkle to healthy periwinkle, a method that also has potential for use in transmitting from other plants into periwinkle. Twelve phytoplasmas including Chrysanthemum yellows, *Rehmannia glutinosa*, ribes in vinca, strawberry green petal, crotalaria saltiana phyllody, sweet potato little leaf, vinca coconut phyllody, plum leptonecrosis, elm yellows, potato witches' broom, brinjal little leaf and apple proliferation were transmitted from diseased periwinkle to healthy periwinkle by inserting 1.5 cm long sections of diseased stem explants into the stem of the healthy plant directly. All phytoplasmas were successfully transmitted to plants and showed typical disease symptoms 6-8 weeks after transmission. Nested PCR and *SecA* gene sequencing were used to confirm that transmission had occurred.

GENETIC DIVERSITY OF *APPLE CHLOROTIC LEAF SPOT VIRUS* (ACLSV) IN FRUIT CROPS

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ACLSV is one of the most common fruit tree viruses worldwide. It infects pome and most of the stone fruits. Based on biological and serological properties, two distinct ACLSV strains (apple and peach) have been identified. Also the comparisons of complete nucleotide sequences of several ACLSV isolates from plum, apple, cherry and peach have showed high sequence variability. Such high genetic variability usually arises from mutations and recombination during the replication of virus genome. Recombination in natural populations has been reported for fruit crop viruses from family *Betaflexiviridae*. The putative recombination events in the CP gene and phylogenetical relationships as well as molecular variability of 25 Latvian ACLSV isolates from several apple (*Malus × domestica* Borkh.), pear (*Pyrus communis* L.) and plum (*Prunus domestica* L.) cultivars were analysed. Phylogenetical analyses were done in MEGA5 and recombination analysis was done using the RDP 3.44. Data regarding pair wise comparison displayed high genetic variability in CP gene among ACLSV isolates. The sequence similarity among full CP of twenty-five ACLSV Latvian isolates was 87.98 – 99.48% at nucleotide and 84.46 – 99.48% at amino acid level. The minimal homology between nucleotide and amino acid sequences retrieved from GeneBank was 66% and 69.4%, respectively. Also several putative recombination signals have detected in the CP encoding gene at N' terminal, where MP overlap CP gene, which previously is described as hypervariable region. Despite of wide molecular variability, the estimates of evolutionary divergence between host groups showed that divergence between *Malus*, *Pyrus* and *Prunus* isolates is low and also phylogenetic tree lost grouping by hostplants. Only some *Prunus* isolates clustered separately from other isolates. It could mean, that for some plum isolates have different evolutionary pathway as other fruit isolates and due to intensive agriculture practices virus have lost its primary host specificity.

A SURVEY FOR VIRUSES AND VIROIDS IN USA CHERRY GENETIC RESOURCES

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A survey of U.S. cherry (*Prunus* spp.) genetic resources for virus infections was conducted during 2009 and 2010. Two hundred sixty samples were collected from cherry tree in the Plant Genetic Resources Unit in New York, the National Clonal Germplasm Repository for Fruit and Nut Crops in California, and the U.S. National Arboretum and the National Park Service National Mall, both in Washington, D.C. The samples were tested by RT-PCR for 14 viruses and by dot-blot hybridization for two viroids. Eleven viruses, *American plum line pattern virus*, *Apple chlorotic leaf spot virus*, *Cherry green ring mottle virus* (CGRMV), *Cherry necrotic rusty mottle virus* (CNRMV), *Cherry virus A* (CVA), *Little cherry virus 1*, *Little cherry virus 2* (LChV-2), *Plum bark necrosis stem pitting associated virus*, *Prune dwarf virus*, *Prunus necrotic ringspot virus* (PNRSV) and *Tomato ringspot virus*, were detected. *Hop stunt viroid* was detected only in a *P. undulata* accession. Virus infections were very common in every collection, except for the Repository in California. A majority of the plants in the other three locations were infected by at least two viruses, with the most common viruses being CVA (85.1%), LChV-2 (48.8%) and PNRSV (18.4%). RT-PCR products from some isolates of the 11 viruses were cloned and sequenced. Sequence analyses showed varying degree of genetic diversity among the isolates of different viruses. The high incidence but low accompanying genetic diversity of CVA in two locations might be due to propagation of clonal scions or rootstocks from infected mother trees.

MONITORING OF PSYLLID SPECIES (*HEMIPTERA, PSYLLOIDES*) IN CONIFEROUS PLANTS IN POLAND

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During 2010-2011, monitoring of the psyllid fauna species overwintering in coniferous plants was carried out at the Botanical Garden in Powsin, Warsaw, Poland. Specimens were collected by using the beating tray method in *Pinus sylvestris* Laponica, *P. tabuliformis*, *Picea pungens* Tomek trees 'Candidatus Phytoplasma pini' affected and in phytoplasma free *Pinus nigra*, *P. mugo* and *Abies alba* trees. In the collected samples in 2011 four species of psyllids were identified. Of these species, three, *Aphalara polygoni* Förster, *Aphalara exilis* (Weber & Mohr) and *Cacopsylla melanoneura* (Förster) are already known to occur in Poland whereas *Cacopsylla affinis* (Löw) was recorded for the first time. Similarly Triozidae was represented by four species. Among these, *Heterotrioza remota* (Förster) was predominant while *Trioza urticae* (L.), *Trioza rhamni* (Schrank) and *Trioza nigricornis* (Förster) were sampled sporadically. Species known as vectors of phytoplasmas belonging to 16SrX phytoplasma group were studied in priority. The infection with phytoplasma was studied by amplification of DNA using polymerase chain reaction with subsequent RFLP analysis in selected individuals. Phytoplasma strain belonging to 16SrX phytoplasma group were detected in two specimens of *C. affinis* and in one individuals of *T. rhamni* collected from *P. sylvestris* Laponica.

FIRST REPORT OF MOLECULAR IDENTIFICATION OF '*CANDIDATUS PHYTOPLASMA PYRI*' IN PEAR TREES IN BELGIUM

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Pear decline (PD) is a quarantine organism for the European Union that has been assigned to the Candidatus taxon, '*Candidatus Phytoplasma pyri*'. In October 2010, nearly 140 samples of leaves showing early yellow or red discoloration were collected in pear production orchards in north-west Belgium. DNA was extracted from 0.5 g of leaf midribs using a simplified extraction method. Samples were then tested for phytoplasma presence by PCR using the universal primer pair fU5/rU3, all yielding PCR products of expected size. BLAST analysis of a sequenced amplicon revealed 100% of identity with '*Ca. P. pyri*' strains (16SrX group). About the 15% of the collected samples were infected. The results of the characterization of these isolates are presented. To our knowledge this is the first report of '*Ca. P. pyri*' in orchard pear trees in Belgium, confirmed by molecular tests.

IDENTIFICATION OF *PEACH LATENT MOSAIC VIROID* (PLMVd) IN MONTENEGRO

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Peach latent mosaic viroid (PLMVd) and *Hop stunt viroid* (HSVd) isolates can cause latent infections, but may also induce dapple fruit symptoms, fruit and stone deformations, fruit cracking, discoloured spots, mosaic, extreme chlorosis, delayed bud break, flowering and ripening, enhanced susceptibility to biotic and abiotic stress, bud necrosis, and tree decline. 13 samples of 12-year-old peach trees (cv. Elegant Lady) were collected in the most important peach-growing area of Montenegro in the vicinity of Podgorica. Some of the trees showed mild or severe mosaic, chlorotic rings, and fruit deformations, but they were also infected with *Plum pox virus*. RT-PCR analyses using RF-43/RF-44 primers for PLMVd resulted with positive amplification of an 348 bp product in nine peach samples, while RT-PCR results with primers VP-19/VP-20 for HSVd were negative for all samples. Seven PCR products of PLMVd were successfully cloned and sequenced. Consensus sequences were deposited in the GenBank under accession numbers JF927892–JF927898. Sequence analyses revealed that PLMVd isolates from Montenegro shared 92.6 to 97.9% identity among each other, 94 to 98% identity with the PLMVd isolate G sequence (EF591868) and 91.8 to 94.4% identity with PLMVd sequence M83545. Since the spread of PLMVd infections can pose a threat to Montenegrin fruit production, additional studies are required to further evaluate the incidence of the pathogen on other locations and other hosts in Montenegro.

POSTER SESSION II
PLUM POX VIRUS

PLUM POX VIRUS SITUATION IN EMILIA-ROMAGNA REGION (ITALY)

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Plum pox virus (PPV) is the causal agent of Sharka, the most dangerous disease of stone fruit trees, reducing fruit quality and yield. In Italy, Sharka has been detected since 1973 but in the Emilia-Romagna region (north of Italy) appeared in 1982 on few apricot and plum orchards. Only after 1995 it became epidemic on peach, even if since 1992 surveys to evaluate the distribution of PPV were conducted in order to contain the epidemic diffusion. Each year, from spring until mid-summer, samples with suspected symptoms were collected from orchards in five different provinces and analysed by ELISA test using both polyclonal and monoclonal antibodies and, randomly, by molecular method (RT-PCR), to distinguish different strains. Strain characterisation showed that infected peach plants hosted PPV-M whereas apricots and plums were infected by PPV-M, PPV-D or PPV-Rec (Grillini *et al.*, 2011). Since 1997 to 2011, 3,800 farms (more than 10,000 hectares) of stone fruit cultivation (orchards and nurseries) were surveyed, georeferenced by GPS and recorded in GIS; 24,600 symptomatic samples were collected and sent to Plant Protection Service (PPS) laboratory for official analysis. In all fields where infected plants were detected, the percentage of symptomatic plants was discriminated between below or over 10%, in order to explant, respectively, individual infected plants or the entire plots. Compulsory phytosanitary measures were refunded to growers by public administration, that, until 2011, employed 5,382,233 euros. Despite of these affords, the infection rate increased each year of about 20% and, in 2011, 263 new PPV foci (16 on apricot, 187 on peach and 60 on plum) were discovered or confirmed and 22,297 plants were removed. PPS established 9 areas where Sharka is present and established, without possibility to eradication, surrounded with an appropriate buffer zone, 1 km large, where systematic inspections are carried on.

EVALUATION OF *PLUM POX VIRUS* SENSIBILITY ON DIFFERENT STONE - FRUIT VARIETIES IN EMILIA ROMAGNA REGION (ITALY)

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During the years between 2003 and 2011, about 200 stone fruit varieties were inoculated by chip budding with PPV M strain, grown in a screen house and then surveyed for symptom expression, on leaves, flowers and fruits during the following season. In particular 126 peach and nectarine, 45 apricot and 28 plum varieties and seedlings (from breeding programs) under evaluation have been assessed in the present work. A large number of varieties showed discoloration and mottling on the young leaves in spring; furthermore all peach and nectarine selections with rosaceous flowers appeared infected by PPV with strong symptoms on petals. Many peach and apricot cultivars and seedlings, but only one plum variety, presented fruit deformations with typical rings and mottling. Only few accessions (2 peaches and 10 apricots) belonging to “ancient” germplasm or to seedlings neither appeared interested by typical symptoms on leaves and fruits, nor resulted positive to PPV by ELISA and Real Time RT-PCR assays. These experimental data evidenced high sensibility of stone fruit germplasm to PPV-M as confirmed by the information gathered in the Emilia-Romagna region, by inspectors of the local Plant Protection Service. In particular, during 15 years of orchard survey activity, 36 varieties of apricot, 37 of plum, 102 of nectarine and 83 of peach (both traditional and promising varieties) resulted infected, showing typical symptoms, as a result of the natural PPV spread in the field.

SUSCEPTIBILITY OF DIFFERENT PRUNUS ROOTSTOCKS TO NATURAL INFECTION BY *PLUM POX VIRUS-T*

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Plum pox virus (PPV) in stone fruit trees in Turkey was reported in 1968 and was endemic till the 2000's, in Central Anatolia, Marmara and Aegean regions, where the PPV-T strain remains widespread. There is no detailed information available about the epidemiology and response of different rootstocks to PPV-T. Thus, susceptibility of different *Prunus* rootstocks to PPV was assessed under natural inoculum pressure in the Izmir-Aegean region. A wide range of rootstocks was evaluated 'Nemaguard' (*P. persica* x *P. davidiana* hybrid seedling), 'Mariana' GF8.1 (*P. cerasifera* x *P. munsoniana*), Docera 6 (*P. domestica* x *P. cerasifera*), Myrobolan 29 C (*P. cerasifera*), GF677 (*P. dulcis* x *P. persica*) and 'Garnem' (*P. dulcis* x (*P. persica* x *P. davidiana*)). The rootstock plants were individually analysed for PPV by 5B-IVIA monoclonal antibody twice (spring and autumn) in 2009, 2010 and 2011. The results demonstrated that the 'Myrobolan 29C' and 'Nemaguard' rootstocks were the most susceptible to natural PPV-T infection with the infection rate of 6,14 % and 4,66 %, respectively. Moreover, typical PPV symptoms were also observed on both rootstocks. Other assayed rootstock species showed no symptoms and were negative by DASI-ELISA. The most abundant aphid species monitored by the sticky-plant method were *Myzus persicaea* and *Hyalopterus pruni*. When PPV isolates obtained from naturally infected rootstocks were analysed by sequencing, all isolates were characterised as PPV-T.

MOLECULAR INVESTIGATION ON RECOMBINANT *PLUM POX VIRUS* ISOLATES IN CENTRAL ITALY

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In the frame of an Italian project financed by Ministry of Agriculture a monitoring was performed to verify the presence of PPV-Rec isolates in Central Italy and to evaluate the molecular variability of these isolates, with the aim of defining the appropriate modalities of containment. Regional Plant Protection Services of Central Italian regions have provided samples from plum and peach trees with PPV symptoms (Abruzzo, Emilia-Romagna, Latium and Marche). A multigene approach was used: three different genomic regions were analyzed to verify the molecular variability of PPV isolates in order to investigate recombinant identification: C terminal of coat protein gene, 3' terminal of Nib gene and the genomic region including P3 and 6K1 genes. Coat protein (CP) gene was analyzed using RT-PCR with primers P1/P2, followed by RFLP analysis after digestion with *RsaI* restriction enzyme. Nib gene was studied using RT-PCR with four specific primers in three different combinations mM5/mM3, mD5/mD3 and mD5/mM3. P3-6K1 genome region was analyzed by RT-PCR with primers PP3/PCI, followed by RFLP analysis after digestion with the restriction enzymes *EcoRI*, *DdeI* and *VspI*. By multigene analysis several isolates were classified as PPV-M or PPV-D strains, whereas some of the isolates coming from Emilia-Romagna region resulted to be anomalous, as they showed an RFLP pattern typical of PPV-M strain both on CP gene and P3-6K1 genomic region, after digestion with *RsaI* and *DdeI* enzymes, respectively. Whereas they resulted belonging to PPV-D strain on the basis of the other molecular tests. The results confirmed the presence of 'anomalous' PPV recombinant isolates in Emilia-Romagna region, but, for the moment, they seem to be confined in these areas. More approaches are needed to improve the knowledge about these isolates and to verify their spread in other Italian regions.

The research leading to these results has received funding from the Italian Ministry of Agriculture Project 'Strateco'

TEMPORAL ANALYSIS OF *PLUM POX VIRUS* IN CHILE

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Plum pox virus (PPV) has been previously reported in Chile, and its presence is related exclusively with Dideron (D) strain. Four stone fruits commercial orchards infected by PPV-D were used to provide information concerning the temporal spread of the virus in Chile. During the spring seasons of years 2008 and 2011 (from September to October) different plant tissues were sampled from a block of about 400 plants at each orchard. These materials were tested by ELISA technique, using the „Realisa Reforzado“ kit (REAL, Spain). In orchard A (Apricot cv. Castelbrite) the prevalence increased from 11.25% in 2008 to 17.25% in 2011, while incidence decreased gradually from 11.25% (2008) to 0.6 (2011). Orchard C (Peach cv. Summer African Gold) showed a prevalence of 12.02% (2008) to 36.06% (2011), and more or less constant values of incidence: 12.02% (2008), 11.2% (2009), 8.62 (2010), 10.44% (2011). In orchard D (Prune cv. D’Agen) we observed a prevalence of 7.0% to 28.46% in four years, and alternate values of incidence: 7.0% (2008), 1.88% (2009), 13.7% (2010), 8.89% (2011). In orchard E (Nectarine cv. Artic Snow) the prevalence values were 13.25% to 26.5%, and incidence decreased gradually from 13.25% to 0.34%. After the first year of evaluation, in orchard A the positive plants were cut, while in orchard E preventive control of aphids migration was implemented. This may explain why the orchards A and E achieved the lowest incidence values at the end of four years, considering that at least one known PPV vector, *Myzus persicae*, has been found in the four orchards. In addition, spatial analysis are currently being carried out to assess the spread pattern of PPV, and to identify disease clusters in each study site. During 2012 will take place the fifth and final ELISA test in the four orchards.

DYNAMICS OF SPREAD OF PPV-REC AND PPV-D IN AN EXPERIMENTAL PLUM ORCHARD

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The three *Plum pox virus* (PPV) strains (PPV-M, PPV-D and PPV-Rec) are present in Serbian plum orchards. Although PPV-Rec is widespread in plum, little is known about its epidemic properties and competitiveness, in particular when confronted to PPV-D. An experimental plum orchard of 400 healthy trees variety Čačanska lepotica was built up in 2008 in order to study the dynamics of spread and competitiveness of PPV-Rec and PPV-D isolates from artificially inoculated trees. The few *Prunus* trees situated in the surrounding of the orchard were precisely located, sampled and tested by ELISA and IC-RT-PCR for the presence of PPV. Contaminated trees were removed when possible and all positive samples were partially sequenced. One PPV-D and one PPV-Rec isolates were selected from a collection of Serbian PPV isolates to be used as artificial inoculum in the orchard (4 trees per isolate). The selection of the isolates was based on the nucleotide differences between the sequences of the 427 bp genomic fragment in order to be able to distinguish the inoculum sources from the isolates spreading in the surrounding of the orchard. The two selected isolates were then fully sequenced and biologically characterized (assessment of the aphid transmissibility). Each year, from 2008 to 2011, all trees in the orchard were visually inspected and tested by ELISA test. All positive samples were strain-typed by IC-RT-PCR method and partially sequenced. Artificially inoculated trees were found infected in 2009. New cases of infection were detected within the orchard, with respectively 2, 18 and 33 PPV-Rec infected trees and 1, 2 and 4 PPV-D infected trees detected from 2009 to 2011. The dynamic of spread of PPV-Rec and PPV-D isolates will be analyzed in the light of the spatio-temporal and viral genomic sequence data, in order to disentangle the influence of both internal and external sources of inoculum. Preliminary results will be presented and further discussed.

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DETECTION OF *PLUM POX VIRUS* IN REGIONS OF BELARUS

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In Belarus, Plum Pox Virus (PPV) was first identified in Minsk region in 2000 on symptomatic plum trees of cultivar Nagrada Nemanskaja. In 2009 and 2010 the virus disease was monitored in plantations of stone fruits of Minsk and Brest regions. In 2011 more than 6 hectares of stone fruits, 8 plantations, was investigated for PPV presence in three regions of Belarus (Vitebsk, Grodno and Mogilev). 73 samples were collected from symptomatic trees on the base of visual observation. PPV detection was carried out by ELISA and PCR analyses. PPV was confirmed in 32.9% out of all tested samples with visual symptoms. The most frequently (19.2% out of all tested samples) the virus was detected in samples from cherry plum cv. Kometa Kubanskaja. The presence of PPV was detected also in samples from plum rootstocks (OD 2-3), sour cherry cv. Vjanok and sweet cherry cv. Eput.

EFFECT OF CO-INFECTION OF *PLUM POX VIRUS* AND *PRUNE DWARF VIRUS* IN *PRUNUS PERSICA*; A QUANTITATIVE ANALYSIS BY REAL-TIME RT-QPCR

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Effect of co-infection of *Plum pox virus* (PPV) and *Prune dwarf virus* (PDV) were studied in *Prunus persica* (GF-305). Plants were inoculated in five types of experiments: i) with PPV-D, ii) with PDV, iii) first with PPV-D then PDV, iv) first with PDV then PPV-D and v) with PPV-D and PDV at the same time. The plants were evaluated after the bud breaking based on symptoms, virus detection and quantification. PPV-D and PDV were detected by ELISA and RT-PCR. The virus titre was quantified by real-time RT-qPCR. GF-305 plants inoculated with both viruses (PPV-D and PDV) were in general more dwarf than single virus (PPV-D or PDV) infected plants. In both type of mixed infected plants (experiment iii and iv) titre of PDV was higher than PPV-D. However, the titre of PDV in these mixed infected trees was as high as solely PDV infected trees. Same is true for the titre of PPV-D. Furthermore, PPV-D was undetectable in all tested plants co-inoculating with PPV-D and PDV at the same time. PDV was detected in some of the above mentioned trees and the virus titre was much lower than solely infected PDV trees. From our results it could be concluded that mixed infection of both viruses has synergetic effect on symptoms development. However, influence of mixed infection in virus titre (PPV-D or PDV) is unclear. Further quantitative analysis of PPV-D and PDV co-infection will confirm the possible effect of mixed infection of these viruses in fruit trees.

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SEROLOGICAL AND MOLECULAR CHARACTERIZATION OF THE ISOLATES OF *PLUM POX VIRUS* FOUND IN LATVIA.

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A survey of *Plum pox virus* (PPV) isolates present in different countries has been initiated in the frame of SharCo project financed by European Union (contract n°204429, FP7/2007-2013). Leaf samples were collected in 2009 in two orchards in Latvia, from symptomatic/ suspected and symptomless plants of 21 plum (*Prunus x domestica*), 2 myrobolan plum (*Prunus cerasifera* L.) and 3 apricot (*Prunus armeniaca* L.) trees. They were tested for the presence of *Plum pox virus* with PPV-Universal DAS-ELISA kit (AMR Lab Consultants, Spain) using monoclonal antibody 5B, as well as with DAS-ELISA using locally prepared polyclonal antibodies. Nine samples, for which positive results have been obtained in these tests, were re-tested with four monoclonal antibodies: PPV-Universal (again), D-specific and M-specific (DASI-ELISA kits purchased from AMR Lab Consultants, Spain) and (subset of D specific) MAb V/8. Three samples from one orchard showed positive reaction with MAb Un, D and V/8; one sample from the same orchard reacted positively with MAb Un and D, but not with V/8. These isolates were preliminary classified to two different serotypes of strain D. Five samples from another orchard showed positive reaction only with PPV - universal monoclonal antibody, and could not be positively strain typed using available antibodies. Two cDNA fragments (600 nts or more) corresponding to P3-CI junction and N1b/CP junction were obtained by IC-RT-PCR method for all isolates and sequenced. The analysis confirmed the identification of the four isolates from the first orchard as D strain/type isolates. Four out of five isolates from another orchard, showed more than 94% nucleotide sequence identity of both cDNA fragments with PPV-W3174 (syn. PPV-Winona). Based on these results, Latvian isolates named LV-140pl, LV141pl, LV-143 and LV-145pl, detected in the plum hybrids 298-70 and Tern 32-34, were classified to PPV-W strain. Further research of Glasa based on full genome analysis showed that those isolates represented 'ancestral' PPV-W.

PARTIAL CHARACTERISATION OF BIOLOGICAL PROPERTIES OF PPV-C ISOLATES FOUND IN BELAROUS AND ESTABLISHMENT OF *IN VITRO* CULTURES OF INFECTED L2 AND OWP-6 ROOTSTOCKS.

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A survey of *Plum pox virus* isolates present in different countries has been initiated in the frame of SharCo project financed by European Union (contract n°204429, FP7/2007-2013). Several trees of *Prunus* hybrids, used as rootstocks for cherry cultivation, have been found in Belarus to be infected with *Plum pox virus*, strain PPV-C. The virus was found in ten plants (>10 years old trees) of L2 (*Prunus lanesianna*) and one plant of OWP-6 (*Prunus cerasus* x *P. padus* or *P. cepadus*). Biological properties of two PPV-C isolates: BY-101 from L2 and BY-181 from OWP-6 have been studied by chip bud inoculation to several potential woody hosts and mechanical inoculation of *N. benthamiana*. All experiments have been performed in contained glasshouse facility. The woody plants tested were: *Prunus avium* x *Prunus pseudocerasus* ‘Colt’ – vegetatively propagated rootstock, *Prunus avium*, ‘Alkavo’ - rootstock propagated from seeds, *Prunus avium*, F12/1 rootstock vegetatively propagated, *Prunus persica* GF305, and Antypka (*Prunus mahaleb*). During one year study we have found that all plants of F12/1 rootstock grafted or chip bud inoculated with PPV-C (BY-101 and BY-181) died soon after new shoots started to develop, although for final conclusion more trials are necessary. Neither GF305 nor *P. mahaleb* became infected with studied PPV-C isolates; all inoculated plants developed normally, showing no symptoms and detectable presence of PPV-C in our experiment. Clear symptoms were observed on all ‘Colt’ and several ‘Alkavo’ inoculated plants. The symptoms on leaves of infected L2 and OWP-6 shoots developing from buds on ‘Colt’ or ‘Alkavo’ rootstock were also quite pronounced. It must be stressed, however, that so far the plants in question have been tested for the presence of PPV, PNRSV, PDV and ACLSV, but not for the presence of other viruses. The extensive testing is needed to eventually claim L2 to be considered as promising indicator plant for PPV-C. It is quite possible, however, that it may be a good host for maintaining PPV-C. *In vitro* cultures has been established from plants infected with PPV-C (L2 and OWP-6 rootstocks) as well as from healthy L2 plants (to be used for maintaining PPV-C isolates and potentially as indicator plant). The presence of PPV has been verified in May 2012 by ELISA. These cultures will be used to maintain a virus in a convenient and relatively safe manner, and they will be suitable for international exchange of reference isolates. Both PPV-C isolates from L2 and OWP-6 induced strong systemic symptoms on inoculated plants of *N. benthamiana*.

MAPPING THE PATHOGENICITY DETERMINANTS IN THE GENOME OF *PLUM POX VIRUS*

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Three major strains of the *Plum pox virus* (PPV), namely PPV-D, PPV-M and PPV-Rec differ by the preference of natural host species and by other biological properties observed in artificial herbaceous hosts. To map the phenotypes reflecting virus-host interactions in the PPV genome we prepared a set of inter-strain-chimeric infectious cDNA clones and analysed their biological properties after biolistic infection of various herbaceous host plant species. All of the seven plant species tested were successfully infected, three of them symptomless and four species showed variable symptoms. In *Nicotiana benthamiana* and *Pisum sativum* neither qualitative nor quantitative aspects of symptomatology were related to any single PPV genome locus. On the other hand, P1/HC-pro genomic region was obviously responsible for the hypersensitive reaction of *Nicotiana occidentalis* and *Nicandra physaloides*. The *in silico* analysis mapped this phenotype determinant to the 3'-proximal part of the P1 gene. To localize the responsible amino acid motif more detailed analysis was performed by application the technique of site-directed mutagenesis. Obtained results will be presented.

OCCURRENCE OF *PLUM POX VIRUS* STRAINS IN MORAVIA (CZECH REPUBLIC)

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Plum pox virus the causal agent of sharka disease of plums, apricots, and peaches is known to be present in all parts of Czech Republic. Previous studies have confirmed the presence three basic strains - *Plum pox virus* strain M (PPV-M), D (PPV-D), and Rec (PPV-Rec) in various *Prunus* species all over the Moravia region. To obtain the detailed information about the distribution of these strains, the survey was done during the period 2005-2011 in this area. In agree with the expectation it was confirmed the mass occurrence of PPV-M in the most of the peach intensive orchard, its presence was noted in two old apricot orchards and sporadically on myrobalans. In contrary to the surprising data were obtained in the case of PPV-Rec strain. The PPV-Rec was localized in two intensive plum orchards originally established from the infected material coming from the Serbia (former Yugoslavia). Spreading of PPV-Rec isolates from this primary source proceeded in two different ways. First of them was the natural transmission mediated by aphids from infected trees mainly to domestic plums. The second way is connected with man as the main vector and in the area traditional using of offsets (unlucky PPV-Rec infected) for multiplication and plum growing in private gardens and small orchards. Thanks to these two ways of PPV spreading, recently the PPV-Rec is occurring in narrow zone close to border with Slovakia long about 80km. PPV-D strain is according to our knowledge the widely occurring in the whole Moravian region and except to above mentioned localities is the dominant strain infecting *Prunus* species.

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SURVEY OF SHARKA DISEASE (*PLUM POX VIRUS*) ON STONE FRUIT TREES IN NORTHERN HUNGARY

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A survey of sharka disease, caused by *Plum pox virus*, was conducted in Slovakian- Hungarian border region during 2011. The species surveyed were peaches, apricots, plums at large scale and small scale farming and blackthorn at natural surroundings. The virus symptoms were visually assessed except of blackthorn, which was asymptomatic, and leaf samples were taken. Obtained samples were subjected to biological assays with indicator plants, serological (ELISA) and molecular (conventional RT-PCR) diagnostics of PPV. The results indicated that PPV is present in the border region in all examined stone fruits. The samples were taken from 31 different places of the border region and the infection rate varied between 0 – 100 %. The apricot orchards in the region are less infected by the virus than the plum orchards. The virus was detected in peaches, apricots and plums. The positive isolates were compartmentalized. The definition of the groups is significant, because the isolates from different groups infected the host plants, and spread in the orchards in different measures. We used three processes to define the groups. All tested PPV isolates belonged to intermediate group according to the symptoms on *Chenopodium foetidum*. Half of the PPV isolates belonged to recombinant group, 45 % belonged to D and 5 % to M group according to the molecular characterization and sequence analysis. We got the same results with restriction analysis by *EcoRI* and *DdeI* restriction enzymes.

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DIVERSITY OF *PLUM POX VIRUS* IN PLUM ORCHARDS IN SERBIA**S. Paunović¹, D. Jevremović¹, S. Dallot²**¹ *Fruit Research Institute, Kralja Petra I 9, Čačak, Serbia*² *INRA - UMR BGPI CIRAD- TA A54/K, Campus international de Baillarguet, Montpellier cedex 5, France**E-mail: spaunovic@yahoo.com*

Serbia is a leading plum producing country in Europe with more than 41 million of bearing trees. For almost 80 years, *Plum pox virus* (PPV) is a threat for the stone fruit production in Serbia. So far, three major out of seven strains were reported: PPV-M, PPV-D and PPV-Rec. Material for this study was collected during three year period (2008-2010). A total number of 265 samples were collected from 84 plum orchards in 12 Serbian districts. Two to four trees showing clear sharka symptoms were randomly chosen and sampled from each orchard. Strain-typing was performed by IC-RT-PCR method with PPV-M and PPV-D specific primers targeting two genomic regions of PPV. Additionally, 39 isolates from different locations were selected for sequencing fragments located in C-ter N1b—N-ter CP part of CP region and C-ter P3-6K1-N-ter CI region. The most prevalent strain in analyzed samples was PPV-Rec (53.5%), followed by PPV-D (27.9%) and PPV-M (5.4%). Mixed infections were found in 13.2% of samples. All types of mixed infections were detected: PPV-M+PPV-D, PPV-M+PPV-Rec and PPV-D+PPV-Rec. The highest incidence among mixed infections was PPV-D+PPV-Rec combination - 67.6%. For the first time, natural triple infection was confirmed in one sample (PPV-M+PPV-D+PPV-Rec). In 37 orchards only one PPV strain was found; in 42 orchards two strains and all three strains were detected in 5 orchards. The most prevalent strain was PPV-Rec that was found in 69 orchards (in single and mixed infections) in all districts. On the contrary, PPV-M strain was found only in 15 orchards. Obtained results confirmed earlier assumption on the long term presence of PPV-Rec strain on plum in Serbia. Phylogenetic analysis of selected isolates confirmed strain-typing results. Further analysis showed the absence of geographical genetic differentiation of isolates that suggest the intensive gene flow between districts through contaminated planting material in the past.

The research leading to these results has received funding from the European Community's Seven Framework Programme (FP7/2007-2013) under Grant Agreement n°204429, SharCo project and project TR-31064 from the Serbian Ministry of Education and Science.

PLUM POX VIRUS ON SOUR CHERRY IN CROATIA

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Plum pox virus (PPV) has been routinely screened in Croatian nurseries since 1988 by ELISA. From 2004 onwards, IC-RT-PCR has been introduced to supplement the detection methods in accordance with the EPPO protocol. So far, the PPV strains M, D, and Rec have been detected in the country with the geographic prevalence of PPV-M, especially in the European plum. Sour cherry cultivation has increasing importance in the last decade. Besides commercially available sour cherry varieties grown in the continental part of the country, the tendency is to promote growing of indigenous cultivar *Prunus cerasus* L. 'Maraska' in Dalmatia including the preservation of Maraska gene pool and domestic planting material production. In one of the nurseries specialized for Maraska sour cherry located near Zadar, a routine PPV screening was performed in 2010. Leaf samples were taken from 30 asymptomatic plants and two were found PPV infected by ELISA and IC-RT-PCR. The amplification product obtained by using PPV-C specific primers SoC1/SoC2 resulted in amplification product of expected size (193 bp). The amplicon was cloned into pTZ57R/T vector and inserts from ten recombinant plasmids were sequenced. The genetic structure of this PPV-SoC isolate was homogeneous with all ten amplicon sequences sharing 100% sequence identity. Phylogenetic analysis of 177 nucleotide-long fragments, obtained after removing the primer sequences from amplicons, showed that PPV-SoC isolate from Maraska clusters with other SoC isolates. Interestingly, it also shares 100% nucleotide identity with the prototype sour cherry isolate (GenBank Acc. No. AY184478) originating from Moldavia. This is the first report of PPV-C strain in Croatia as well as the first report of PPV in sour cherry.

LARGE SCALE *PLUM POX VIRUS* SURVEY OF CULTIVATED AND WILD CHERRY IN ROMANIA

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Plum pox virus (PPV) is the causal agent of Sharka disease, one of the most destructive diseases of stone fruits in the world. Seven strains of PPV (D, M, EA, C, W, Rec and T) have been reported so far. Intensive research in the last years revealed that D, M and Rec are the most common strains. Other four minor strains have limited geographical distribution. PPV-Cherry (PPV-C) is the only strain known to naturally infect sweet and sour cherry. PPV-C has been detected on occasion in a few European countries (Italy, Republic of Moldavia, and Romania). Nevertheless, potential introduction of PPV-C into new areas could have serious impact on cherry industry. In Romania, PPV-C was previously reported in a sweet cherry orchard from Bistrita area which was promptly removed. To investigate if PPV-C has accidentally introduced or infection persists, a large survey (349 samples) was conducted in the cherry orchards surrounding the initial plot where PPV-C was found. The survey was extended to orchards in five additional counties of Romania. One hundred ninety samples (190) were collected from Iasi, an important cherry growing area, and 21 samples from Sibiu, Valcea, Olt and Constanta counties. Leaves showing diffuse or ring chlorotic spots, and asymptomatic leaves were collected from sweet, sour and wild cherry. A total of 560 samples were tested. Serological tests were conducted using the AGDIA ELISA system and molecular detection was mainly performed by IC-RT-PCR using hPPV(3')NTR/cPPV(3')NTR primer pair. All symptomatic and a few symptomless samples of cherries were additionally subjected to IC-RT-PCR with the P1/P2 primer pair. All samples tested proved to be negative, even samples showing PPV-like symptoms. The survey results suggest that the occurrence of PPV-C in Bistrita area ten years ago was accidental and confirmed very limited distribution of PPV in cherry trees in Romania.

The project was financially supported by a research agreement with USDA-APHIS-PPQ-CPHST.

CHARACTERIZATION OF *PLUM POX VIRUS* ISOLATES FROM DIFFERENT PEACH VARIETIES IN MONTENEGRO

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Although *Plum pox virus* (PPV) is considered the most devastating viral pathogen in stone fruits in Montenegro, data about occurrence of its strains in the most important peach-growing area is scarce. In September and October 2011, chlorotic ringspots, veinal chlorosis, netting, blotches and deformations, were observed at peach trees in the vicinity of Podgorica. In this study, 58 symptomatic leaves samples originated from 8 different varieties (Rita Star, Morsiani, Caldesi, Adriana, Gloria, Spring Belle, May Crest, Spring Crest, Maria Marta) were collected. Presence of PPV was ascertained in 35 samples (60.3%) using Real-Time PCR. The highest PPV infection rate was detected in Maria Marta and Spring Belle (83.3%), followed by Spring Crest (75%), Adriana and May Crest (66.7%), Gloria and Morsiani (62.5%) and Rita Star (55.5%). PPV was not detected in Kaldesi variety. In order to determine molecular variability of PPV isolates, four highly infected samples were selected from four varieties (Maria Marta, Gloria, Morsiani and Rita Star). RT-PCR method with primers targeting CP region, was followed by cloning and sequencing of 1276 bp DNA fragment. Sequence analyses revealed that all peach isolates from Montenegro shared 92.2 – 99.1% nucleotide identity with corresponding PPV-M strain sequences deposited in GenBank. The Montenegrin isolates 374/11 from Rita Star and 399/11 from Gloria were most closely related (99.1 and 97.1%) with isolate SK68 (M92280.1), while isolates 377/11 from Morsiani and 424/11 from Maria Marta shared most identity (97.6 and 98.1%) with previously reported Montenegrin isolate Godinje 1 (HQ452396) from region of Bar. Considering the high percentage of infected trees in the most important peach-growing area, a severe control related to importation and production of virus-free propagation material, as well as an eradication of infected trees is of the great importance in Montenegro.

POSTER SESSION III
DIAGNOSIS AND
NEXT GENERATION SEQUENCING

VIRUS SANITATION AND DEEP SEQUENCE ANALYSIS OF FIG

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Fig (*Ficus carica* L.) is one of the most widespread fruit tree in the Mediterranean basin. Although the relevance of the crop in some suitable areas, sanitation status is rather deteriorated as several new viruses affecting this perennial woody host were recently discovered and characterized. No certification programme for the multiplication of virus-free propagative material was currently applied in fig-growing countries. In order to create sanitarily-improved commercial orchards using virus-tested primary stocks, sanitation of some accessions (mainly showing fig mosaic symptoms and verified to be affected by *Fig mosaic virus*, FMV, and *Fig Latent Virus-1*, FLV-1) was applied through sanitation treatments (*in vitro* heat therapy and shoot tip culture, alone or in combination). RT-PCR analysis was done before and after treatments. Furthermore, a real time RT-PCR protocol, SybrGreen-based, was developed for the detection of FLV-1. Double stranded RNA, extracted from the accession F5P5 (from which FLV-1 genome was firstly sequenced), was used to construct a cDNA library for deep sequencing (by HiScanSQ Illumina technology) to analyze the whole viral population. Several contigs of 6 different viruses, belonging to different taxa, were identified after alignment to sequence database and their presence was further validate by molecular tools. This study enabled (i) the production of fig clones in which FMV and FLV-1 were eliminated; (ii) the development of a new sensitive tool for FLV-1 detection to be applied for phytosanitary controls; and (iii) the first application of a whole genomic analysis technique to check virus presence and new species identification on fig.

DEVELOPMENT OF QUANTITATIVE REAL-TIME RT-PCR FOR THE DETECTION OF *HOP STUNT VIROID*

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Hop stunt viroid (HSVd) is a worldwide pathogen able to infect a wide range of hosts. It affects latently grapevine and almond, whereas in hop, plum, peach, apricot, cucumber and citrus it causes specific disorder like hop stunt, dapple fruit on plum and peach, fruit rugosity on apricot and plum, pale fruits on cucumber and cachexia on citrus. In some cases different molecular variants were associated with a peculiar symptoms expression as the cachexia isolate on citrus. Because of the diffusion and the wide range of hosts of HSVd, its detection requires the development of a diagnostic protocol able to identify all viroid isolates from every host. For this reason, a new protocol for HSVd detection has been developed designing a new primer pair and a probe in order to perform a real-time RT-PCR assay based on TaqMan™ chemistry. The protocol was tested for its sensitivity and specificity. Sensitivity was evaluated with dilution series of *in vitro* transcripts from whole cloned HSVd genome. Dilutions were used for the production of a standard curve able to quantify the viral template in infected samples. The method was also evaluated for its specificity using target controls from plum, peach, citrus (cachexia and not cachexia isolates) and grapevine isolates and non-target from healthy peach, apricot, plum, pear, citrus, almond and grapevine and other viroids (*Apple scar skin viroid*, *Peach latent mosaic viroid*, *Pear blister canker viroid* and *Potato spindle tuber viroid*). According to the results obtained in quantification of the viral pathogen the protocol gave an high-quality value of efficiency. These results underline the goodness of the new diagnostic protocol for HSVd detection and quantification.

APPLICATION OF HIGH RESOLUTION MELT (HRM) ANALYSIS FOR SIMULTANEOUS DETECTION OF *CHERRY GREEN RING MOTTLE VIRUS* AND *CHERRY NECROTIC RUSTY MOTTLE VIRUS*

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Cherry green ring mottle virus (CGRMV) and *Cherry necrotic rusty mottle virus* (CNRMV), unassigned members of the family *Flexiviridae* are flexuous, filamentous plant viruses with a single-stranded, positive-sense RNA genome. Both viruses have a very similar morphology and genomic organization and share about 60% identity at the nucleotide level over the entire genome. The purpose of this study was to develop a rapid and sensitive method for simultaneous detection of CNRMV and CGRMV by high resolution melt (HRM) RT-PCR. The CNRMV and CGRMV isolates originated from sweet and sour cherry cultivar collections maintained in Research Horticulture Institute and from commercial orchards in Poland. Seven sources of CNRMV, eight of CGRMV and two containing mix infection were used in this study. Total nucleic acids were isolated from the leaves using the silica capture method. Real-time PCR and HRM analysis were performed using Light Cycler 480 II system (Roche Diagnostics, Mannheim, Germany). Detection of CNRMV and CGRMV was performed simultaneously using a probe free, multiplex reaction that included one specific primer set for each virus or one primer pair specific for both of them. These two strategies allowed us to detect virus infection in all tested samples. In addition, HRM analysis made it possible to differentiate clearly between CNRMV and CGRMV by HRM curves. When the HRM profiles were displayed in normalized melting curve format, three distinctly different patterns could be seen corresponding to one for each individual virus and one for the virus mix. Monophasic curves were generated from the samples positive for one virus only, while biphasic curves were observed from mixed templates. Moreover, sequence variations among CNRMV and CGRMV isolates have been observed from the HRM peaks, as confirmed by sequencing. This first application of HRM RT-PCR in plants reveals a new potential for rapid and sensitive detection of multiple pathogens.

DETECTION OF *PLUM POX VIRUS* WITH IMMUNOSENSOR UTILIZING ANTIBODIES IMMOBILIZED ON GOLD NANOPARTICLES

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Immunosensor has been developed for fast and sensitive detection of *Plum pox virus* (PPV) in plant extracts. It was based on gold electrodes modified with: 1,6-hexanedithiol, gold nanoparticles, anti-PPV polyclonal antibody and bovine serum albumine (BSA). It could be used for determination of the presence of the virus in the extracts from plum (*Prunus domestica*) and tobacco (*Nicotiana benthamiana*) leaves. PPV particles trapped from the solution by antibody immobilized on the electrode formed an insulating layer with resistance dependent on the quantity of virions deposited. This phenomenon could be monitored by the electrochemical impedance spectroscopy (EIS). As measured in a series of experiments, the immunosensor displayed very good detection limit about 10 pg/ml and wide dynamic range from 10 pg PPV/ ml to at least 200 pg/ml. The presence of extract from plant materials had no influence on immunosensor response. The immunosensor was capable of discriminating between samples from healthy plants and samples containing 0.01% of extract from infected plant material. These results make the immunosensor a promising device for fast, reliable and simple PPV detection. With the use of the antibodies specific for other viruses the potential range of applications in phytodiagnosics seems to be vast, although many details need to be worked out and practical suitability needs to be carefully validated. The work on miniaturization of immunosensor presented is in progress.

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DETECTION OF TWO STRAINS OF “*CANDIDATUS PHYTOPLASMA ASTERIS*” FROM PEACH AND APRICOT USING LOOP-MEDIATED ISOTHERMAL AMPLIFICATION

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Phytoplasmas are associated with more than 700 diseases in several hundred plant species and cause significant damage to agricultural crops. ‘*Candidatus Phytoplasma asteris*’, one of the largest group of phytoplasma species, infects many crops, including fruit trees. Since there is no effective way to cure phytoplasma diseases, early detection and the removal of infected plants is important to prevent their spread. PCR is the most commonly used method for detecting phytoplasmas due to its high sensitivity and specificity; however, it is slow and requires expensive equipment, including a thermal cycler and electrophoresis apparatus, which makes it difficult to apply it in the field. In this study, we developed a LAMP-based detection kit for phytoplasmas (‘*Ca. P. asteris*’ and ‘*Ca. P. japonicum*’). The LAMP kit targets the *groEL* gene, a highly conserved bacterial housekeeping gene (> 93.8% nucleotide identity among ‘*Ca. P. asteris*’ strains). Using this kit, we attempted to detect the ‘*Ca. P. asteris*’ PYR and AY-A strains, which were transmitted to periwinkle from peach yellows and apricot chlorotic leafroll infected peach and apricot trees, respectively. First, we purified total DNA from PYR- and AY-A-infected periwinkle leaves and from healthy periwinkle, peach and apricot leaves. Using these DNA samples, the LAMP reaction was performed at 61°C for 60 min. Both phytoplasma strains were specifically detected. Using a real-time LAMP assay, we confirmed that amplified DNA from the PYR- and AY-A-infected samples could be detected in about 30 and 36 min, respectively. A LAMP-based detection kit would be useful for the easy, rapid detection of ‘*Ca. P. asteris*’.

DEVELOPMENT OF A MINI-OLIGO ARRAY FOR THE ANALYSIS OF *PLUM POX VIRUS* VARIABILITY

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The SharCo project (European Community's Seven Framework Programme (FP7/2007-2013) under Grant Agreement n°204429) involves multidisciplinary approaches to provide strategies and tools for *Plum pox virus* (PPV) containment. In this context, one of the tasks aimed at the development of a mini-oligo array approach for the rapid, genome wide analysis of PPV isolates. A first generation of the oligochip containing 32 oligoprobes was produced and allowed discrimination among PPV strains. The development of a second generation of PPV oligochips (containing 10 replicates of 90 probes from 18 to 22 nucleotides each) then successfully allowed to analyse and probe the intra-strain variability. A complete protocol including spotting of validated probes, post-spotting procedures, hybridization, evaluation of signals using a GenPix 4000B scanner and final signal analysis, was generated. A rapid characterization of PPV diversity and rapid PPV typing based on mini-oligo arrays has been successfully assayed in different laboratories. The advantages and disadvantages of this technology are discussed in comparison with other available technologies such as deep sequencing.

VALIDATION OF DIAGNOSTIC PROTOCOLS FOR THE DETECTION OF '*CANDIDATUS PHYTOPLASMA MALI* AND '*CANDIDATUS PHYTOPLASMA PRUNORUM*'

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The plant pathogens that cause significant economic damage to agricultural crops are subjected to phytosanitary regulations aimed at protecting the sanitary and quality of propagative material and production. The availability of official diagnostic protocols, therefore, is necessary for the uniformity of results obtained in different laboratories and to give them a legal value, essential for the application of the phytosanitary measures. Moreover, in this direction is moving the entire international community in order to standardize the diagnosis. The Italian Project ARNADIA, funded by the Ministry of Agriculture Food and Forestry, had the aim to produce diagnostic protocols officially recognized at national level for the identification of major plant pathogens. One of the sub-tasks of ARNADIA Project planned the production of reference and validated protocols for the diagnosis of '*Candidatus Phytoplasma mali*' and '*Candidatus Phytoplasma prunorum*'. For this purpose a Working Group has been established, including the main Italian scientific institutions which are involved in the study of both phytoplasmas, Several diagnostic protocols and reagents have been compared in five different laboratories, using the same target and no-target reference samples. Protocols of PCR and real time PCR have been selected and developed to determine the performance characteristics for the validation under the standard UNI CEI EN ISO/IEC 17025 and EPPO PM7/84 and PM7/98. The results showed that the accuracy and analytical sensitivity of PCR performed with specific primers and nested PCR are comparable, whereas real time PCR results to be more reliable. A ringtest among seven Italian laboratories of Phytosanitary Regional Services is running to evaluate the concordance parameter of all selected protocols.

DETECTION OF *PLUM POX VIRUS* IN VARIOUS TISSUES USING FIELD TESTS, SEROLOGICAL AND MOLECULAR TECHNIQUES

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Intensive testing using fast, reliable and inexpensive diagnostic tools is needed for successful containment of sharka. We have tested various tissues of apricots, peaches and plums infected with *Plum pox virus* (PPV) isolates belonging to PPV-D, PPV-M or PPV-Rec. Field tests (AgriStrip, BIORREBA AG, Switzerland and Immunochromato, NIPPON GENE, Japan), DAS-ELISA (BIORREBA AG, Switzerland), two step RT-PCR and real time RT-PCR were used. In the beginning of April flowers and small leaves were sampled. Leaves without symptoms, symptomatic parts of the leaves and asymptomatic parts of the leaves with symptoms were tested in the beginning of May and August. Stalks of symptomatic and asymptomatic leaves were also sampled in the beginning of May as well as dormant buds in the beginning of August. Flowers of apricots and plums in full bloom proved to be a very good source for detection of PPV. The infection could be detected with all used techniques. Detection in mature leaves using field tests and DAS-ELISA depended on the presence of symptoms. PPV could be detected with all tested techniques in symptomatic parts of leaves even in the beginning of August, but was not detected in asymptomatic leaves using field tests, DAS-ELISA and partly also molecular techniques. PPV was detected only in some of the samples of asymptomatic parts of the leaves with symptoms and of stalks by field tests and DAS-ELISA. Reliable detection in buds is very important for testing of graftwood. Unfortunately, infection could not be confirmed in buds in August using field tests or DAS-ELISA. We conclude that field tests are useful for confirmation of the PPV infection in symptomatic leaves. If symptoms are not present, DAS-ELISA should be combined or replaced by molecular techniques.

DEEP SEQUENCE ANALYSIS OF VIRAL SMALL RNAs FROM A GREEN CRINKLE-DISEASED APPLE TREE

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Apple green crinkle disease was described in 1934 in Japan. Though it is suspected to be a viral etiology, the causal agent of the disease is unknown. In a search for a virus or viruses associated with green crinkle, we have undertaken a deep sequence analysis of small RNAs from a green crinkle-diseased apple tree. Low-molecular weight RNAs were extracted from skin tissues of bumpy young fruits of a diseased apple. Small RNAs were separated by polyacrylamide gel electrophoresis and then the libraries of small RNA populations were constructed for sequencing with an Illumina HiSeq2000 system. Twenty to 26 nt tags were obtained from Illumina reads by cutting off the 3' adapter sequence. The similarity search between these tags and the RefSeq and GenBank virus data was performed by BLASTN analysis. The data disclosed the presence of *Apple stem grooving virus* (ASGV), *Apple stem pitting virus* (ASPV), *Apricot latent virus* (ApLV), *Apple chlorotic leaf spot virus* (ACLSV), *Apricot pseudo-chlorotic leaf spot virus* (ApCLSV) and *Citrus exocortis viroid* (CEVd) in a diseased apple. Assembling of reads into larger contigs and reconstruction by aligning contigs on databases-retrieved sequences are now in progress.

POSTER SESSION IV
HOST-PATHOGEN INTERACTION

GENOME SEQUENCE VARIABILITY OF A *PLUM POX VIRUS* ISOLATE RELATED TO ITS LONG-TIME MULTIPLICATION IN DIFFERENT HOST SPECIES

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To study spontaneous adaptations of the Plum pox virus (PPV) to various *Prunus* host species, the PPV-M isolate VAR-2 (originally isolated from peach) was chip-budded on peach, apricot and plum trees in 2006. Five years later the virus was isolated from all these sources to perform the complete genome sequencing. Obtained subisolate sequences were compared to the VAR-2 sequence determined from the original source, as well as from its passage in *Nicotiana benthamiana*. PPV-M tends to infect mainly peaches under field conditions and we presume that this host preference is genetically determined. Therefore a long-term-maintenance in atypical host species could lead to selection and fixation of mutations which enable to overcome this limitation and escape the original host preference. Results of sequence comparisons will be presented, together with conclusions outlining the factors affecting specific host-PPV interactions.

SCREENING OF TURKISH LOCAL APRICOT VARIETIES AND CROSSINGS BY MOLECULAR MARKERS LINKED TO *PLUM POX VIRUS* RESISTANCE

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Turkey is the most important producer and exporter country of apricot, *Prunus armeniaca*, which is an important temperature fruit. The production of apricots for fresh market relies on foreign cultivars grown on Mediterranean and Aegean regions while Malatya is the most important region for production of dry apricot but base on a several local varieties. The sharka diseases caused by the *Plum pox virus* (PPV) has been reported in several parts of Turkey, although there are no reports for its presence in Malatya. However, when today's production conditions are considered, it may be assumed that it is just a matter of time for the PPV to reach Malatya. Because of that, breeding PPV resistant cultivars having the pomological characteristics of local varieties became an important objective from an economical point of view. In order to breed new cultivars resistant to PPV, marker assisted selection based on markers linked to PPV resistance were used for screening 19 local apricot genitors and progenies obtained from crossings between the PPV resistant cv. Stark Early Orange (SEO) and loca

APPLE LATENT SPHERICAL VIRUS VECTORS FOR CROSS PROTECTION AGAINST ZUCCHINI YELLOW MOSAIC VIRUS

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Apple latent spherical virus (ALSV), classified into a genus *Cheravirus*, was originally isolated from an apple tree in Japan. The virus can experimentally infect a broad range of plant species including cucurbits and legumes without causing symptoms. We previously reported that ALSV-based vectors could effectively induce stable virus-induced gene silencing (VIGS) for long periods in plants. Here, we show that a genetically engineered ALSV vector containing a partial genome sequence of *Zucchini yellow mosaic virus* (ZYMV) display a high degree of cross protection against ZYMV. A wild-type ALSV and a ALSV vector containing ZYMV sequence (ca 200 bp) were inoculated to the cotyledons of cucumber. Five to ten days after inoculation, the infected plants were challenged with ZYMV expressing GFP. Protection was evaluated by symptom development on plants and fluorescence microscopy and ELISA monitored the accumulation of the challenging viruses. The results showed that no symptoms were observed on any plants infected with ALSV vector containing viral genome sequence for a month after challenge inoculation. The accumulation of ZYMV was strongly reduced in the plants. Thus, it is thought that the infection of ALSV vector containing ZYMV genome sequences induced VIGS to target the ZYMV genomic RNA, resulting in strong cross protection against ZYMV.

POSTER SESSION V
SMALL FRUITS:
VIRUSES, VIROIDS AND PHYTOPLASMAS

DETECTION OF VIRUSES AFFECTING *SAMBUCUS* SPP. PLANTS IN POLAND

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Sambucus spp. plants are cultivated in Poland for fruit production and as the ornamental shrubs. Fruits of elderberries are used for processing in preserves and for wine-making, and many different elderberry parts are used for the production of pharmaceutical components. Studies conducted in the U.S. and Western Europe indicated that the greatest losses in the elderberry production cause viruses. Some of those viruses may cause diseases of stone fruit cultivars. In 2009-2010 virus-like symptoms including chlorosis, rings or line patterns on the leaves and retarded growth of the whole plants were observed on *Sambucus nigra* and *S. racemosa* grown in commercial plantations as well as natural habitats. Samples from symptom-showing leaves were tested for the presence of 11 viruses using biological test, ELISA or RT-PCR. Out of 46 plants tested, 17 were infected with *Cherry leaf roll virus* (CLR), one with *Arabis mosaic virus* (ArMV), two with *Tomato black ring virus* (TBRV) and four with *Elderberry latent virus* (EILV). Mixed infection of two viruses: TBRV and CLR or TBRV and ArMV were found in two wild elderberry plants whereas commercial *S. nigra* cv. Aurea and *S. racemosa* cv. Sutherland Gold were infected with CLR and EILV. To our knowledge, this presentation is the first report on EILV in *S. nigra* and *S. racemosa* plants.

EPIDEMIOLOGICAL STUDIES ON LETHAL REDNESS DISEASE OCCURRING IN STRAWBERRY CROPS IN ARGENTINA

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In strawberry (*Fragaria x ananassa* D. cv camarosa) plants, the lethal redness symptom is characterized by stunting, curled leaves and redness on the abaxial face, followed by a generalized decline, necrosis and subsequent death of the plant. These symptoms cause yield losses by preventing proper fruit development. Previous work reported the association of those symptoms with at least two different phytoplasmas. The strawberry read leaf phytoplasma (16SrXIII, subgroup E) and the strawberry X-disease phytoplasma (16SrIII) which have been reported exclusively associated with strawberry in Argentina. The aim of this work is study the relationship between those and other phytoplasmas, with the symptoms described and to determine the incidence and prevalence of the described symptom and the percentage of diseased plants, in strawberry crops in Argentina. During the period 2010-2011 40 plots of strawberry fields located in Lules (Tucumán province, Northwest Argentina), the most important production region in Argentina, were sampled. Incidence ($I = \text{N}^\circ \text{ of plants with symptoms} / \text{N}^\circ \text{ total of plant counted}$), prevalence ($P = \text{presence of at least 1 plant with symptoms per plot} / \text{N}^\circ \text{ total of plots analyzed}$) and percentage of infected plants ($\text{No. of PCR-positive samples for phytoplasmas} / \text{No. of symptomatic plants analyzed}$) were determined. In each sampled plot, transects constituted by 100 plants (300 x ha) were randomly laid. Each symptomatic plant was analyzed by PCR. Our result shows that the strawberry lethal redness is present with a 62% of prevalence and low incidence (0.45%) in strawberry production areas. We also demonstrate that the described symptoms are not always associated with phytoplasma infection, since only 7.96% of them were positive by PCR, suggesting the presence of other pathogens or abiotic stresses involved. It is necessary to continue the surveys in production areas and it would be important to study these symptoms in nurseries where strawberry seedlings are produced.

A VIRUS COMPLEX RESPONSIBLE FOR INCREASED EXPRESSION OF CRUMBLY FRUIT SYMPTOMS IN RED RASPBERRY ‘MEEKER’

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Raspberry crumbly fruit is a widespread disease most commonly caused by a virus infection. In the last decade, the severity of symptoms caused by the disorder has increased considerably in commercial areas of the Pacific Northwest (PNW) of the United States and British Columbia (BC), Canada. *Raspberry bushy dwarf virus* (RBDV), a pollen-borne virus, has long been attributed as the causal agent of crumbly fruit. However, the identification of two new viruses, *Raspberry leaf mottle virus* (RLMV) and *Raspberry latent virus* (RpLV), present at high incidence in the PNW and BC, suggested the existence of a new virus complex responsible for the increased severity of the disease. A field experiment consisting of ‘Meeker’ plants infected by single or mixed infections of RBDV, RLMV, or RpLV revealed that treatments co-infected with RBDV-RpLV-RLMV and RBDV-RLMV had a 71% and 76% reduction, respectively, in primocane growth during the establishment year. Also in the first fruiting year, plants co-infected with RBDV-RpLV-RLMV and RBDV-RpLV had the lowest berry weights, firmness and number of drupelets. Comparisons of virus titers across treatments revealed that the titer of RBDV was increased approximately 400-fold in plants co-infected with RLMV or RLMV-RpLV relative to the titer in plants infected with RBDV alone. The significant increase in titer of RBDV in presence of RLMV in the field was similarly found in greenhouse experiments, suggesting that environmental conditions do not have an impact on the virus interaction. Neither RBDV nor RpLV had an impact on titers of co-infecting viruses. Taken together, these findings suggest that growth reduction and severe crumbly fruit disease in ‘Meeker’ are caused by a co-infection of RBDV and RLMV or RpLV. The implications of these findings, at the applied level, are discussed in this report.

PLANT RNA ISOLATION AID™ HELPS TO EXTRACT HIGHER QUANTITIES OF TOTAL RNA FROM RASPBERRIES

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Nucleic acid extraction from many plant species is still a big challenge. Most difficult are woody plants, since they contain high concentrations of secondary metabolites, which can be purified together with nucleic acids. Many of these substances are known inhibitors of PCR. Raspberries and blackberries are known to be very difficult plants for extraction of good quality RNA. In our study we investigated the influence of Plant RNA Isolation Aid™ (Life Technologies) on quantity of extracted total RNA (totRNA) from different plants, including red raspberries. TotRNA was extracted from red raspberry leaves on MagMAX™ Express Magnetic Particle Processor using MagMAX™-96 Total RNA Isolation Kit (Life Technologies) with and without addition of Plant RNA Isolation Aid™. Extracted totRNA was reverse transcribed and its quantity was checked using 18S rRNA qPCR assay. The Ct values of totRNA extracted without Plant RNA Isolation Aid™ ranged between 11 and 32. The Ct values of totRNA extracted with addition of Plant RNA Isolation Aid™ in the lysis buffer ranged between 8 and 16. The results of our study show that Plant RNA isolation Aid™ greatly improves totRNA extraction from red raspberry leaves.

STUDIES OF *RUBUS* VIRUSES IN SLOVENIA

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In the years 2009-2011 several samples of cultivated red raspberry from different, mostly private gardens and some samples of wild blackberries were tested for the presence of viruses using ELISA and RT-PCR. ELISA was used for the detection of several nepoviruses and *Raspberry bushy dwarf virus* (RBDV), RT-PCR was used for the detection of viruses which can not be detected by ELISA. No nepovirus infections were found in analyzed samples. As expected, RBDV was the most prevalent virus, followed by *Rubus yellow net virus* (RYNV). RBDV was found only in red raspberry samples, while RYNV was found in red raspberry and blackberry samples. *Black raspberry necrosis virus* (BRNV) was found only in three samples of red raspberry from one location. These samples were also infected with RBDV, but according to the information provided by the owner, they did not show symptoms on leaves or fruits. Nine out of ten red raspberry samples from another location were infected with RBDV and RYNV. The leaves of doubly infected plants showed chlorosis and yellowing and the plants had crumbly fruit. The studies of incidence and distribution of *Rubus* viruses are still ongoing under the national research programme P2-0133.

SURVEY OF SLRSV, RPRSV, SMYEV AND SCrV IN STRAWBERRY FIELDS IN BELGIUM

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In order to assess the prevalence of strawberry viruses in Belgium, a survey was carried out since January 2011. Two aphid-transmitted viruses (SMYEV, *Strawberry mild yellow edge virus* and SCrV, *Strawberry crinkle virus*) and 2 nematode-transmitted viruses (RpRSV, *Raspberry ringspot virus* and SLRSV, *Strawberry latent ringspot virus*) were targeted through simplex and duplex PCR, derived from the protocols of Wei *et al.* (2008) and Thompson *et al.* (2003). Samples were collected mainly in strawberry fields (279 samples) but also in *Rubus* sp. (59 samples), *Ribes* sp. (32 samples) and *Prunus* sp. (95 cherry samples). SCrV analyses were limited to the genus *Fragaria*. Until now, no virus transmitted by *Xiphinema* sp. and/or *Longidorus* sp. was detected. Additionally, 5 strawberry leaf samples were infected by SMYEV two of which were also positive for SCrV. A monitoring of the infected fields has been set up.

SPREAD OF *HOP LATENT VIROID* (HLVd) IN HOP GARDEN

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Hop (*Humulus lupulus* L.), as a vegetative propagated monoculture grown for many years at the same location, is severely endangered with virus and viroids infections. Productive potential is decreased by these pathogens which is observable not only on the yield but on the quality of hop cones as well. Cones are used mainly in beer production. Very important is content of hop resins and essential oil for aroma and taste of beer. It is the reason why many countries have begun a recovery process. Viroids are the smallest autonomously replicating pathogenic agent yet describe. In hop plant are present two viroids: *Hop latent viroid* (HLVd) and *Hop stunt viroid* (HSVd). Analyses of commercial hop gardens in Czech Republic shown very high infection of HLVd, and viroid infection decreased content of alpha bitter acids in cones on average by 40 %. In Czech Republic the main variety is Saaz semi-early red-bine hop, which is the typical fine aroma hops, grown in the regions of Žatec (Saaz), Ústěk (Auscha) and Tršice (Trsitz) as Osvald's clone 31, 72 and 114. In period 2004-2010 we collected samples from experimental hop garden planting from virus-free and viroid-free rootstocks and analyzed spreading of reinfection by HLVd in cones of Osvald's clone 31, 72, and 114. No morphological symptoms were observed on infected plants. These results are important for elimination of HLVd and production virus and viroid free planting material of hop in Czech Republic.

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A NEW *FLEXIVIRUS* IDENTIFIED IN BLACKBERRY

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Two symptomatic smooth blackberry (*Rubus canadensis*) plants, collected during the survey on plant viruses in the Great Smoky Mountains, contained high-molecular-weight dsRNAs indicative of virus infections. Purified dsRNAs were used as a template for shotgun cloning and sequencing. Analysis of clones revealed the presence of *Black yellow vein-associated virus* (BYVaV) and *Blackberry virus S* (BIVS), but also a putative new flexivirus. This prompted further molecular characterization of this virus, tentatively named *Rubus canadensis virus-1* (RuCV-1). The RuCV-1 genome is 8.3 kb, polyadenylated and codes for five ORFs: replication-associated polyprotein (RAP), triple gene block (TGB) and viral coat protein (CP). Phylogenetic analyses of the entire RAP indicated that RuCV-1 is closely related to members of the genus *Foveavirus* (fam. *Betaflexiviridae*). However, identities with extant foveaviruses are below the current ICTV species demarcation thresholds, suggesting that RuCV-1 is a novel member of the taxon. A virus-specific RT-PCR based method has been developed and study on the incidence/importance of this virus in cultivated blackberries in Southeastern United States is ongoing.

A NEW *EMARAVIRUS* DETECTED IN BLACKBERRIES AFFECTED BY YELLOW VEIN DISEASE

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Blackberry yellow vein disease complex (BYVD) is a major concern to fresh market blackberry production in the United States. The disease is caused by the synergistic effects of multiple virus infections. The identity of the viruses infecting plants is usually insignificant and symptom severity is closely linked to the number of viruses infecting plants. A new, multipartite negative sense RNA virus, a member of the genus *Emaravirus*, was identified in BYVD plants showing leaf mottling, chlorotic ringspots and curved midribs. RT-PCR detection protocols have been developed and used to evaluate virus distribution in the Southeastern United States in both wild and cultivated blackberries. The infection rate was higher in the former, indicating that the virus is well established in the area. Studies on the population structure of isolates collected from five US states showed geographically-related clustering, suggesting that virus movement because of infected propagation material is rather minimal.

SENSITIVE DETECTION OF THREE BERRY FRUIT VIRUSES BY TAQMAN® QPCR

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Several isolates of berry fruit viruses have been studied recently, giving unprecedented insight into the population structure of berry viruses. The data generated can be used for the development of 'universal' primers, able to detect the vast majority of virus isolates in United States and elsewhere. TaqMan® quantitative reverse transcription-PCR (qRT-PCR) assays with absolute quantification have been developed for *Blackberry chlorotic ringspot virus* (BCRV), *Strawberry necrotic shock virus* (SNSV) and *Blackberry yellow vein associated virus* (BYVaV). RNA extracted from virus-infected leaves was used for reverse transcription with specific and/or random primers. Standard curves were constructed and showed that the assays can detect as low as 30 copies of the respective viruses. The assays, reproducible for both Ct values and calculated copy numbers, have been successfully tested on several rosaceous hosts.

GENOME ORGANIZATION AND SEQUENCE DIVERSITY OF A NOVEL BLACKBERRY *AMPELOVIRUS*

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Blackberry is an important small fruit crop in the United States (US), and recently several new viruses have been identified associated with blackberry yellow vein disease. One of the new viruses was discovered in Mississippi in 2010. Experiments were conducted to characterize the virus, determine its distribution and population structure in the US. Genome sequences were obtained by “next-generation” sequencing and random primed cloning of double stranded RNAs extracted from symptomatic plants. Phylogenetic analysis revealed the close association of the virus with *Grapevine leafroll virus-3*, the type member of genus *Ampelovirus* in the family *Closteroviridae*. The virus genome is approximately 18 Kbp long and consists of 12 open reading frames (ORFs). Screening of survey samples collected during 2008-2011 from various states in the US revealed the presence of the virus in Arkansas, Georgia, Mississippi, North and South Carolinas. Nucleotide and amino acid diversities were analyzed for three genomic regions; polyprotein (region between methyl transferase and helicase domains), heat shock protein70 homologue (HSP70h) and coat protein (CP). Considerable variations were observed in the polyprotein (77-100%) and CP (86-100%) regions compared to the HSP70h (99-100%). Sensitive detection protocols are developed based on the information obtained in this study and are able to detect a wide range of isolates.

POSTER SESSION VI
CONTROL STRATEGIES

**PRELIMINARY RESULTS ON STUDIES OF RESISTANCE TO
PLUM POX VIRUS-D IN *PRUNUS* IN ARGENTINA**

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Sharka, caused by *Plum pox virus* (PPV), is the most detrimental viral disease of stone fruit in the world. It is characterized by its difficulty to control, due to its rapid transmission by aphids, uneven distribution through the plant and low concentration. The use of resistant cultivars seems to be the only definitive method to control the disease. In Argentina, PPV was detected on plum (*Prunus salicina* cv. Red Beaut) and apricot (*P. armeniaca* cv. Bulida) at November 2004. After seven years of survey, it had not been found any positive peach. In this study was evaluated, by artificial inoculation, the ability of two peach cultivars (*P. persica* cv. Pavía Catherine and Loadel) to allow a long distance movement of PPV strain D isolated through their vascular tissues. The virus xylem and phloem transport was evaluated using GF 305 under controlled insect-proof greenhouse conditions. The presence of PPV symptoms on leaves of GF 305 rootstocks, cultivars and GF 305 scions on the top, was evaluated during one cycle of study. DASI-ELISA and AC-RT-PCR were performed to each part of the studied plants. The genotypes studied allowed the virus movement through their vascular system in both directions at the first cycle of study. No PPV symptoms were observed on leaves of any of the cultivars, although they were positives by ELISA and PCR. GF 305 always showed clear symptoms and gave positives reactions by the performed tests. The results presented at this work are preliminary; more growing cycles would be needed to determine the tolerance of Pavía Catherine and Loadel peach cultivars to PPV-D Argentinean isolate.

MONITORING OF FRUIT TREE PHYTOPLASMA VECTORS IN AN OVERWINTERING SITE

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Psyllids – vectors of fruit tree phytoplasmas – were studied in overwintering site, locality Olšany (south Moravia, Czech Republic) over the years 2006-2010. The population dynamics of *Cacopsylla pruni* ('*Candidatus* Phytoplasma prunorum' vector), *C. picta* and *C. melanoneura* (both '*Candidatus* phytoplasma mali' vectors) was monitored as well as incidence of phytoplasma positive individuals was determined by PCR/RFLP analysis. Using a sweeping net on a long stick total 803 individuals of above mentioned vectors were collected. It was confirmed that conifers (*Picea abies* as the dominant species in the area) growing in the investigated highland site of middle Moravia harbour *C. pruni*, *C. picta* and *C. melanoneura* during the winter season. It was enlarged knowledge about seasonal migration of three studied psyllid species. Sum of captured individuals varied from year to year depending on population density, seasonal migration of psyllids and their activity, and weather conditions. *C. picta* was less frequently captured among the three phytoplasma vectors studied. The presence of the phytoplasma positive individuals reached 22% for *C. pruni* ('*Ca. P. prunorum*'), 15% for *C. picta*, and 14% for *C. melanoneura* (both '*Ca. P. mali*').

RECOVERY PHENOMENA IN APRICOT TREES CV. BERGERON INFECTED BY EUROPEAN STONE FRUIT YELLOWS IN THE PROVINCE OF TRENTO (ITALY)

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Recovery phenomena, have been intensively studied for apple proliferation and grapevine yellows. Despite the physiological basis of recovery is not yet completely understood, several recent findings in apricot orchards in Friuli-Venezia Giulia (Italy), have indicated the importance of this strategy also for the control of European stone fruit yellows (ESFY) infection, associated with ‘*Candidatus Phytoplasma prunorum*’, transmitted by *Cacopsylla pruni* (Scopoli) presence. The aim was to investigate the occurrence of recovery in two experimental apricot fields of “Bergeron” on “Wavit”, in the province of Trento (Italy), originally planted using ESFY-free material, where the disease has been constantly spreading since 2000 leading to partial or total tree dieback causing major economic losses to growers. The investigation was carried out with visual inspections for typical symptoms (early bud-break during dormancy, premature leaf-roll, fruit deformation and dieback) and a diagnostic method based on Spot Real-Time Reverse Transcription PCR assay. Plants originally infected were considered recovered only after a minimum of three consecutive years without symptoms. The results obtained showed that three different groups of plants infected by ESFY were present in the experimental fields: many plants with typical symptoms and positive to PCR analysis, some recovered infected plants and some asymptotically infected plants. The research will continue with the aim to quantify the concentration of the pathogen in the recovered plants. To date no plant genotypes showing resistance to ESFY are available, moreover previously results, obtained in the same area, showed that most of the insecticide applications have no efficacy in controlling the disease. Both use of pathogen-free propagation material and resort to natural recovery could be adopted as integrated approach for the control of this phytoplasma disease.

POSTER SESSION VII
CERTIFICATION

CAV'S LABORATORY ACTIVITY WITHIN THE ITALIAN CERTIFICATION SCHEME OF PLANT PROPAGATION MATERIAL

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CAV (Centro Attività Vivaistiche) is an association of nurserymen founded in 1982. Its main goal is to improve the quality of the nursery production by the achievement of genetic and phytosanitary certified propagating material. With more than 800 primary sources raised in its screen-houses, CAV represents the main Nuclear and Propagation Centre in Italy, officially recognized by the Italian Ministry of Agriculture (Mipaaf) to supply pome fruits, stone fruits, strawberry and olive prebasic and basic propagating material, to the Italian nurseries as well as to other international customers. To increase the guarantee on its procedures and released products, CAV obtained the ISO 22005:2008 certification for row traceability. In order to fulfill all the technical requirements listed in the Italian certification scheme, CAV manages infrastructures (glass houses) and a modern laboratory, accredited by Mipaaf, equipped for the detection of several pests (Viruses, Bacteria, Fungi, Phytoplasmas, Viroids, Mites and Insects) and for the assessment of cultivar trueness-to-type (fingerprinting) for candidate primary source, prebasic, basic and certified material. The techniques currently used include biological (indexing), serological (ELISA, IFAS), molecular (PCR, Real-Time PCR), microbiological tests (isolation and identification) and microscopy observations (optical/fluorescence). CAV's laboratory is also equipped to obtain virus free clones of fruit varieties by several methods i.e. heat therapy and tissue culture.

The 2011 activities include:

N. 2296 biological assays

N. 2802 serological tests for primary source, pre basic and basic material

N. 11.918 serological tests for certified material

N. 1233 molecular tests for primary source, pre basic and basic material

N. 149 selected/sanitized primary sources submitted for registration in the National certification scheme

The increasing needs coming from the internationalization of its activities and the improving of the quality management system pushed CAV to request in 2009 the accreditation in agreement with ISO/IEC Standard 17025 *General requirements for the competence of testing and calibration* (credit ILAC/ACCREDIA. No. 0896). In the same years, CAV obtained the official recognition by MAF BIOSECURITY NEW ZEALAND for testing Malus and Pyrus "high healthy material" to be exported to New Zealand.

CIVI-ITALIA, THE ITALIAN NURSERY ASSOCIATION AND ITS ROLE WITHIN THE FRAMEWORK OF THE ITALIAN CERTIFICATION SCHEME

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CIVI–Italia, the inter-professional centre for nursery activities, is the national consortium among nursery groups and growers' union associations. It is officially recognized by the Italian Ministry of Agriculture (Mipaaf) with a ministry decree issued on December 2nd, 1993, as the exclusive national association to represent the nursery sector for promoting and carrying out the plant propagating certification programme in Italy. CIVI-Italia plays an important role in the framework of the Italian voluntary certification scheme by having the main statutory objectives to enhance nursery production in order to qualify vegetable, fruit, olive and citrus yields. For the tasks assigned to it, CIVI-Italia facilitates the connections among the several structures that constitute the National Certification Scheme which is composed by

9 Centers of conservation for premultiplication, located in 7 different Regions;

10 Centers of premultiplication, located in 8 different regions;

26 Centers of Multiplication (Mother blocks), extended over 150 ha, located in 10 different regions;

Over 100 nurseries which manage nursery plots extended over 2.500 Ha

1.511 Primary sources of the varieties more requested by the market.

Upon completion of administrative and field checks, the competent Regional Phytosanitary Service notifies certification admission to the nurseryman and to the body charged of printing the labels-certificates, together with the authorisation to print and affix the label-certificate. Labels/certificates are made and distributed by CIVI-Italia, which send the final schedules showing the numbers assigned to the labels-certificates produced to the locally competent Regional Plant Health Services and to the Ministry for Agriculture and Forestry Policies every year. In the last season 2011/12, the volume of certified plants produced in Italy by 102 nurseries was constituted by: 6,83 pome fruit; 1,24 stone fruit; 22,3 fruit rootstocks; 190 strawberry (million of plants).

The Certification program in Italy, following the new course set up in 2003, constitutes a positive experience implemented under the coordination and control of the Ministry and Regional Phytosanitary Services, in strong cooperation with the nursery private sector.

THE VOLUNTARY CERTIFICATION SCHEME FOR FRUIT PLANT PROPAGATING MATERIAL IN ITALY

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In Italy, the qualification of plant propagating material, by the establishment of certification programmes were carried out at regional level in several Regions for different species since 1981. The adopted rules were quite different even if referred to very similar protocols and in agreement with the International regulations. The Italian National Service for the Voluntary Certification was founded in 1989 by the Ministry decree. During the years, some Regions attended to the national certification programs for pome and stone fruit, olive, strawberry and citrus. Due to the occurrence of new pests and diagnostic techniques, and on the basis of these experiences, the necessity to modify the rules was clear, also in the light of the European Directive 92/34/EEC, which was carried out in Italy since April 1997. Furthermore, the Regional Governments in Italy were assigned the tasks previously fulfilled by the Central Administration. During the years from 2003 to 2006, new decrees reorganized the National Certification Service and in 2006 updated technical protocols for each species were issued. According to these rules, the National Certification Service is established at the Ministry for agriculture food and forestry policies (Mipaaf). It is in charged the national coordination.

It is composed of: Technical Committee, Operating Secretariat and Regional Phytosanitary Services.

The Regional Phytosanitary Services are in charge of the certification (sanitary and trueness to type controls) in their territory. 1.511 are the primary sources officially recognized in the Italian certification scheme, in representation of classic and most recent varieties licensed by the national and international breeding programs. In the last season 2011/12, the volume of certified plants produced in Italy by 102 nurseries was constituted by: 6,83 pome fruit; 1,24 stone fruit; 22,3 fruit rootstocks; 190 strawberry (million of plants).

