

A DNA MICROARRAY-BASED ASSAY FOR THE DETECTION AND IDENTIFICATION OF PLUM POX VIRUS AND GENOTYPING OF ITS ISOLATES TO THE VIRAL STRAINS. M. Barba¹, G. Pasquini¹, F. Faggioli¹, A. Hadidi², H. Czosnek³, K. Çağlayan⁴, H. Mazyad⁵ and G. Anfoka⁶. ¹CRA, Centro di Ricerca per la Patologia Vegetale, Via C. G. Bertero 22, 00156 Rome, Italy. ²Agricultural Research Service, United States Department of Agriculture, Beltsville, MD 20705, USA. ³Institute of Plant Sciences and Genetics in Agriculture, Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot 76100, Israel. ⁴Department of Plant Protection, Faculty of Agriculture, Mustafa Kemal University, 31034 Antakya-Hatay, Turkey. ⁵Plant Pathology Research Institute, Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Giza 12619, Egypt. ⁶Department of Technology, Faculty of Agricultural Technology, Al-Balqa' Applied University, Al-Salt 19117, Jordan. E-mail: marina.barba@ispave.it

In recent years, DNA microarray technology has become one of the most widely used tools for functional genomics. The utilization of this technology for plant pathogen detection relies on its advantage of simultaneous detection and identification of many pathogens or their strains in a single assay. Current detection methods of plant pathogens do not have this capability. The nucleotide sequences of several *Plum pox virus* (PPV) isolates and strains have been deposited in the GenBank. Thus, it is feasible to design long (70 nts) species- or strain-specific probes for the detection and identification of the virus and genotyping of its isolates to their respective strains using the DNA microarray technology. Our experimental results revealed that several 70-mer oligonucleotide probes were specific for the detection and genotyping of individual PPV isolates to their respective strains. Other probes were specific for the detection and identification of two or three PPV strains. One probe (PPV species specific = universal), derived from the genome highly conserved 3' non-translated region, detected all individual strains of PPV. This universal probe combined with probes specific for each known strain could be used for the discovery of new PPV strains. Finally, by indirect fluorescent labelling of PPV cDNA with cyanine in a separate step after cDNA synthesis, we were able to enhance the sensitivity of the PPV microarray assay, thus eliminating the PCR amplification step of cDNA. By using the PPV microarray, we were able to efficiently detect and identify PPV strains in PPV-infected peach, apricot and *Nicotiana benthamiana* leaves. The microarray-based PPV detection method is versatile and has the potential to make the simultaneous detection of plant pathogens using microarray technology feasible and easier.

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DEVELOPMENT AND EVALUATION OF GENETIC VIRUS RESISTANCE IN PRUNUS FOR THE ERADICATION OF PLUM POX VIRUS IN CANADA. D.C.W. Brown¹, B. Miki¹, H. Sanfaçon², A. Svircev³, L. Tian¹ and A. Wang¹. ¹Agriculture and Agri-Food Canada (AAFC), London, Ontario, Canada, N5V 4T3. ²AAFC, Ottawa, Ontario, Canada. ³AAFC, Summerland, British Columbia. ³AAFC, Vineland, Ontario, Canada. E-mail: broundc@agr.gc.ca

Generation of germplasm resistant to PPV through the use of new technologies will provide an effective and practical long-term strategy to eradicate PPV from peach orchards and protect the tender fruit as well as ornamental/nursery industry in Canada. Six Agriculture and Agri-Food Canada laboratories are co-operating and developing a consumer acceptable approach for resistance to PPV in *Prunus*. This approach is centered on gen-

eration of PPV resistant germplasm with established gene silencing technology, development of new approaches for genetic resistance, and development of biosafety approaches that can be used to produce transgene-free fruits with PPV resistance. We previously reported that high level resistance to PPV (near-immunity) can be engineered into the model host *N. benthamiana* by expressing PPV-specific intron-spliced hairpin RNA's targeting the HC-pro, P1 and CP regions of the PPV genome. We have transferred the technology to *Prunus* (plum cv. Stanley). Similarly to *N. benthamiana*, transgenic plums displayed a high level of resistance to PPV. Diagnostic PPV-specific patterns of the 22 and 26 bp siRNAs were found in both species confirming that RNA silencing had been activated in these lines. Both the 22 and 26 bp siRNAs were present at all times in the resistant lines; whereas, only the 22 bp siRNA was found in susceptible lines and only after PPV infection. These results confirm that the engineered PPV-resistance is related to a natural antiviral defense mechanism operating against PPV and suggest that transgenic resistance differs from the natural antiviral defense in that it is expressed constitutively and provides high level of resistance or immunity to PPV. Progress and research results will be reported on (i) the generation of resistant germplasm using gene silencing technology, (ii) new approaches for genetic resistance, (iii) biosafety approaches to produce transgene-free fruit, and (iv) platform technologies for use in *Prunus* sp.

TOLERANCE OF AUTOCHTHONOUS TURKISH STONE FRUIT VARIETIES TO SHARKA. K. Çağlayan, Ç.U. Serçe and M. Gazel. Mustafa Kemal University, Faculty of Agriculture, Plant Protection Department, Antakya, Turkey. E-mail: caglayan@mku.edu.tr

Turkey is the most important producer and exporter of apricot (*Prunus armeniaca* L.), a major temperate fruit grown for fresh or dry consumption. Turkey's table apricot production is largely based on the use of foreign cultivars (Précoce de Thyrinthe, Précoc de Clomer, Canino and Ninfa) grown in the Mediterranean and Aegean provinces of the country. By converse, autochthonous cultivars like Hacıhaliloglu, Kabaası, Hasanbey, Cataloglu, Soganci, Cologlu, Alyanak, Sekerpare, Kurukabuk and Ismailaga prevail in Malatya, which is the most important Turkish area for dry apricot production. Studies for determining the reaction of autochthonous Turkish apricot varieties to *Plum pox virus* (PPV) infection are few. Apricot cvs Cologlu, Hacıhaliloglu, Hasanbey, Karacabey and Tokaloglu were reported to be sensitive to PPV-M and PPV-D (Dosba *et al.*, *Acta Hort.* 309: 211-219, 1992). Likewise, cvs Aprikoz, Cologlu, Hacıhaliloglu, Tokaloglu and Sam from the Bursa province, grown outdoors in the province of Ankara, proved to be sensitive to natural PPV infection during four years of observation (Elibuyuk and Erdiller, *Acta Hort.* 384: 549-552, 1995). Recently, some local Turkish cultivars that were field-tested in Hungary under heavy inoculum pressure gave some promising results for PPV tolerance (Çaglayan *et al.* *European Meeting on Plum Pox*: 17, 2004).

DIAGNOSIS IN DORMANT PERIOD FOR PREDICTION OF SHARKA DISEASE: PRACTICAL PARAMETERS. N. Capote, A. Olmos, E. Bertolini, A. Moreno, E. Vidal and M. Cambra. Centro de Protección Vegetal y Biotecnología. Instituto Valenciano de Investigaciones Agrarias (IVIA). Carretera Moncada-Náquera km 5, 46113 Moncada, Spain. E-mail: mcambra@ivia.es

Legal and illegal transport of plant material for vegetative

propagation is the main cause for *Plum pox virus* (PPV) spread over long distances. Plant material is usually commercialized during the dormant period and for this reason it is necessary to assess the reliability of available methods of diagnosis to avoid that "subliminal" infections may escape detection. This evaluation was done by analysing practical parameters (sensitivity, specificity, positive and negative likelihood ratios and probability post-test), after PPV detection assays of 285 adult *Prunus* trees sampled in winter and in the following spring. Symptoms of sharka, as indicator of disease, were recorded in spring. The following detection methods were compared using the same plant extract: DASI-ELISA (5B-IVIA), IC-PCR (P1/P2), NASBA-FH and Spot Real-time PCR (using crude extracts spotted on nylon membranes). The most sensitive method was Spot Real-time PCR (0.94 ± 0.01), followed by NASBA-FH (0.93 ± 0.02), IC-PCR (0.92 ± 0.02) and DASI-ELISA (0.87 ± 0.02). The most specific techniques proved to be Spot Real-time PCR and DASI-ELISA, which had the same specific value (0.94 ± 0.01), followed by IC-PCR (0.89 ± 0.02) and NASBA-FH (0.87 ± 0.02). Likelihood ratios were calculated for all techniques to predict the probability of post-test disease development based on the Bayesian approach. Thus, if a positive response were obtained in the dormant period, the probability of disease appearance (i.e., sharka symptoms) in the next spring was similar for Spot Real-time PCR (97.41%) and DASI-ELISA (97.21%), followed by IC-PCR (95.34%) and NASBA-FH (94.42%). On the contrary, if negative results were obtained in winter, the probability that sharka would develop in the following spring was 14.14% for Spot Real-time PCR, 17.05% for NASBA, 18.48% for RT-PCR and 25.47% for DASI-ELISA. This comparison showed that none of the detection techniques used is satisfactory for routine testing. In particular, DASI-ELISA showed an overall behavior comparable to that of assayed molecular techniques. However, Spot Real-time PCR was most reliable, sensitive and specific for PPV detection in winter time. In addition, this high throughput technique does not require nucleic acids extraction nor the immunocapture phase which makes it a rapid and user-friendly method.

PRODUCTION OF MONOCLONAL ANTIBODIES AGAINST PLUM POX VIRUS ISOLATE W3174 AND COMPARISONS OF ELISA AND A BIOPLEX BEAD-BASED SUSPENSION ASSAY FOR SPECIFIC DETECTION. H. Croft¹, T. Malinowski², L. Krizbai³, I. Mikec⁴, V. Kajič⁴, A. Varga¹ and D. James¹. ¹Centre for Plant Health, Canadian Food Inspection Agency, 8801 East Saanich Road, Sidney, British Columbia, Canada V8P 4L6. ²Research Institute of Pomology and Floriculture, Pomologiczna 18, 96 100 Skierniewice, Poland. ³Central Service for Plant Protection and Soil Conservation, Budaorsi ut 141-145, 1118 Budapest, Hungary. ⁴Institute for Plant Protection, Svetosimunska 25, 10 000 Zagreb, Croatia E-mail: jamesd@inspection.gc.ca

Plum pox virus (PPV) isolate W3174 was characterized recently and identified as representing a new strain of PPV. In this study, the N-terminus region of the coat protein of PPV-W3174 was identified as unique and perhaps suitable for the production of PPV-W specific monoclonal antibodies (MAbs). This would provide a useful tool for simple and rapid identification of isolates of this strain. Recombinant peptides for use as immunogens were expressed in *Escherichia coli* transformed with the vector pCOLD containing the appropriate construct. The expressed proteins were His-tagged to facilitate purification with Ni-NTA agarose columns. A panel of MAbs were produced and screened for the detection of PPV-W3174, in ELISA and Western blot analysis. Isolates of PPV strains D, M, C, and EA were included also in the evaluation. TAS-ELISA was compared to a Bioplex

bead-based suspension assay for rapid and reliable detection of PPV-W3174.

QUASISPECIES COMPLEXITY AMONG PPV-D AND PPV-REC ISOLATES IN THE CZECH REPUBLIC. S. Gadiou, D. Safarova and M. Navratil. Palacky University, Faculty of Science, Department of Cell Biology and Genetics, 783 71 Olomouc, Czech Republic. E-mail: gadiou@prfnv.upol.cz

Plum (*Prunus domestica*) and myrobalan (*Prunus cerasifera*) trees showing typical PPV symptoms of diffuse spots and oak leaf mosaic were sampled from September 2005 to October 2006. Leaf samples were collected from 7 plum orchards in the Czech Republic in the following localities, Bohutice I, Bohutice II, Brtev, Hlohovec, Hrusky, Lipov, and Tyn and Becvou. PPV-D isolates were identified in all orchards. PPV-Rec isolates were identified in only two plum orchards at Lipov and Bohutice I. In order to widen the knowledge on the differences within the PPV-Rec and PPV-D isolates coming from the different Czech orchards under study, SSCP analysis of HC-Pro region was done. All PPV-Rec patterns were variants of PPV-D pattern. Five distinct patterns were obtained for PPV-Rec isolates and 11 distinct patterns for PPV-D isolates.

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EVALUATION OF APRICOT CULTIVAR SUSCEPTIBILITY TO PLUM POX VIRUS INFECTION UNDER GREENHOUSE CONDITIONS. P. Gentil¹ and J.M. Lemontey². ¹Ctifl, Centre de Lanxade BP 21, 24130 La Force, France. ²SEFRA, Quartier Marcellas, 26800 Etoile-sur-Rhône, France. E-mail: gentil@ctifl.fr

Although a number of papers deal with the susceptibility of apricot cultivars to *Plum pox virus* (PPV) infection, only a few deal with the reactions of new varieties to this virus. With this study, we wanted to investigate the behaviour of a range of new apricot cultivars in relation to PPV infection, to help the industry in the choice of the best germplasm and to improve the extant strategy for limiting the spread of Sharka disease. In 2006 and 2007, during the construction of our future high containment facility, we have tested our protocols using artificial inoculations with *Apple mosaic virus* (ApMV, strain P1LA12). Two methods were tested: inoculation by direct grafting in the greenhouse and inoculation by dormant buds of seedlings produced in a nursery, then potted and transferred to a screenhouse. Preliminary results obtained in 2007, showed that despite the low percentage of successful graft take (16% to 73% depending of the cultivar) symptoms were expressed during the first vegetative cycle by the subjects inoculated by direct grafting. Dormant buds gave a 100% growth but with no symptom expression during the first vegetative cycle. After laboratory controls, all tests will be repeated in the second vegetative cycle. At the end of 2008, when the experiments with ApMV will come to an end, and the building of the new high containment facility will be completed, the experiment with PPV will be initiated.

MOLECULAR ANALYSIS OF PEACH VIRUS ISOLATE AGUA AND ELUCIDATION OF SOME SEROLOGICAL CROSS-REACTIONS INCLUDING CROSS-REACTIONS TO PLUM POX VIRUS ANTISERUM. D. James, A. Varga and H. Croft. Centre for Plant Health, Canadian Food Inspection Agency, 8801 East

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Peach virus isolate Agua (PcVA) cross reacts with polyclonal antisera against *Plum pox virus* (PPV, genus *Potyvirus*, family *Potyviridae*), and *Apple stem pitting virus* (ASPV, genus *Foveavirus*, family *Flexiviridae*). Cross-reactions were observed in several serological assays including ELISA, Western blot, and immunosorbent electron microscopy. Preliminary analysis indicated that PcVA was not an isolate of PPV. PcVA genome consists of 9005 nucleotides, excluding a poly(A) tail at the 3' end, and has a genome organization similar to ASPV, with 5 open reading frames. When the sequence of PcVA was compared to ASPV, identity levels of 53% and 37% were observed for the nucleotide (nt) and amino acid (aa) sequences, respectively. ASPV is the type member of the genus *Foveavirus*, and PcVA is a new and distinct member of this genus. Alignments of the amino acid sequence of PcVA and ASPV revealed a number of conserved peptides 4-8 aa residues long common to both viruses that may constitute linear epitopes responsible for the cross-reactions observed. Similar peptides were not observed when PcVA was compared to PPV. However, a series of conserved amino acid residues located at regular intervals were common to both viruses. It is possible that these aa residues contribute to the formation of conformational or discontinuous epitopes in PcVA that are recognized by the PPV antiserum.

NEW VIEWS ON PATHOGEN-DERIVED RESISTANCE. M. Khan¹, D. Mendonça², E. Borroto-Fernandez¹, F. Maghuly¹, G. Marzban¹, H. Kättinger¹ and M. Laimer¹. *Plant Biotechnology Unit, IAM, Department of Biotechnology, BOKU, Muthgasse 18, 1190 Vienna, Austria.* ²*Departamento de Ciências Agrárias, Universidade dos Açores, 7900 Angra do Heroísmo, Portugal. E-mail: m.laimer@iam.boku.ac.at*

Strategies for engineering resistance to viruses involve three types of transgenes: (i) pathogen-derived transgenes, (ii) plant-derived transgenes including PR and R-genes, (iii) non-plant, non-pathogen-derived transgenes, e.g. antibodies and antiviral proteins (Laimer M. 2006. In: Fladung M. and Ewald D. (eds) *Transgenic Trees*, pp. 181-199. Springer, Vienna). Viral coat protein (CP) sequences have been used as sense, antisense, full length, truncated or untranslatable constructs, that confer protection levels from immunity to delay and attenuation of symptoms. Meanwhile, PDR has been shown to be RNA-mediated and based on mechanism of co-suppression and post-transcriptional gene silencing (PTGS) or homology-related gene silencing, pointing to the existence of a natural defense mechanism of adaptive protection against viruses (Baulcombe, *Nature* **43**: 356-363, 2004). Furthermore, many plant viruses encode proteins suppressing PTGS, suggesting a co-evolution of defense and counter-defense between the host and the invading virus. In the case of transgenic fruit trees, initially the use of translatable and non-translatable CP sequences yielded both immunity and recovery in model plants. However both the number of protected lines and the level of protection against homologous virus strains seemed worthwhile improving. A further driving force for the modification of constructs were safety considerations concerning (i) selection of viral sequences reducing the potential risk of recombination or (ii) mutations of the CP gene suppressing particle assembly, heterologous encapsidation, and complementation. To abolish the transfer of functions to challenging viruses via recombination, a number of constructs containing a translatable or non-translatable CP-gene sequence of PPV NAT with and without the 3' NTR, were first tested on herbaceous hosts and finally trans-

formed into *Prunus sp.* (Mendonça, Ph.D. Thesis, University of Azores, 2005). PTGS has been achieved with high efficiency in transgenic plants expressing self-complementary hairpin RNAs having two complementary regions that form a double stranded region separated by a short loop. This approach has also been described in herbaceous plants for PPV (Pandolfini *et al.* *BMC Biotechnol.* **25**: 1-7, 2003). A further advantage is represented by temporally and spatially inducible gene expression by the use of tissue specific promoters, e.g. pathogen inducible promoters. Inducibility of the *uidA* gene under the control of the Mal d 1 promoter by PPV in *N. benthamiana* was analysed (Mendonça, 2005). Truncated CP-sequences have been described to confer resistance, thus suggesting the use of partial CP sequences for new constructs, possibly omitting the N-terminal region involved in aphid transmissibility and reducing the 3'NTR potential recombination events. The use of these fragments as inverted repeat constructs with the ST-LS1 intron (E. Maiss, personal communication) is currently under investigation.

IDENTIFICATION AND EXPRESSION ANALYSIS OF GENES DIFFERENTIALLY EXPRESSED IN *PRUNUS DOMESTICA* L. DURING *PLUM POX VIRUS* INFECTION. K. Kowalczyk, D. Wójcik, T. Malinowski and L. Michalczyk. *Research Institute of Pomology and Floriculture, 96100 Skierniewice, Poland. E-mail: kkowal@insad.pl*

Sharka, caused by *Plum pox virus* (PPV), is the most devastating disease of plum (*Prunus domestica* L.). At present, it is believed that the only effective control of PPV spread can be achieved by growing resistant cultivars. Most plum cultivars are highly susceptible to PPV. Therefore many research programmes aiming at breeding resistant or tolerant plum cultivars are currently carried out worldwide. Unfortunately, plum breeding for resistance through conventional methods is difficult because available sources of resistance to PPV are rare. Identification of gene products involved in host plant response to PPV infection could help understanding the mechanisms of resistance to this virus. These genes could also be used as molecular markers in marker-assisted selection (MAS) in plum breeding programmes. Unfortunately, the knowledge on PPV-*Prunus* spp. interactions at the molecular level is very limited. The aim of this study was to identify candidate genes linked to plum reaction to PPV infection. The experiment was done using plum cvs Čačanska Najbolja, relatively resistant to Sharka and Čačanska Rodna, very susceptible (both cultivars are half-sibs, having cv. Stanley as a parental cultivar). Pools of mRNA from leaves of infected and healthy plants were compared using cDNA-AFLP. So far, 95 primer combinations have been tested in cDNA-AFLP analysis. Fifty two cDNA fragments, 32 from cv. Čačanska Najbolja and 20 from cv. Čačanska Rodna, corresponding to genes differentially expressed during PPV infection were cloned, sequenced and subjected to BLAST analysis. Of cloned cDNA fragments, 18 showed significant homology to genes encoding known proteins such as ABC family proteins (*Arabidopsis thaliana*), 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR) (*Lycopersicon esculentum*), ribonuclease II family protein (*Arabidopsis thaliana*), protein kinase family protein (*Arabidopsis thaliana*), phosphoethanolamine-N-methyltransferase (*Brassica napus*) and GTP-binding protein tyxA (*Arabidopsis thaliana*). Further validation of identified candidate genes was assessed by confirming their expression pattern during PPV infection. The first screening was conducted using semi-quantitative RT-PCR. The expression pattern of every candidate gene was examined in both tolerant and susceptible cultivars. Results confirmed that some of the identified genes displayed a differential expression in healthy and in-

fectured plants. For these genes, putatively linked to reaction of plum to PPV infection, further expression analysis is being performed using quantitative real-time RT-PCR.

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SEROLOGICAL AND GENETIC DIVERSITY OF PLUM POX VIRUS EL AMAR STRAIN. S. Matic¹, I. Elmagraby², V. Law³, A. Varga³, A. Myrta² and D. James³. ¹Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi and Istituto di Virologia Vegetale, Sezione di Bari, Via Amendola 165/A, 70126 Bari, Italy. ²Istituto Agronomico Mediterraneo, Via Ceglie 9, 70010 Valenzano, (BA), Italy. ³Centre for Plant Health, Canadian Food Inspection Agency, 8801 East Saanich Road, Sidney, British Columbia, Canada V8P 4L6. E-mail: jamesd@inspection.gc.ca

Sixteen isolates of *Plum pox virus* (PPV) were collected in Egypt from various orchards at Sinro (Fayum Oasis) and El Amar (Nile Delta). The hosts were mainly *Prunus armeniaca* (apricot, cvs Amar and Balady), as well as an isolate from *Prunus salicina* (Japanese plum, cv. Balady). The various isolates of PPV-EA were screened against a panel of monoclonal antibodies (MAb) including: the universal MAb 5B; MAb AL (M specific); MAb 4DG5, and 4DG11 (D specific); MAb AC (C specific); and a panel of MAbs produced against PPV-EA, including MAb EA24, which is specific for PPV EA isolates. Some variability of MAb binding response of the various isolates in ELISA was observed. All isolates were positive with MAb 5B, and negative with MAbs specific for strains D, M, and C. There were three very interesting isolates. Isolate APR 50 did not react with the EA-specific MAb EA24, but reacted with all other PPV EA-derived MAbs. Isolate APR 53 reacted with EA24, but only with four of the other 11 PPV EA-derived MAbs. Isolate APR 48 has an 11 amino acid deletion (33 nt) at CP position aa 70-80, but reacted with all EA-derived MAbs except for EA11. It is likely that MAb EA11 recognizes an epitope in this region of the CP. Sequencing and phylogenetic analysis of the coat protein region confirmed that all 16 isolates are PPV EA, including isolate APR 50. Amino acid changes in two regions of the N-terminus of APR 50 were found, that may contribute to the lack of recognition by MAb EA24.

PLUM POX VIRUS INDEXING IN THE UNITED KINGDOM. S. Matthews-Berry. Central Science Laboratory, Sand Hutton, York, YO41 1LZ, UK. E-mail: s.matthews-berry@csl.gov.uk

Plum pox virus (PPV) was first identified in the UK in 1965 and an eradication programme was implemented. However, by 1974 it was clear that PPV had established in England and was spreading within orchards and some nurseries. The possibility of complete eradication now seemed very low and the focus of the campaign moved from eradication to containment. The main effort was concentrated on nurseries producing certified material and those using imported material, where infected trees were destroyed. In 1993 with the introduction of the EU single market, 'plant passporting' of *Prunus sp.* for internal movement was required. Passports are issued for material which has been: (i) certified under the official certification scheme (PHPS), or (ii) derived from material which has been tested within the last three years (non-certified material). To meet the requirements for testing of non-certified material, indexing of regulated host species susceptible to PPV used for propagat-

ing, on premises authorised to issue plant passports, must be tested for PPV at least every three years. If PPV is confirmed infected plants must be removed and any further passporting or sale of plants susceptible to PPV is prohibited. Further propagation is only permitted from plants which have been tested and found free from PPV, at least once in the last three complete cycles of growth but they cannot be passported. This can only resume once there have been no symptoms caused by PPV on plants at the nursery or on susceptible plants in the immediate vicinity within the last three growing seasons. Indexing of *Prunus sp.* in the UK has shown that incidences of PPV in propagating material are low. From 1994-2006 only 37 positive were identified from 18320 samples tested. Strain typing of all PPV positives has been carried out since 1998 and only the D strain has been identified. While no official surveys of fruit orchards have been carried out incidences of PPV in orchards are low. It has not been possible to eradicate PPV in the UK but it has been successfully contained. To contain PPV it has been necessary to establish a supply of virus free planting material. This has been achieved through the PHPS and plant passporting, with their effectiveness being dependent on the implementation of a regular and reliable inspection, testing and removal of infected trees.

EXPERIMENTAL TRANSMISSION OF D AND M STRAINS OF PLUM POX VIRUS BY PRUNUS PERSICA. A. Moreno, N. Capote, M.C. Martínez, M.T. Gorris and M. Cambra. Centro de Protección Vegetal y Biotecnología. Instituto Valenciano de Investigaciones Agrarias (IVIA). Carretera Moncada-Náquera km 5, 46113 Moncada, Spain. E-mail: mcambra@ivia.es

Plum pox virus (PPV) can be transmitted by vegetative multiplication and by aphids in a non-persistent manner. The transmission rate depends on the species of the aphid vector, the PPV isolate and the plant host. Experimental transmission of PPV-M (Gladys 5M-IVIA) from donor plants (GF305 peach seedling and peach cv. Gladys) to receptor plants (GF305 seedlings and peach cvs Calante, Mercil and Royal Glory) by *Myzus persicae*, was performed. Donor and receptor plants were prepared by grafting on GF677 seedlings. Highly successful transmission of PPV was obtained when GF305 seedling was the donor (41.7%) or the receptor (43.3%) host. However, when the donor was cv. Gladys or the receptor was one the other peach cultivars tested, the transmission efficiency was lower (15.0% and 23.3%, respectively). These results indicate that peach seedlings used as rootstocks are more readily infected than peach cultivars, at least by PPV-M. The same experiment was performed using adult GF305 trees inoculated with PPV-D from nectarine and Japanese plum (RB 3.30). No transmission of PPV-D to adult GF305 nor to any peach cultivar assayed was obtained using mature GF305 as donor. These results suggest that the assayed PPV-D isolates cannot be efficiently transmitted to peaches. This is in agreement with the observed epidemiology of PPV-D in Spain where this strain has not been observed to spread to peach cultivars that were growing next to heavily infected plots of apricot or Japanese plum, in which Spanish PPV-D isolates spread normally. Additional transmission assays were performed using Japanese plum trees (*P. salicina*) cv. Sun Gold infected with D, M or both D+M-PPV isolates as donor plants. These plants had similar viral titres as shown by DAS-ELISA (5B-IVIA) and quantitative real-time RT-PCR assays done prior to transmission trials. In these tests, receptor plants were GF305 seedlings ca. 10 cm high. Two consecutive experiments using 20 receptor plants/PPV donor plant, were made in each assay. The segregation of populations of both viral strains and the interference between them in the transmission process was analysed. Results show that the higher transmission rate was obtained with PPV-M infected donor plant with 74.28% followed by the 66.6%

transmission rate obtained with PPV-D donor plant. When the donor plant was infected with both isolates, the transmission rate obtained was lower than in the case of a simple infection of a donor plant. When the receptor plants were analysed by qRT-PCR, in the case of the 3 treatments, all PPV positive samples were infected with PPV-M isolated but not with PPV-D.

SPREADING OF PLUM POX VIRUS STRAIN M IN SOUTH MORAVIA. M. Navratil, D. Safarova and S. Gadiou. *Palacky University, Faculty of Science, Department of Cell Biology and Genetics, 783 71 Olomouc, Czech Republic. E-mail: milan.navratil@upol.cz*

Plum pox virus (PPV), the causal agent of Sharka disease of plums, apricots, and peaches, is present in most of the stone fruit growing areas of the Czech Republic but little is known on its epidemiological behaviour. During 2005-2007, a survey conducted in south Moravia to determine the distribution of PPV, confirmed the occurrence of strains PPV-D, PPV-M, and PPV-Rec, PPV-D being the most widespread. By contrast, PPV-M and PPV-Rec were found only in two orchards. One of these, located in Pasohlávky, was an intensive plantation infected by PPV-M, which was established in the 1980s with uncontrolled peach material imported directly from former Yugoslavia and Hungary. The second orchard, located in Hrušky, was an apricot stand established with PPV-M-infected material imported from Hungary in 1987. The study of the spatial and host distribution of PPV-M in both localities is in progress.

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AGRISTRIP, A RAPID ONE-STEP ASSAY FOR THE DETECTION OF PLUM POX VIRUS. T. Oberhänsli, P. Munch, J. Barnikow and W. Bitterlin. *BIOREBA AG, Cbr. Merian-Ring 7, 4153 Reinach BL1, Switzerland. E-mail: oberhaensli@bioreba.ch*

To maintain high crop quality, growers and inspectors need reliable tools for fast identification of plant pathogens on-site. A test format that meets this requirement is lateral flow immunochromatography. Specific antibodies that have been validated in our ELISA tests for the detection of *Plum pox virus* (PPV) were used for the development of such a lateral flow test (called AgriStrip). This test was compared for sensitivity, speed of detection and accuracy to the laboratory methods DAS-ELISA and RT-PCR with serial dilutions of extracts of leaves from infected or healthy plum trees. The AgriStrip lateral flow assay detected the virus at dilutions down to 1:160-1:640 within minutes, whereas one day or more was needed for DAS-ELISA and RT-PCR, respectively. Even though the laboratory test formats were two-fold (DAS-ELISA) to over ten-fold (RT-PCR) more sensitive than the on-site test AgriStrip, the latter proved its reliability for detecting and confirming PPV infections in peach, apricot and plum tissues. Simplicity, speed (results within minutes) and economic on-site application of AgriStrip largely compensate for the lower sensitivity.

SEROLOGICAL AND MOLECULAR DETECTION OF PLUM POX VIRUS STRAINS FROM PLUM, APRICOT AND SWEET CHERRY CULTIVARS IN THE MUNTENIA AREA OF ROMANIA. C. Plopa, M. Calinescu and M. Butac. *Research Institute for Fruit Growing Pitesti, Marului Street, 117450 Maracineni, Arges, Romania. E-mail: catitasarpe@hotmail.com*

The present study was carried out using leaf samples collected in August-September from Romanian and imported plum, apricot and cherry cultivars infected by *Plum pox virus* (PPV). Tested plum cultivars, selected from the plum genotype collection of the RIFG Pitesti-Maracineni were: Late d'Agen, Agen 707, Vânat românesc 300, Stanley, Negre de toamna and Laxton Cropper. Apricot cultivars were: Mamaia, Litoral, Cea mai buna de Ungaria, whereas sweet cherry cultivars were: Negre de Bistrita and Timpurii de Bistrita. A first set of tests, done by DAS-ELISA, showed that plum and apricot but not sweet cherry accessions were infected by PPV. The second round of testing was by RT-PCR using specific primers, which allowed to distinguish the different PPV strains.

THE INCIDENCE OF PLUM POX VIRUS ON PLUM TREES IN THE SOUTH CARPATHIAN AREA OF OLTENIA. S. Preda¹ and M. Isac². ¹*Fruit Growing Research Extension Station Vâlcea, Calea lui Traian Street 464, 240273 Vâlcea, Romania.* ²*Research Institute for Fruit Growing Pitesti, Marului Street 402, 117450 Maracineni, Arges, Romania. E-mail: predasilvia@yahoo.com*

Plum pox virus (PPV) is the worst plum pathogen because of its virulence and ability of quick spreading in the field. The most conspicuous effects of viral infections are a marked decrease in productivity, smaller and tasteless fruits, and a generalized decline of the trees. Investigations carried out over a 10-year period have disclosed that PPV occurs in the majority of plum orchards of the Carpathian area of Oltenia. Laboratory testing showed that the prevailing viral strains are M and D alone or in combination. Over 63 plum cultivars and rootstocks were surveyed for PPV infection in natural and isolated conditions. The results were that the following cultivars: Andreea, Scoldu-1, Mirabelle de Nancy, Vânat românesc-4 and Miroval were apparently not infected, whereas cvs Centenar, Valor, Diana, and Dâmbovita, showed clear-cut symptoms of the leaves.

SAFE AND DURABLE RESISTANCE DISPLAYED BY TRANSGENIC RESISTANT PLUMS. M. Ravelonandro¹ and R. Scorza². ¹*UMR GDPP, INRA-Bordeaux, BP 81, 33883 Villenave d'Ornon, France.* ²*Appalachian Fruit Research Station, ARS-USDA, Kearneysville, WV 25430, USA. E-mail: ravelona@bordeaux.inra.fr*

Pathogen-derived resistance (PDR) is an interesting technology that permits to interfere with virus replication in plants. This biotechnological tool is still considered as new and has raised concern for the unknown impact that could derive to agriculture and environment from its practical application. In the attempt to control Sharka disease induced by *Plum pox virus* (PPV), a segment of the viral genome coding for the coat protein was engineered into plums, and the outcome of this operation was assessed under greenhouse and open field conditions for more than 10 years. The results of this evaluation have shown, above any reasonable doubt, that transgenic plums are agriculturally and environmentally safe and efficient in restraining PPV infections.

PRELIMINARY RESULTS ON INHERITANCE OF RESISTANCE TO PLUM POX VIRUS IN APRICOT cv. HARLAYNE. J. Salava¹, J. Polák¹, B. Krška². ¹*Crop Research Institute, Drnovská 507, 161 06 Prague 6, Czech Republic.* ²*Faculty of Horticulture, Mendel University of Agriculture and Forestry, Valtická 337, 691 44 Lednice, Czech Republic. E-mail: salava@vurv.cz*

Resistance to *Plum pox virus* (PPV) was studied in 150 seedlings resulting from controlled pollination between the resistant cv. Harlayne and the susceptible cv. Marlén. Each seedling was inoculated at the base with a chip bud from a symptomatic plum tree infected with the M strain of PPV. Symptoms on the leaves of the plum used for inoculation and the hybrid seedlings were observed for six times during three growth periods and ELISA was used to confirm the presence or absence of the virus in the leaves of the hybrid seedlings. Substantial differences were observed in disease progress among seedlings. One hundred and thirty-four seedlings showed symptoms and reacted positively in ELISA. Simple Chi-square tests for good-of-fit, based on an assumed genetic model, were used to evaluate the segregation ratio obtained (134 susceptible :16 resistant). The simplest explanation for these results is that three complementary genes confer resistance to PPV in this genetic background. Progeny is being screened to confirm the hypothesis on inheritance resistance. The possible use of cv. Harlayne in apricot breeding programs to obtain resistant cultivars is discussed.

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MOLECULAR CHARACTERIZATION OF TURKISH PLUM POX VIRUS ISOLATES. Ç.U. Serçe, K. Çağlayan and M. Gazel. *Mustafa Kemal University, Faculty of Agriculture, Plant Protection Department, Antakya, Turkey. E-mail: culubas@mku.edu.tr*

Turkish *Plum pox virus* (PPV) isolates show discrepancies between serotyping results obtained with monoclonal antibodies and molecular typing determined by either RFLP analysis or subgroup-specific PCR assays targeting the CP region of PPV. PPV isolates from twelve apricot and four plum trees were considered to be strain M based on PCR, but possessing both D- and M-specific epitopes. To secure better information on the allocation of Turkish virus isolates in the current classification of PPV subgroups or strains, several isolates were characterized serologically and molecularly. All isolates tested reacted with the M-specific MAb AL, but some of them reacted both with M- and D-specific MAbs. PPV isolates that typed simultaneously as M and D in serotyping turned to be M in both PCR and RFLP-based typing. Comparison of typing results obtained using MAb- and PCR-based techniques indicates that the use of MAbs alone is not reliable for typing Turkish PPV isolates. Rather, they should be typed using both serological (MAbs) and molecular (PCR) assays.

PLUM VARIETY TESTING FOR REACTION TO PLUM POX VIRUS UNDER NATURAL CONDITIONS. A. Stoev¹ and P. Iliev². ¹*Plant Protection Institute, Kostinbrod 2230, Bulgaria.* ²*Experimental Plum Growing Station, Dryanovo 5370, Bulgaria. E-mail: anton_stoev@yahoo.com*

More than 70 years after the first record of sharka (plum pox) disease, the use of cultivars resistant or tolerant to *Plum pox virus* (PPV) remains the main strategy for the well-being and development of the plum industry in Bulgaria. At the beginning of the 21st century, Bulgarian farmers can dispose of a sufficient range of plum varieties ensuring economic returns from newly planted orchards. This is the outcome of a number of investigations for the development and introduction of new cultivars which retain a satisfactory quality and quantity of the yield in the case of PPV infection. Special attention in Bulgarian varietal testing was paid to cultivar type Kyustendilska sinja sliva (*Prunus domestica* ssp. *domes-*

tica). More than 40 varieties of the above mentioned type, including those known as Vengerka in Russia, were checked for their reaction to PPV. Under natural conditions of aphid-mediated infection, Vengerka krupnaya sladkaya was tolerant, whereas Vengerka 43 was severely affected (sensitive). Variety Kabardinskaya rannaya proved to be weakly sensitive.

PLUM POX VIRUS: DETECTION OF D AND M STRAINS IN PLUM ORCHARDS OF EMILIA-ROMAGNA, ITALY. V. Vicchi¹, A. D'Anniballe¹, P. Fini¹, P. Grillini¹ and M. Pozza². ¹*Servizio Fitosanitario Regionale, Via di Saliceto 81, 40128 Bologna, Italy.* ²*Consorzio Fitosanitario Provinciale, Via G. Andreoli 13, 41100 Modena, Italy. E-mail: vvicchi@regione.emilia-romagna.it*

Plum pox (Sharka) was first discovered in northeast Italy more than thirty years ago. In the Emilia-Romagna region, one of the most important stone fruit-growing areas of Italy, the disease was detected in 1982 in apricot and plum orchards of the eastern areas and, in 1987, in plum stands of the middle-west. Subsequently, there were no new infection foci until 1997 when Sharka was discovered in peach trees of the eastern provinces of the region. *Plum pox virus* (PPV) strains detected were M in peach and D in plum and apricot. Since then, a systematic survey of plum, apricot and peach orchards and nurseries was carried out on a regional scale by the Regional Plant Protection Service. Notwithstanding the eradication plan initiated twenty years ago in the province of Modena, one of the most important regional plum production areas, plum pox is still spreading. During 2005-06 samples of different plum varieties with typical PPV symptoms were collected from 26 orchards in the said area. PPV detection and characterization in plant tissues was done by DAS-ELISA or DAS-ELISA using commercial kits, and by molecular tests (IC-RT-PCR and RFLP). Laboratory analyses showed that both PPV-D and -M occur in plum orchards.

EPIDEMIOLOGY OF PLUM POX VIRUS IN NURSERY BLOCKS. E. Vidal, A. Moreno, E. Bertolini, N. Capote, M. Gil, C. Collado and M. Cambra. *Centro de Protección Vegetal y Biotecnología, Instituto Valenciano de Investigaciones Agrarias (IVIA). Carretera Moncada-Náquera km 5, 46113 Moncada, Spain. E-mail: mcambra@ivia.es*

The biological behaviour of *Plum pox virus* (PPV) in adult trees in the orchards has been studied, but there is a lack of information on its epidemiology in *Prunus* rootstocks grown in nurseries. In this work, the most important parameters governing the epidemiology of PPV in nursery blocks were studied in Liria, Valencia (Spain) during 2006-2007. This included the assessment of susceptibility to PPV-D of the most widely used rootstocks, and the identification of aphid species and their activity period. Furthermore, the efficiency of oil treatments was evaluated as a control strategy to reduce the spread of PPV in nursery blocks grown in the open field. Susceptibility of different *Prunus* rootstocks to natural PPV infection was studied in an area with a high PPV incidence. The virus was detected by DAS-ELISA (5B-IVIA) and real-time RT-PCR. The highest PPV infection rate was detected in blocks of Mariana GF8-1 (10.8%), followed by Adesoto 101 (6.2%), Nemaquard (5.7%), and Myrobalan 29C (3.6%). PPV was not detected in blocks of Cadaman, Garnem and GF677. The sticky shoot method and yellow water-pan traps placed on the orchards were used to monitor winged aphid populations visiting the nursery. Aphid species were identified and preserved in alcohol. The main aphid species landing on nursery

blocks were *Aphis spiraeicola* (56.4%) and *A. gossypii* (4.1%). Aphid populations were at their peak in May. The efficiency of oil treatments in the PPV spread was evaluated in experimental blocks of Mariana GF8-1 and Nemaguard rootstocks (both very sensitive to natural PPV infection) established in an area with a high PPV incidence. Mineral oil (Sunspray Ultrafine 1%) treatments were performed every 10-12 days in these blocks following a statistical design, and PPV prevalence was assessed in treated and non-treated blocks. PPV infection in Mariana GF8-1 (10.8%) differed significantly from that of Nemaguard (5.7%) and was reduced in both cases (7.8 and 3.5%, respectively) when oil treatment was applied.

IDENTIFICATION OF *PLUM POX VIRUS* IN UKRAINE. L. Yusko, H. Snihur, O. Afonina, S. Petrenko and V. Polischuk. *Virology Department, Taras Shevchenko Kyiv National University, Volodymyrska Street 64, 01033 Kyiv, Ukraine. E-mail: virus@biocc.univ.kiev.ua*

Sharka, is the most devastating virus disease of stone fruits elicited by *Plum pox virus* (PPV). Therefore, our research was focused on the monitoring of Ukrainian stone fruit orchards for the presence of PPV during 2004-2006, with special reference to those of the Zakarpatsky region. The surveyed area was chosen because historically the presence of Sharka was documented therein since the 1960s. To compare the sanitary status of stone fruit plantings in the lowlands, foothills and mountain areas of Zakarpatsky region, we first conducted visual inspections for the presence of PPV symptoms. These were observed in the leaves 3-4 week post flowering and consisted of blurred spots along the veins or of light-green to yellow-green ringspots on the whole leaf surface. Symptoms were observed on the trees of 11 of the 13 areas surveyed, except for Mizhgirska and Volovetska which are in mountain agroclimatic zone. The highest disease rate was recorded at Irshavsky (foothill) and Mukachevsky and Uzhgorodsky (lowland). Leaf samples with and without symptoms were collected from trees in 8 surveyed areas, and PPV was identified by ELISA using a commercial kit (Loewe Biochemica, Germany). Serological assays showed that 6 of the 8 areas were infected. PPV was more frequently found in Hustsky and Irshavsky (foothill), in agreement with the outcome of visual observations. ELISA showed that about 85% of symptomatic samples contained the virus, and that approximately 10% of those collected from apparently healthy plants were also infected. Besides the Zakarpatsky region, PPV was identified also in the Kyiv region, where the percentage of infected trees was significantly lower (14%) as compared with 85% registered at Zakarpatsky. It is worth noting that PPV could be detected during all period of vegetation (March

through October) in shoots, buds, flowers, leaves and fruits. Detection was highest in May-June, when fully developed leaves were used for analysis. Younger leaves contained less virus. Intriguingly, leaves of diseased apricots were often symptomless, while ripe fruits exhibited typical Sharka symptoms. Different indicator plants, including *Nicotiana benthamiana*, *N. glutinosa*, *N. clelandii*, and *Pisum sativum* were used for biological testing. In summary, this work has confirmed the widespread presence of PPV in stone fruit plantings of Ukraine and has allowed to draw a map of PPV distribution in the Zakarpatsky region.

MOLECULAR CHARACTERIZATION OF *PLUM POX VIRUS* ISOLATES FROM MONTENEGRO. J. Zindovic¹, M.V. Marn² and I. M. Pleško². *¹Biotechnical Institute of Podgorica, Bulevar Svetog Petra Cetinskog bb, 81 000 Podgorica, Montenegro. ²Agricultural Institute of Slovenia, Hacquetova 17, 1001 Ljubljana, Slovenia. E-mail: jelena.zindovic@yahoo.com*

Plum pox virus (PPV) isolates have been assigned to two major (PPV-D and PPV-M) and two minor groups (PPV-EA, PPV-C). Recently, a fifth group (PPV-REC) has been identified, which represents a homogenous ensemble of frequently occurring natural recombinants between PPV-M and PPV-D strains. All PPV-REC isolates share the same recombination breakpoint on the N1b polymerase gene, therefore conventional CP gene-based molecular and serological methods are not able to discriminate between PPV-M and PPV-REC isolates. In our study, samples were collected in plum orchards in the surroundings of Niksic (Montenegro). Sixteen samples, that tested PPV-positive by DAS-ELISA (Bioreba, Switzerland) were selected for further analysis. This was done by PCR using: (i) subgroup-specific primer pairs P4/P3-M (PPV-M specific) and P4/P3-D (PPV-D specific) (Dallot *et al.*, 2008. *Acta Hort.*, in press), positioned downstream the recombinant crossover, and (ii) subgroup-specific primer pairs C1f/CI-M (PPV-M specific) and C1f/CI-D (PPV-D specific) (Glasa *et al.*, *Eur. J. Plant Pathol.* **108**: 843-853, 2002), positioned upstream the recombination breakpoint. The results disclosed the presence of PPV-D type isolates in four samples, which were PPV-M negative when tested by TAS-ELISA (Agritest, Italy). The other 12 samples were PPV-M positive by TAS-ELISA. Seven of them proved to be of PPV-REC type by RT-PCR using the above mentioned primers. For the remaining five samples the type of strain could not be determined, therefore they were further analyzed using primer pairs mM5/mM3, mD5/mD3 and mD5/mM3 (Šubr *et al.*, *Acta Virol.* **48**: 173-176, 2004.), designed around the recombination breakpoint. All these latter samples were positive only with primer pair mD5/mM3, thus were identified as PPV-REC.

