Twenty strains of the ESFY phytoplasma, which on the basis of graft-inoculation experiments greatly differ in aggressiveness, were examined by sequence analyses of several PCR-amplified non-ribosomal genes in order to identify molecular markers linked to virulence. These strains, which were maintained in various stone fruit genotypes, were indistinguishable with techniques for routine phytoplasma differentiation and characterization such as sequence and RFLP analyses of PCR-amplified rDNA. Also, the virulent ESFY strains maintained in periwinkle, namely GSFY1, GSFY2 and ESFY1 as well as an avirulent strain of the same phytoplasma, maintained in apricot, which was identified in recovered apricot trees, in France, and used there as a cross protecting agent, were included in the work for comparison. For PCR amplification, primers were designed from a number of genes distributed over the chromosome of strain AT of the apple proliferation phytoplasma. Visible PCR products were only obtained with primer pairs derived from the tuf gene which encodes the elongation factor Tu (EF-Tu), *rps*C (*rps*3) gene encoding the ribosomal protein S3, tlyC gene which encodes hemolysins, a membrane-damaging agents that serve as important virulence factors for many bacteria, the imp and fol genes encoding an immunodominant membrane protein and an enzyme involved in the folate biosynthesis, respectively. Nucleotide sequence comparisons revealed that the highest genomic variability occurred within the imp gene sequence with dissimilarity values ranging from 0.2 to 4.6%. For the remaining genes, the strains examined proved to be identical o nearly identical. Within the tuf gene, the presence of an additional TaqI restriction site, which had already known to occur in the strain GSFY1, was not identified for the other strains. The genetic differences observed among the strains examined are neither suitable markers for strain differentiation nor linked to pathological traits.

Hypo and hyper-virulence in apricot trees infected by European stone fruit yellows

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An apricot orchard, located in an area of northeast Italy under high European stone fruit yellows (ESFY) infection pressure, has been monitored starting from the year of planting (1990). By time, most of the plants showed symptoms or resulted infected by PCR analyses. Particularly interesting resulted two groups of apricots: some asymptomatically infected and others recovered from the symptoms but not from the pathogen. With the aim to isolate strains of the phytoplasma characterised by different virulence, each group was used as mother plants and propagated. The new plants were used to constitute experimental orchards, where the plants were observed for the presence of symptoms and in part tested by PCR starting from 2003. The obtained results confirmed the presence of strains of the pathogen characterised by different virulence. The strains originally present in infected apricots recovered from the symptoms of ESFY resulted hypo-virulent; all the propagated infected plants never showed symptoms of the disease. Surprisingly, the strains present in asymptomatic mother plants of apricot resulted hyper-virulent and the propagated plants always showed severe symptoms. In the propagated plants, the transmission of the pathogen resulted higher for the hyper-virulent strains in comparison with the hypo-virulent ones. A graft transmission trial carried out in the greenhouse using some of the identified hypo and hyper-virulent strains, confirmed the results obtained in open field. Real time PCR analyses showed that in the plants infected by hypo-virulent strains the colonisation of the pathogen was lower than in the plants infected by the hyper-virulent ones. It is possible to affirm that the hypo-virulent strains were present in the originally recovered mother plants of apricot. The research will continue with the aim to verify the possibility of cross protection among the identified hypo and hyper-virulent strains.

Poster Presentations

First survey on blueberry viruses and phytoplasma in the Czech Republic

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First survey on the occurrence of viruses and phytoplasma in blueberry Vaccinium corymbosum (highbush blueberry) and naturally occurring Vaccinium species is currently conducted in the Czech Republic. Plantations, germplasma collections, propagation materials and wild plants are monitored for

symptoms, assayed on differential host plants, by commercial ELISA kits for *Blueberry scorch virus* (BBScV), *Blueberry shock virus* (BlShV), *Blueberry shoestring virus* (BSSV), *Blueberry leaf mottle virus* (BLMoV), and by PCR for *Blueberry red ringspot virus* (BRRV) and phytoplasma. First results will be presented. Granted by the Czech Ministry of Education OC09022.

Tomato ringspot nepovirus (ToRSV) in wild blackberry (*Rubus fruticosus* L.) in Hatay province of Turkey

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During observations of virus-like symptoms in wild blackberry (*Rubus fruticosus* L., Rosaceae) some stunted plants growing in the border of stone fruit orchards in Hatay were found showing severe yellow blotching and deformity of the leaves. Samples (shoots and leaves) were collected in September 2008 from wild blackberries plants growing at the border of apricot orchards and neighboring stone fruit nurseries in Hatay province in Eastern Mediterranean Region of Turkey. Each of 12 wild blackberry samples taken from 7 symptomatic and 5 symptomless plants was tested for virus by mechanical inoculation of sap to herbaceous plants. Sap was inoculated on *Chenopodium amaranthicolor, C. quinoa, Cucumis sativus, Gombherena globosa, Nicotiana benthamiana, N. clevelandii, N. glutinosa, N. rustica* and *Petunia hybrida*. Sap from six symptomatic plants induced symptoms as necrotic or chlorotic lesions, ring spots on test plants. No symptoms were induced in these test plants by sap from symptomless blackberry plants. A sap-transmissible virus was obtained from each of symptomatic plants and identified as Tomato ringspot nepovirus (ToRSV) by double-antibody sandwich enzyme-linked immunosorbent assay (DASELISA). Results of biological indexing were also confirmed by serological assays (DAS-ELISA). Cuttings of symptomatic plants were rooted in pots and kept in insect-proof growing room for symptom observations and other studies. Investigations on the virus in wild and cultivated *Rubus* spp. and its vector/s have been in progress. Further studies are necessary to investigate distribution and natural transmission of the main virus diseases in cultivated *Rubus* spp. due to its recent increasing numbers of plantations and economical importance in Hatay.

To our knowledge, ToRSV were reported for the first time in wild blackberry in Turkey.

Detection of Blueberry red ringspot virus in highbush blueberry cv. Coville

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Blueberry red ringspot virus (BRRSV) is a member of genus Soymovirus in the family Caulimoviridae. It is known to infect Vaccinium corymbosum, V. formosum, V. australe and probably V. macrocarpon. Many blueberry cultivars are sensitive to BRRSV, cv. Bluecrop is reported to be field-resistant and cv. Jersey is field-immune. BRRSV causes red ringspots or red blotches on one year old stems or older. In mid- to late summer reddish-brown spots develop on older leaves. The spots are prominent on the upper surface of the leaf. In some cvs. fruit symptoms, circular areas of light colour and/or fruit deformations can be seen. The disease can significantly reduce yield. The disease is present in the USA. On one plant of highbush blueberries in introduction plantation at Brdo pri Lukovici, symptoms indicating BRRSV infection were observed. Red rings appeared on some of the stems and also red rings or spots were observed on some leaves. At the harvest time spots of light colour were observed in ripening fruits. DNA was isolated from symptomatic tissue in spring 2008. Primers RR13 and RR14 (Glasheen et al., 2002) were used in subsequent PCR. Obtained amplification product of approximately 490 bp was sequenced and the sequence confirmed the infection of blueberry plant with BRRV.

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Comparison of Raspberry bushy dwarf virus isolates from Hungary and Slovenia

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In 2006 and 2007 samples of wild and cultivated *Rubus* species were collected on 7 locations in Hungary and on 16 locations in Slovenia in the frame of a bilateral project. 7 varieties of raspberry from one Hungarian collection orchard were found to be infected with *Raspberry bushy dwarf virus* (RBDV). In Slovenia the presence of RBDV was confirmed only in 3 samples of wild *Rubus* out of 43 samples collected in the woods. Serological characterisation with three monoclonal antibodies (R2, R5 and D1) was performed on positive samples. Selected positive samples were partially sequenced. The results of serological and molecular analyses were compared with the results of raspberry and grapevine isolates obtained in Slovenia in the frame of other projects.

Occurrence of small fruit viruses in Belarus

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Phytosanitary state of small fruit plantations was studied in Belarus by DASELISA. Objects of research were cultivars of Rubus idaeus L. and Ribes sp. L. According to “Statute of fruit plant material production in Belarus” following viruses were identified: CMV, ApMV, SLRV, RRV, RBDV, ArMV, TBRV and ToRSV. A high level of virus infection in commercial raspberry cultivars (Alyonushka, Balsam and Meteor) was detected: 52.9% of SLRV, 41.0% of RRV, 38.2% of RBDV, 32.3% of ApMV, and 20.5% of ArMV. CMV, TBRV and ToRSV were absent from all tested plants. Upon the average only 5.8% of samples were free from viruses tested, 32.3% were infected by one virus, 35.2% had two viruses, 23.5% contained three viruses and 2.9% of samples had four viruses. The phytosanitary inspection of black currant collection plants determined the high level of virus infection: 100% of RRV,

97.5% of TBRV, 100% of SLRV and 81.8% of ArMV, and only 5.8% of plants were infected by CMV. All black currant cultivars tested were 100% infected with RRV and SLRV, as well as with TBRV, except cultivar Katyusha (83.3% of the tested plants were infected). Not less intensive infection was detected in black currant mother plantations: RRV – 100%, TBRV – 93.3%, SLRV – 71.1%, CMV – 62.2%. The most common red currant viruses appeared to be RRV (47.9%) and TBRV (34%). CMV was detected in 2.1% of tested plants, and ArMV - in 4.3 of ones correspondingly. Only 28.7% of samples were free from viruses tested (RRV, TBRV, SLRV, ArMV, CMV, TomRSV), 47.9% were infected by one virus, 20.2% had two viruses, 3.2% contained three viruses.

Virus survey in strawberry production fields in the United States and Canada

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In an effort to determine the incidence of viruses in strawberry production fields in the US and Canada, approximately 1500 samples were collected and either brought back or shipped to the USDA-ARS laboratory in Corvallis, Oregon between 2002 and 2007. RNA was extracted from leaf tissue and archived at - 80C for subsequent uses. During the same time, RT-PCR tests were developed for most known strawberry viruses. For this study a subset of 275 samples, representing the major strawberry production areas in the US and Canada were tested for: Beet pseudo yellows (BPYV), Fragaria chiloensis latent (FClLV), Strawberry crinkle (SCV), Strawberry latent ringspot (SLRSV), Strawberry mottle (SMoV), Strawberry mild yellow edge (SMYEV), Strawberry necrotic shock (SNSV), Strawberry pallidosis (SPaV) and Strawberry vein banding (SVBV) viruses, as well as a housekeeping gene as an internal control by RT-PCR. The Pacific Northwest had the highest rates of infection with the aphid-borne viruses but was virtually free of the whitefly transmitted viruses. In contrast, California, southeastern US, northeastern US, midwestern US and Ontario had aphid and whitefly transmitted viruses in about equal numbers. The midwestern US had the lowest incidence of virus infection. BPYV was only found in samples from CA and southeastern US, but has been detected from Maryland in previous studies. In the Pacific Northwest, fields with aphid control had very low incidence of virus infection compared to nearby fields without aphid control. Also the disease pressure was much lower in Oregon than in northern Washington and British Columbia. As a result of this information, management strategies can be designed for the major viruses and vectors that occur in a given area. As an example, management efforts in the Pacific Northwest should be targeted toward control of aphids, whereas in other areas, whiteflies are important vectors of a number of viruses.

Infectious agents associated whit strawberry decline in Italy

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Symptoms of decline have been observed in different strawberry cultivated areas and since 2003-2004 they have spread in north Italy and especially along the cost of the Emilia-Romagna region, where most of the nurseries are present. In the Spring the plants showed loss of vigour, small and distorted leaves, yellowing and/or light yellow edge. In the Summer and Fall groups of plants showing decline, reddish and curled leaves, poor development of the central crown leaves with an evident edge chlorosis, distributed in patches in the fields, were observed. Plant samples with the most representative symptoms were collected, and analyses, based on molecular diagnostic protocols, have been applied to detect viruses transmitted by aphids, by whitefly or without known vectors and prokaryotes transmitted by planthoppers. The results obtained from the analyses done in 2007 and 2008 on plants with decline and chlorosis symptoms, have showed the presence of *Strawberry mottle virus* (SMV), *Strawberry mild yellow edge virus* (SMYEV) and *Strawberry crinkle virus* (SCV), already known to be present in our cultivated areas, and *Strawberry vein banding virus* (SVBV) and *Strawberry chlorotic fleck virus*, (SCFV), never found before in Italy. On a total of 86 tested samples, 35 (40%) were infected with one or

more of the above mentioned viruses. Fifty plants with evident decline symptoms in the Fall, were collected and 28 (56%), after molecular analysis, were found to be affected by two different prokaryotes: *Candidatus phytoplasma solani* (Stolbur) and a γ 3-proteobacterium similar to that identified on sugarbeet in France. The presence of these viruses and prokaryotes had quite often a spot-like distribution. However it should be controlled, since these pathogens, due to their transmission mode, can spread in a short time causing epidemies with significant economic losses.

Characterisation of mixed virus infections in *Ribes* species in Switzerland

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Various symptoms of viral infections are frequently observed in Ribes sp. in Switzerland. Although viruses infecting *Ribes* sp. were described in several countries, the situation in Switzerland remains poorly documented. Therefore, symptomatic plants from diverse origins were analysed by electron microscopy (EM), immuno-precipitation electron microscopy (IPEM), Western blot and (RT)- PCR. By EM, at least four different particles types, often in combination, were observed. (1) Bacilliform particles were typical for the *Badnavirus* genus with dimensions of 145 x 28 nm. This wirus was further identified by PCR as the *Gooseberry vein banding associated virus* (GVBaV). (2) Filamentous particles were mainly observed on black currants with downward rolling of leaves with interveinal reddening in late summer and fall. We tentatively named this unknown virus *Blackcurrant leafroll-associated virus 1* (BCLRaV-1). In phylogenetic analysis of HSP70h nucleotide sequences, BCLRaV-1 felt in the *Closterovirus* genus. In Western blot analysis, one dominant protein with an estimated molecular weight of about 28 kDa was detectable. Nevertheless, this virus was shown to be different from the *Raspberry mottle closterovirus* (RMoV) by IPEM and RT-PCR. (3) RT-PCR and sequencing of products also clearly demonstrated the presence in our *Ribes* samples of *Rubus chlorotic mottle virus* (RuCMV), a *Sobemovirus* recently descriebed in Scotland. This finding correlates with the presence of the 30 nm particles observed by EM. (4) Still another entity with isometrical particles of 60 nm could not yet be attributed to a particular genus. Altogether, our data suggest the presence of multiple virus infections in *Ribes* sp. in Switzerland and emphasize the need for an efficient sanitary selection process.

Evaluation of the reliability of lateral flow immunochromatography strips for detection of *Plum pox virus*

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The need for fast (preferably cheap) and reliable diagnostics of plant virus and virus-like disease is especially high in the case of vector-transmitted pathogens like *Plum pox virus*. For some applications, fast diagnostics 'in the field' may be critical for effective control of the pathogen. Diagnostic kits based on lateral flow immunochromatography are offered by several companies for detection of *Plum pox virus* as well as many other pathogens. The result of such test is usually based on visual checking for the presence of two bands developed on the strip. The question of sensitivity and reliability of such assay performed by untrained users was addressed in this simple study. Plum and peach samples containing different amounts of PPV (estimated by ELISA) were tested with AgriStrip (Bioreba) assay. The set of strips have been presented to a number of persons with the task of selecting them into three groups: 'positive', 'negative', 'don't know'. Although there were differences in the evaluation of the strips corresponding to lower amounts of the virus, consistent evaluation results were observed for relatively high and medium concentrations of the virus. These results indicate that immunostrip assay may be a valuable tool in the hands of nurserymen/nurserywomen and farmers for rapid checking the suspicious plants. On the other hand, some samples with very low amount of virus may need verification performed with other techniques in the specialised laboratory.

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Transient expression of the coat protein of Apple chlorotic leaf spot virus inhibits the viral RNA accumulation in *Nicotiana occidentalis*

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The coat protein of Apple chlorotic leaf spot virus(ACLSV-CP) plays a crucial role in infectivity and efficient viral RNA accumulation in host cells (J. gen. Virol, 88, 2007). In this study, the effect of ACLSV-CP on viral RNA accumulation in *Nicotiana occidentalis* was investigated. The wild type CP (wtCP), CPm40 (an amino acid (aa) substitution of Ala to Ser at aa position 40), CPm75 (a substitution of Phe to Tyr at aa position 75) (both CPm40 and Cpm75 are fatal to viral infectivity and RNA accumulation), and CPm40m75 (two aa substitutions at positions 40 and 75) of ACLSV-P205 were transiently expressed in *N. occidentalisleaves* by agroinfiltration. Immunoblot analysis showed that wtCP and CPm40m75 were stably accumulated in infiltrated tissues, in contrast to proteins of CPm40 and CPm75 which were not detected, indicating that the stable accumulation of CP is important for effective viral RNA accumulation. However, co-agroinfiltration of an infectious cDNA clone of ACLSV (pBICLSF) or pBICLSF-based CP mutants (pBICLCPm40, pBICLCPm75, pBICLCPm40m75) with wtCP showed that no viral genomic RNA accumulations were found in any tissue infiltrated with pBICLSF, pBICLCPm40m75, pBICLCPm40, or pBICLCPm75. The inhibition of ACLSV-RNA accumulation was found only in leaves co-expressed with CP protein, but not with a frame-shift mutant of CP or P50 movement protein of ACLSV. These results suggested that the stable accumulation of ACLSV-RNA may be regulated by the level of CP accumulation and/or the timing of CP expression.

Highly efficient method of inoculation of apple viruses to apple seedlings

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Virus inoculation to original plants is an important steps in research, for example, to satisfy Koch's postulates, to test resistance to viruses in a breeding program, and to analyze gene function by virus vectors etc. However, it is generally difficult to inoculate viruses to woody fruit trees like apple, and an efficient inoculation method has not been developed thus far. In this study, we showed that a biolistic inoculation of total RNAs from infected tissues resulted in a high infection rate in apple seedlings. Total RNAs were extracted from *Chenopodium quinoaleaves* infected with Apple latent spherical virus (ALSV) or Apple chlorotic leaf spot virus (ACLSV). The RNAs were biolistically inoculated to the cotyledons of apple seedlings using a Helios Gene Gun System (20~30 ug RNA per plant). The inoculated seedlings were grown in a growth chamber at 25 C and then analyzed by Northern blot hybridization two weeks after inoculation. The results indicated that 61 out of 66 plants (92%) inoculated with ALSV and 6 out of 7 plants (86%) inoculated with ACLSV were found to be infected with each virus. Thus, the biolistic inoculation of total RNA from infected tissues to apple seedlings is an efficient inoculation method of apple viruses and the method can be applied to other virus-fruit tree combinations.

Nucleotide analysis of pome fruit virus isolates detected in apple and pear samples from Italy and India

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In the frame of a joint research project between Italy and India field surveys were done in different pear and apple growing areas of North of India and Central and Southern Italy. Samples were collected from plants belonging to common and local varieties and molecularly analyzed for the detection of the main pome fruit viruses (*Apple stem pitting virus*, *Apple stem groving virus*, *Apple chlorotic leaf spot virus*, *Apple mosaic virus*) by using harmonized diagnostic protocols. In order to evaluate phylogenetic relationship among isolates of different geographical origin positive samples of each virus were sequenced and the obtained sequences were compared with those retrieved in gene bank. The sequence homology were evaluated and a phylogenetic tree was built.

Detection of pear vein yellows disease caused by Apple stem pitting foveavirus (ASPV) in Hatay province of Turkey

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Apple stem virus (ASPV, genus Foveavirus) cause *Pear vein yellows disease* (PVYD) in pear (*Pyrus communis*). The purpose of this study was to investigate the relation of ASPV with PVYD in pear trees are exhibited severe leaf symptoms in Hatay province of Turkey. A survey was carried out in 9 pear orchards and 3 nurseries to investigate PVYD and to inspect the symptoms associated with the disease on pear in Hatay. A few number of quince trees planted in the inspected pear orchards were also detected for the disease. Leaf symptoms consist of yellow vein banding, reddening and flecking along veins were observed during the summer till winter (dropping leaves) on pear. Symptoms of PVYD were not found on quince trees. ApMV and ACLSV which are important viruses of pome fruits were also investigated on pear orchards and quince (*Pyrus cydonia*) trees in pear orchards. The shoot and leaf samples were taken randomly from inspected trees in orchards and seedlings in nurseries in July for ApMV, ACLSV and for (ASPV) in September in 2008. A total of 47 samples from local pear cultivars (Mustafa Bey and Ankara cvs.) and 6 from quince trees (unknown cultivar) were collected. All samples were detected for presence of ApMV and ACLSV by ELISA. ApMV, ACLSV infections were not found in detected samples. Fifteen samples randomly selected from 25 symptomatic and all of 5 asymptomatic pear samples were also tested for ASPV by biological indexing. Sap extracts were mechanically inoculated on herbaceous test plants. Symptoms including vein clearing and leaf necrosis were observed on *G. globulosa* and *N. occidentalis* test plants. Twelve samples collected from symptomatic and asymptomatic pear samples were found to be infected with the disease by sap transmission tests. ASPV found present in 60,0% of the tested samples (12/20). This preliminary study demonstrated that a high rate of ASPV infections were presence on local pear cvs. in the province.

Determination of the effects of APPLE stem grooving virus on some commertial apple cultivars

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The studies to determine the effects of *Apple stem grooving capillovirus* (ASGV) on external and physical characteristic of some commercial apple cultivars were carried out in Adana Plant Protection Research Institute's screen house facility in Turkey during 2006-2008. The selected cultivars for this aim were Jerseymac, Fuji, Golden Delicious, Summerred, Granny Smith, Vista Bella, Galaxy Gala and Starking. The selection of the cultivars was based on their common use by growers in the country. All cultivars were grafted on M9 rootstock and potted in screen house. Turkish io-50 ASGV isolate, which had been obtained from previous works from an Anna apple tree, was used for inoculation by chip budding, and the success of inoculation was confirmed by DAS-ELISA. The trial was evaluated two years after inoculation, based on six external and two physical parameters of the inoculated trees. The results demonstrated that ASGV has no statistically important effects on length of tree, number of the branches, average and total length of the branches, and leaf dry matter. However, ASGV decreased the trunk diameter about 18%, and the woody dry matter in a statistically significant rate, whereas the angle of the branches from the trunk increased in an average about 22% by ASGV infection. The cultivars reacted differently to the virus inoculation and stem grooving symptoms were observed on some tested cultivars.

Virus diseases of pomes fruit trees in Belarus

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Virus diseases diagnostics of fruit crops were begun in 1998 in Belarus. Before that time, only fragmentary researches of apple-tree viruses using woody test were carried out. The purpose of our work was monitoring of contamination by viruses and production of certified varieties and vegetative propagated rootstocks of *Malus*, *Pyrus* and *Cydonia*. Tests on woody indicators in the field and in the glasshouse and serological tests (DAS-ELISA) were used for virus disease detection. Currently apple trees are infected mainly by *Apple chlorotic leaf spot trichovirus* (ACLSV, 59,32 %), however, data about ACLSV strongly differ for various plantings, varieties and rootstocks. So, the virus presented in 66,38 % of varieties and rootstocks propagation material while in fructifying plantings it was revealed in 54,75 % of the samples. *Apple stem grooving virus* was detected in propagation material (21,74 %) and in fructifying plantings (8,89 %). This virus infects completely many old varieties, cultivated for a long time. Apple mosaic ilarvirus was detected only in 3,50 % of samples from apple trees. *Apple stem pitting virus, Apple proliferation phytoplasma*, virus-like diseases and viroids weren't observed using woody tests during 4 years. ACLSV was identified on the average in 59,09 % of *Pyrus* varieties and rootstocks propagation planting and in *Cydonia* rootstocks planting. Fructifying plantings were infected by this virus very differently – numbers of varieties were free from the virus (new cultivars), another had 38 – 100% infected plants. According to our researches, plants with a negative test result for all pathogens listed in Belarussian Certification scheme, were transferred to the nuclear stock collection (16 apple varieties, 7 pear varieties, 4 rootstock forms).

Viruses of pome fruits in Bosnia and Herzegovina

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During autumn 2005 and summer 2006, field surveys were carried out to assess the sanitary status of pome fruit trees in Bosnia and Herzegovina. Inspections were done in the main pome fruit growing areas including 10 orchards, 2 nurseries and one varietal collection. A total of 65 apple and 50 pear cultivars were tested by biological indexing for the presence of *Apple chlorotic leaf spot virus* (ACLSV), *Apple stem pitting virus* (ASPV), *Apple stem grooving virus* (ASGV) and *Apple mosaic virus* (ApMV). The average infection level was 81%. Both species showed similar infection rate (83% for apple and 78% for pear). The most frequent viruses of apple were ACLSV (72%) and ASPV (69%), whereas for pear ASGV (69%) and ACLSV (64%). The same number of samples were additionally tested by ELISA, but biological indexing showed as more reliable than ELISA for virus detection. Multiplex RT-PCR results of 20 randomly selected apple cultivars were in line with biological indexing. Results of our surveys report for the first time the presence of ACLSV, ASPV, ASGV and ApMV on pome fruits in Bosnia and Herzegovina.

Detection and Identification of Apple Stem Pitting and Apple Stem GroovingAffecting Apple and Pear in Egypt

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Apple stem pitting virus (ASPV) and *Apple stem grooving virus* (ASGV) are important apple, pear and other fruit crops viruses. They infect many commercial apple and pear cultivars and occur either individually or mixed infections causing yield loses. Young green bud and/or base of petiole, were collected from naturally infected apple and pear trees from different location in Egypt. Both viruses were detected frequently in apple and pear samples. A total of 420 trees from 9 different orchards were tested; 13% ASPV-infected and 17% ASGVinfected trees were recorded. Mixed infection with ASPV and ASGV was recorded in 4.% of the trees. Double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) has been used as a routine method for the detection of ASPV and ASGV and for screening of virus-free materials generated from elimination programs. However, this method involves more steps and thus time consuming. Total RNA was isolated from 100 mg fresh affected apple and pear leaf tissue using Qiagen RNeasy plant mini-kit (Qiagen, Crawley, UK), according to the manufacturer's instructions. The one step-RT-PCR method was performed using ASPV and ASGV - specific primer for each virus. A 316 bp fragment for ASPV and 524bp fragment for ASGV were amplified and indicated the presence of ASPV and ASGV in affected apple and pear. Southern blot hybridization of the amplified products to digoxigenin (DIG)-labeled cDNA probe for ASPV and ASGV were used to confirm the detection results No product was detected in amplified extracts from uninfected apple and pear samples. The detection of ASPV and ASGV by one step-RT-PCR assay were successful and appear useful for testing pome fruit germplasm in quarantine or budwood certification programs.

Current Status of Apple Mosaic Virus in Turkey

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Apple mosaic virus is one of the most important virus infection of apple and hazelnut production in Turkey. It distributes especially with pollens and infected plant material especially with scions. The patogen is present almost all of the hazelnut orchards placed on Black Sea coast , besides in apple production the ApMV was present only on stunted Granny Smith plantations. The other common and local varieties seem to be resistant to the patogen to some extent. The presence of patogen first was confirmed by DAS-ELISA tests and RT-PCR. Rna extraction was succeded in apple tissues but it failed by hazelnut tissues. Hazelnut specimens were subjected to paasage to *Phaseolus vulgaris* (bean) plants and then,RNA will be extracted from bean and will be subjected to RTPCR.

First survey of pome fruit viruses in Morocco

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A preliminary assessment of the presence of pome fruit viruses in Morocco was carried out. Twenty orchards and nurseries were surveyed in the regions of Midelt, Meknès and Azilal. A total of 100 samples (apples and pears) were collected and tested. Biological indexing was made in a climatized greenhouse using the following indicators: *Mallus pumila* 'Spy 227' 'Radiant', 'R 12740 7A' and *Pyrus communis* 'LA/62'. All samples were also tested by ELISA for the presence of *Apple chlorotic leaf spot virus* (ACLSV), *Apple stem grooving virus* (ASGV), *Apple stem pitting virus* (ASPV) and *Apple mosaic virus* (ApMV). The prevailing viruses of apple were ACLSV (71%) and ASPV (58%), whereas ASGV was found in 12 tested trees. The same viruses were present, but less frequently, in pear: ACLSV (61), *Pear Vein Yellows Virus* (PVYV) (25%) and ASGV (18%). Only four apple trees were found to be infected by ApMV. Additional RT-PCR testing confirmed the high incidence of ACLSV and ASPV. This was the first report of pome fruit viruses in Morocco, indicating the high infections rate worsened by the recent report of the presence of fire blight (*Erwinia amylovora*) in the country. Moreover, a total

of 168 apples and 81 pears were sampled and tested for pome fruit viroids *Apple scar skin viroid* (ASSVd), *Apple dimple fruit viroid* (ADFVd) and *Pear blister canker viroid* (PBCVd) by tissue printing hybridization. No viroids were detected.

The evaluation of presence and the symptomology of viruses in commertial quince orchards in Turkey

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Turkey is the biggest quince (*Cydonia oblonga*) producer country in the world with its production about 120.000 tons/year. Virus diseases *Apple stem pitting foveavirus* (ASPV), Apple *stem grooving capillovirus* (ASGV), *Apple mosaic ilarvirus* (ApMV) and *Apple chlorotic leaf spot trichovius* are known as viral pathogens that can affect quality and quantity of the quince production. This study was carried out in Mediterranean region of Turkey between 2006 and 2008. The study was based on the survey activity, the symptomatological observations and the detection of viruses by DAS-ELISA and/or RT-PCR techniques. During the survey activity, 33 commercial orchards in five different counties were visited and 115 samples were collected and examined. Laboratory results showed that 17.39% of the samples were infected by either single or mixed infection of any tested viruses. Single infection of ASPV, ACLSV and ASGV were found 12,17%, 5,21% and 2,60% whereas mixed infections of ASPV+ASGV, ASPV+ACLSV, and ASPV+ASGV+ACLSV were 2,60%, 3,47% and 1,73% respectively. However ApMV was not found in any tested samples. Infected trees were marked and observed monthly during the whole vegetation period for two years. The observed symptoms were evaluated in accordance with the laboratory results. During the study; leaf mosaics, leaf deformation, fruit malformation, gummy fruit, dwarfing of the tree, bud-union abnormalities and trunk deformations were observed.

Incidence of Ilarviruses in Latvian Fruit Orchards

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Apple mosaic virus (ApMV), Prunus necrotic ringspot virus (PNRSV) and Prune dwarf virus (PDV) are most notable Ilarviruses that affect fruit trees. ApMV is named after the disease it causes in apple, the first host in which it was described. Many plant species of *Rosacea* family are susceptible to ApMV, including *Prunus domestica* and *Pyrus communis*. PNRSV was earlier classified as isolate of ApMV but now it is reclassified as separate virus. PNRSV is distributed worldwide, infecting *Prunus* spp. and causing necrotic spots and shot holes in leaves. PDV causes chlorotic and necrotic spots on leaves and stunting of trees. It frequently occurs in mixed infections with other *Ilarviruses* – PNRSV and ApMV. In order to study the incidence of *Ilarviruses* in orchards the samples from apple, pear and plum trees of different varieties were collected from commercial gardens during spring 2007 and 2008. Polyclonal antibodies were used for DAS ELISA test for large scale screening. Totally 890 samples from apple, 252 samples from pear and 655 samples from plum were tested. Preliminary results obtained with ELISA test showed that all tested *Ilarviruses* were present in orchards. With ApMV were infected 1% of apple samples and 2 % of plum samples, but not any pear samples gave positive result. PNRSV was detected in 14% and PDV in 12% of plum samples. Mixed infections of *Ilarviruses* were observed in 4% of tested plum. Frequently observed mixed infection was PNRSV with PDV which appeared in 2% of tested plum samples. The obtained data of ApMV incidence in apple and pear trees with ELISA were compared with RT-PCR results. The RT-PCR results showed that the ApMV incidence in apple orchards is 22%, but in pear orchards 20%. ApMV sequences from different apple, pear and plum isolates are going to be compared for further studies.

Detection of a divergent variant of Plum bark necrosis and stem pitting associated virus (PBNSPaV) in Prunus domestica with peach red marbling disease symptoms

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In order to study the etiology of the peach red marbling disease (PRMa), GF305 peach plants were inoculated by grafting with various sources of PRMa. Double stranded RNAs (dsRNA) were extracted from symptomatic leaves and submitted to random amplification with highly degenerated primers. DsRNAs of high molecular weight were detected in one PRMa source from a Prunus domestica. After cloning and sequencing, seven clones were found to contain viral sequences displaying homology with different regions of the genome of Plum bark necrosis and stem pitting associated virus (PBNSPaV), a member of the *Ampelovirus* genus in the family *Closteroviridae*. The nucleotide identity levels observed ranged between 84% (625 bp fragment in the HSP90 gene and a 203 bp fragment in the HSP70 gene) and 87% (320 bp fragment in the helicase domain of ORF1a and 475 bp fragment in the major CP gene). At the amino acid level, amino acid sequence identity levels in the different regions are borderline with the 10% divergence species demarcation criteria in the *Ampelovirus* genus. Based on these preliminary observations, the molecular characterization of the PRMa-virus was pursued by sequencing the gaps between the different regions already available to yield a continuous ca. 8kb partial genomic sequence. The comparison of this sequence with that of PBNSPaV will allow the clarification of the taxonomic position of this agent. In parallel, the association of this virus with the PRMa disease will be evaluated by looking for this virus in various PRMa sources.

Biological characterization of Apricot latent virus (ApLV)

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Apricot latent virus (ApLV, ALV) was the first described in Moldova on symptomless apricot (*Prunus armeniaca*) cv. Silistra introduced there from Bulgaria in 1993. Based on the complete coat protein gene sequence, this novel virus was classified in the *Foveavirus* genus. The virus naturally infects apricot trees with no apparent symptoms. ApLV, however, causes yellow spot symptoms on leaves of graft-inoculated peach seedlings. Considering that, up to now, little information on biological properties of ApLV was available, the present study was focused on the identification of new potential woody hosts, further on the study of the associated symptomatology and the complete virus biology. The results of our findings are reported herein. Virus-free plants consisted of peach, apricot, cherry and plum varieties were infected by graft inoculation in 2004. Chip buds from ApLV infected peach seedlings (Apr-47, Palestinian isolate) were used in this study. The 200 bp ApLVspecific cDNA fragment was obtained from all 33 tested Prunus cultivars. Due to these results, the ApLV woody host range was extended by new varieties of *P. persica*, *P. avium*, *P. armeniaca* and for the first by japanese and european plum cultivars. Clear cut symptoms associated to ApLV were exhibited by the peach varieties and by one apricot cultivar. Tracking the virus in the different stone fruit species were carried out during the growing season, from these results the best period for reliable diagnosis of ApLV was suggested. Finally, ApLV occurence in the different plant organs, such as flower, fruit, leave and bark tissues, was determined.

Occurrence of Little cherry virus-1 on *Prunus* ssp. in Baden-Württemberg

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A survey on Little cherry virus-1 (LChV-1) has been performed at three different sites between 2004 to 2006 in the State of Baden-Württemberg, including a commercial growing site, a state nursery and a garden for evaluation of trueness of variety. Ten Varieties of *Prunus avium* and a single one of Prunus serrulata were proved partly or totally infested. None of the infested trees has shown distinctive disease symptoms. In addition, testing of different varieties and types of certified rootstocks gave only negative results. The patched distribution pattern of trees of various infestation longevities within both the commercial nursery and the variety trueness evaluation garden can hardly justify the involvement of insect vectors in LChV-1 transmission in the field. At a scattered orchard of Baden-Württemberg LChV-

1 was detected on a local variety of sweet cherry and a wild tree of *Prunus avium*. Despite the occurrence of virus disease symptoms on the local variety, no relationship can be drawn to LChV-1. Further testing proved the presence of apple mosaic virus in addition, which is known to induce the observed symptoms. Studies on the shoots of some infested cherry trees being used for scion propagation showed an uniform dispersal of LChV-1 in all over the tree. Recent studies are conducted to verify the responses of young trees of the variety ‚Regina' to an artificially infestation with either LChV-1 or LChV-2. First year results indicate that - in complete contrast to LChV-2 - there are no adverse effects of LChV-1 on the single fruit weight, fruit yield, fruit size nor on circumference.

Transmission of Little cherry virus -1 (LChV1) by Cuscuta europea to herbaceous host plants

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Cuscuta europea as vector for transmission of *Little cherry virus*-1 (LChV-1) to *Nicotiana occidentalis* '37B'. Little cherry disease has been associated with two different long flexuous filamentous viruses of the family Closteroviridae. *Litte cherry virus*-1 (LChV-1) is an unassigned member in the family while *Little cherry virus*-2 (LChV-2) has been assigned to the genus Ampelovirus. Both viruses have been characterized on molecular level. The viruses can be found both alone and in mixed infections. The disease is distributed worldwide in ornamental and sweet cherry and has a great impact on fruit quality of infected trees. Symptoms produced by infected trees consist of small angular and pointed fruit that do not fully ripen and are imperfectly colored. Fruit have reduced sweetness and are unsuitable for consumption. There is evidence that some strains of LChV-1 on sensitive cultivars are either latent or symptoms are less severe compared to those caused by LChV-2. The disease is readily graft transmissible to cherry. There is no known vector associated with LChV-1, however, LChV-2 is transmitted by the apple mealybug (*Phenacoccus aceris*). Both viruses can be detected by RT-PCR. In order to identify alternative hosts different *Cuscuta* species were investigated in transmission trials. LChV-1 and -2 were graft inoculated onto Prunus avium F12 rootstocks and parasited by *Cuscuta europea*. *N. occidentalis* '37B' served as receptor host plant and could be infected systemically with LChV-1. Virus detection from *Cuscuta* and *N. occidentalis* tissue was done by RT-PCR. Virus transmission was not successful for LChV-2. Propagation of LChV-1 by mechanical transmission on *N. occidentalis* failed, however, the virus was serially transferred by grafting.

First report of Little cherry virus 1 in cherry in Turkey

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Little cherry disease (LChD) is a serious virus disease of sweet (*Prunus avium*), sour (*P. cerasus*) cherry and several ornamental cherry trees. One Ampelovirus species, *Little cherry virus* 2 (LChV-2) and one unassigned species in the Closteroviridae, *Little cherry virus* 1 (LChV-1) have been associated with LChD. Symptoms produced by the infected trees consists of small, pale colored fruits with reduced sweetness and the interveinal areas of upper leaf surface turn red-violet or become bronze colored, while the midrib and the main veins retain their green color. The trees in an orchard located in Osmaniye, Turkey had bronze leaves on the upper shoots in July-August of 2007-2008 and no fruit set. In October 2008, the trees had out of season flowers with pink petals and bronze sepals. Flowers from seven cherry trees cv Napoleon and shoots from one rootstock cherry tree cv Mahaleb were collected from the orchard. Total nucleic acids from these samples were extracted as described Foissac et al. (2001) and used as template for reverse transcription. PCR were performed using primer sets specific for LCV-1 or LCV-2 (Rott and Jelkmann, 2001). Wheras all samples were negative for LCV-2, one cherry cv Napoleon and mahaleb samples gave a 419 bp fragment of LCV-1. The PCR product of mahaleb sample was sequenced and sequence analysis showed 89 % nucleotide identity to GenBank Accession Nos. Y10237 and X93351. To our knowledge, this is the first report of LChV-1 in Turkey.

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Foissac, X. Svanella-Dumas, L., Dulucq, M.J., Candresse, T. and Gentit, P., 2001.
Acta Horticulturae 550:37-43.

Susceptibility of a new range of apricot cultivars to apple mosaic ilarvirus

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Seven apricot cultivars were examined for their susceptibility to *Apple mosaic virus* (ApMV) in greenhouse during one year. Observations were carried out in order to determine if it is possible to use this test for selection of new cultivar regarding their suceptibilty to some viruses before planting them in a region infected by a determine virus. All the cultivars were sensitive to ApMV with different level of symptoms.

Serological Identification for some Important Viruses on Stone Fruits in Saudi Arabia

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To assess stone fruit viruses in spring 2007, field surveys were carried out in the stone fruit growing area (Al Juof - North of Saudi Arabia). Several virus symptoms including green mottle, vein clearing, necrotic spots, chlorosis and/or discoloration and symptoms less were collected and tested for the presence of *Plum pox virus* (PPV), *Prune dwarf virus* (PDV) and *Prunus necrotic ringspot virus* (PNRSV) using double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA). Total of 67 leaf samples (38 peach and 29 apricot) were tested. Result showed that 28 out of 67 leaf samples were infected with one and/or more viruses. The most common viruses were (PNRSV) with rate of 12/67, followed by PDV 9/67 and PPV 7/67. Detecting of mixed infection of PPV+PDV+PNRSV were 3 samples, PPV+PDV also were 3 samples and PDV+PNRSV were 2 samples. Further investigations are in need for other commercial orchards and nurseries. The serological result demonstrates that this is the first report of the detection of PPV, PDV and PNRSV in Saudi Arabia.

Keywords: ELISA, PPV, PDV, PNRSV, virus, detection, Saudi Arabia Abstract:

First occurence of Cherry virus A (CVA) in Czech Republic

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Cherry virus A (CVA), a recently described member of the genus *Capillovirus*, was the first reported in *Prunus cerasus* in Germany. The virus does not appear to cause any obvious symptoms in the plants, but when combined with other viruses it may effect the severity of symptoms, or it may have some influence on graft incompatibility in susceptible combinations of scion and rootstock. The CVA is widely distributed in Europe and North America. In a survey for the assessment of the sanitary status of sour and sweet cherry crops in in the Czech Republic, leaf samples from 200 trees were collected in the Research and Breeding Institute of Pomology Holovousy Ltd. in 2008. Positive amplification in reverse transcription polymerase chain reaction (RTPCR), with one set of specific primers, was used to detect virus. Sequenced analyses of the RT-PCR products identified *Cherry virus A*. This is the first record of the occurence of CVA in the Czech Republic. This work provides the starting point for research on the occurence of the virus in planting material.

Occurrence of Prunus necrotic ringspot virus and Prune dwarf virus in sweet cherries in locality Veleherad (South Moravia, Velehrad)

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Old extensive orchard of wild cherry (*Prunus avium* L.) located in Velehrad (South Moravia, Czech Republic) was examined for *Prunus necrotic ringspot virus* and *Prune dwarf virus* during two years. DAS-ELISA detection kits (Bioreba AG) were used to detect both viruses according instructions of manufacturer. Occurrence of both viruses was confirmed. The problems of sampling and detection are discussed.

Identification of ilarviruses in almond and cherry fruit trees using nested PCR assays

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Stone fruits are known to be susceptible to ilarvirus infections. A nested PCR has been recently developed for the generic detection of *Ilarviruses* amplifying a 371 bp RdRp fragment. Using this method a survey was conducted on a number of almond and cherry trees and revealed high rates of *Ilarvirus*-related infections. For further identification of the viral agents involved in these infections the nested PCR step of the generic assay was modified to specifically detect *Prune dwarf virus* (PDV), *Prunus necrotic ringspot virus* (PNRSV) and *Apple mosaic virus* (ApMV). For the detection of each virus specific downstream primers were designed from conserved RdRp regions and used in respective nested PCR assays. The application of the same thermocycling profile allowed all amplifications to run in parallel. Ilarvirus isolates from different hosts were used for the evaluation of the detection range of the assays. A total of 265 almond and 196 cherry samples were collected from different districts of Greece. In almond trees the incidence of PNRSV and PDV was 41% and 21.5%, respectively. Both viruses were detected, though in lower rates (10%), in wild almonds. In cherry orchards the opposite was observed with PDV (56.6%) being the prevalent virus followed by PNRSV (19.4%). Mixed infections with both viruses were also encountered in approximately 10 and 17% of cherry and almond trees, respectively. ApMV was not detected in any of the samples tested. This is the first extensive survey conducted in Greece in order to monitor the distribution of these viruses using molecular assays.

Molecular characterization of the 3' part of the genome of divergent Cherry virus A isolates and development of a polyvalent CVA-specific PCR detection test

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Previous studies on *Cherry virus A* (CVA) diversity based on the sequence of an internal fragment of the RdRp (the PDO fragment) have shown the existence of five phylogenetic groups, with up to 19% genetic divergence (Marais et al., 2008). Moreover, the prevalence of CVA in asymptomatic cherry trees, including certified material, was found to be very high (around 80%). Remarkably, outside of the major phylogenetic cluster, available detection assays based on PCR or on molecular hybridization failed to detect all the tested CVA isolates. In order to develop a more polyvalent test allowing the detection of all CVA isolates, the 3' half of the genome of representative members of each CVA cluster was sequenced. The comparison of the obtained sequences allowed the design of three primer pairs that were evaluated in RT-PCR assays. The two first primer pairs differed from each other by the location of the reverse primer; they are located in the region of overlap between the RdRp and the movement protein genes and allow the amplification of cDNA fragments of respectively 302-bp or 443-bp. The third couple of primers permit the amplification of an approximately 340-bp fragment comprising the 3' end of the CP gene and a part of the 3' UTR. In a two step RT-PCR assay, the three primer pairs allowed the detection of all the CVA isolates tested, including members of the divergent cluster corresponding to the CVA isolates from non-cherry hosts. (Marais et al. (2008). Acta Horticulturae, 781, 37-45)

Effects Associated with Graft-transmissible Agents Found in the Peach Variety 'Ta Tao 5'

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The peach variety 'Ta Tao 5' is host to at least three graft-transmissilble agents. Extraction and characterization of these agents indicates that they are variants of *Peach latent mosaic viroid* (PLMVd), *Apple chlorotic leaf spot virus* (ACLSV) and an uncharacterized *Foveavirus* referred to as *Asian prunus virus* (APV). Each of these agents is used separately, in defined combinations and in concert in a field

study designed to identify their graft-transmissibility and consequent contribution to phenological changes in the peach varieties 'Springprince' and 'Juneprince.' Field data record significant changes in bloom date, vegetative growth and fruiting in both varieties tested. Further, such phenological variations associated with 'Ta Tao 5' differ significantly from artificial combinations of inoculants. The use of 'Ta Tao 5' as an inoculant source to manipulate growth and development of peach trees is unique when compared with other sources.

Assessment of the main stone fruits viruses and viroids in Algeria

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In order to improve the sanitary status of the propagating material of stone fruits, a field survey was conducted to assess the main viruses and viroids affecting stone fruits in selected growing areas and their distribution according on the collected material by using serological and molecular detection methods. Serological assays were carried out to detect: *Plum pox virus* (PPV), *Prunus necrotic ring spot virus* (PNRSV), *Prune dwarf virus* (PDV), *Apple mosaic virus* (ApMV) and *Apple chlorotic leaf spot virus* (ACLSV). Moreover, tissue-print hybridization was performed to detect *Peach latent mosaic viroid* (PLMVd) and Hop stunt viroid (HSVd). Among nearly 2000 trees tested, no PPV infection was detected, while 14% of them reacted positively to at least one virus. The highest infection rate (17%) was reported in both nurseries and commercial orchards. PNRSV was the most detected virus (9%), followed by ApMV (3%) and PDV (1.5%). Cherry was the most infected species (20%). As for viroids, a high infection rate was recorded for PLMVd (9%)and HSVd (5%); the highest infection rate was reported in mother blocks and varietal collections.

Surveying Viruses on Ornamental Trees and Shrubs in Hungarian Botanical Gardens

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In Hungary the occurrence of *Plum pox virus* (PPV) in ornamental and wild *Prunus* sp. has been surveyed since 2002. In 2005 this program was extended to other viruses occurring on woody plants kept in botanical gardens and collection. So far the following species were found to be infected with different viruses (*Prune dwarf virus* PDV, *Prunus necrotic ringspot virus* PNRSV, *Apple mosaic virus* ApMV, *Cherry leafroll virus* CLRV): *Prunus yedoensis* 'Moerheimii', *P. serrulata* 'Tai Haku', *P. serrulata* 'Pink Perfection', *P. serrulata* 'Ychio', *P. spinosa* 'Purpurea', *P. spinosa* 'Plena', *P. mume* L., *Lonicera sataliensis*, *Lonicera kaukazika*. Biological indexing and serology (ELISA) were used to detect the viruses.

Survey for PPV and PNRSV in nurseries and orchards in the northwest region of Iran

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In survery for *Plum Pox virus* (PPV) and *Prunus Necrotic Ringspot Virus* (PNRSV), 313 samples with the following symptoms were collected from nurseries and orchards in the northwest part of Iran. Chlorotic ring spot, necrosis and vein yellowing on apricot; mosaic and midrib crinkling on peach, calico leaf on almond; chlorotic ringspot in sour cherry; vein yellow, leaf distortion, mosaic and small fruit on cherry were evident. Samples suspected to be infected by PPV were inoculated on *Nicotiana* spp., pea, *Chenopodium quinoa*, beans, *Gomphrena globosa* and broad bean plants. Samples suspected to have infection with PNRSV were inoculated on cucumber plant. Infections with the viruses were revealed in the collected samples and the inoculated plants were by DAS-ELISA and/or DASI-ELISA by the use of respective antibodies. PPV- related symptoms developed on the inoculated plants: chlorotic leaf spot on *N. benthamiana* and beans and purple spots on *G. globosa* leaves. On cucumber plants, dwarfing and

chlorotic yellow spots occurred. Also, dsRNA was extracted from PPV-infected plants and subjected to IC-RT-PCR which ended up in amplification of the expected 243 bp fragment. Thus, PPV was detected with ELISA and IC-RT-PCR; PNRSV by ELISA and glass inoculations.

Health status of the pome- and stone fruit planting material imported to serbia

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The paper summarizes the results of the analysis of the pome- and stone fruits planting material intended to be imported to Serbia on the presence of quarantine and economically important viruses and phytoplasmas during 2004- 2009. Examinations have been done on the officially inspected samples according to the phytosanitary law regulations of the Republic of Serbia. A total of 325 samples have been analyzed: 89 rootstock samples (*Malus domestica*, *Pyrus communis*, *Cydonia oblonga*, *Prunus cerasifera*, *P. persica*, *P. armeniaca*, *P. avium* and *P. mahaleb*); 215 samples of apple, pear, plum, peach and nectarine, apricot, sweet and sour cherry varieties; and 21 samples of seed (*P. cerasifera*, *P. persica*, *P. armeniaca*, *P. avium*, *P. mahaleb* and *P. amygdalus*). On the presence of viruses samples were analyzed by ELISA test, while the PCR test was performed for the detection of phytoplasmas. Depending on the fruit species, type of the sample, vegetation season, category of the planting material and country of origin laboratory tests were performed on the adequately viruses (*Plum pox virus*, *Prune dwarf virus*, *Prunus necrotic ringspot virus*, *Cherry leaf roll virus*, *Arabis mosaic virus*, *Strawberry latent ringspot virus*, *Raspberry ringspot virus*, *Tobacco black ring virus*, *Tomato ringspot virus, Apple mosaic virus*, *Apple stem pitting virus*, *Apple stem grooving virus* and *Apple chlorotic leafspot virus*). Four apple samples were tested on the presence of '*Candidatus* Phytoplasma mali' and 5 pear samples on the presence of '*Candidatus* Phytoplasma pyri'. Plant viruses were detected in 5 samples (1.54%). One rootstock sample *Prunus avium*, originating from Hungary, was found to be infected with Prune dwarf virus. Two plum samples, also from Hungary, were infected with *Plum pox virus*. *Apple mosaic virus* was found in one apple sample from Belgium, and one apple sample from Italy was infected with *Apple chlorotic leafspot virus*. No phytoplasmas were found in tested samples.

Investigations on the phytosanitary status of the main stone fruits nurseries and mother plants in Albania

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To assess the virus and viroids infections of the most important stone fruits in Albania, surveys were carried out in nurseries, mother plots and commercial orchards in the main fruit trees-growing areas. The presence of virus and viroids was assessed by visual inspections and laboratory tests. During field surveys, more than 5,000 trees were individually inspected for symptoms expression. A total of 749 were tested, showing to be highly infected (27%) by one or more viruses; in particular, Sharka infection was detected in all the selected areas and in plants of different origin (nurseries 29%, mother plants 14% and orchard 12%). *Prunus necrotic leaf spot virus* (PNRSV) and *Apple chlorotic leaf spot virus* (ACLSV) infections were frequent in peach and plum, while *Prunus dwarf virus* (PDV) was more frequent in cherry. Regarding viroids, 740 samples were tested for *Peach latent mosaic viroid* (PLMVd); the infection rate was, as for viruses, quite high (23%), particularly on peach (60% of tested samples). This study highlighted the quite alarming situation, especially due to the presence of PPV infection in nurseries; urgent measures should be taken to avoid a serious crisis and deterioration of fruit trees industry in Albania.

Detection of systemic pathogens in tissue culture

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Access to genetic material is crucial to enable plant-based industries to remain competitive, for example by introducing traits such as disease resistance, improved yield or taste. New genetic material can be

moved internationally in a variety of forms such as nursery stock, seed or pollen. However, clonal material such as cuttings or tissue culture must be traded if specific genotypes are required. Tissue culture is of lesser biosecurity risk than other forms of nursery stock and generally has fewer phytosanitary requirements. However, bacteria and viruses may infect tissue culture without showing obvious disease symptoms. Testing is often required to ensure disease freedom but there is uncertainty as to whether plants must first be deflasked. There is concern that detection efficacy might be compromised if the pathogen concentration were smaller in tissue-cultured plants than in deflasked plants. However, allowing plants to remain in tissue culture throughout quarantine would be beneficial because international trade would be faster and cheaper. Research has been initiated to investigate whether two important and commonly regulated pathogens, *Plum pox virus and Xylella fastidiosa* can be detected reliably in tissue culture plants of *Prunus and Vitis*, respectively.

Investigation on rose mosaic disease of rose in Hatay-Turkey

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Several viruses including *Prunus necrotic ringspot virus* (PNRSV) and *Apple mosaic virus* (ApMV) are associated with rose mosaic disease (RMD). Field inspections were carried out during the years of 2008 and 2009. Characteristic symptoms include chlorotic line patterns (zigzag pattern), vein-banding and mottles in leaves were observed during spring. Symptoms were also evident during summer on leaves produced until early summer. Flower abnormalities as phyllody were also exhibited during autumn. Distortion and reduction in flower size and early leaf drop have been observed on symptomtomatic plants in winter period. Leaf samples taken from 15 rose plants include different cvs. neighboring stone fruit orchards tested by mechanical inoculation tests on herbaceous plants and enzyme-linked immunosorbent assay (DAS-ELISA) for presence of *Prunus necrotic ringspot ilarvirus* (PNRSV), *Apple mosaic ilarvirus* (ApMV) and *Arabis mosaic nepovirus* (ArMV) which are the viruses related to RMD. *Catharanthus roseus*, *Chenopodium amaranthicolor*, *C. quinoa*, *Cucumis sativus*, *Nicotiana clevelandii*, *Phaseolus vulgaris*, *Vigna unguiculata* test plants were incubated after mechanical inoculation for symptom appearance at a temperature range between 25±2°C in an insect-proof room. Symptoms include chlorotic local lesions, systemic necrosis, stunting and yellow mottling were began to appear on *C. quinoa* and *Cucumis sativus* 2-3 weeks after sap inoculation. Serological tests of test plants are in progress. According to preliminary results of investigations on symptomatic rose plants, the causal agent of RMD is PNRSV. The rose plants exhibited symptoms in home gardens are going to re-tested for the viruses in spring period of 2009 by ELISA.

The development of resistance to cucumber mosaic virus using intrabodies specific for the viral replicase

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Recombinant antibodies expressed in plants have been used recently with success in conferring resistance against plant viruses without the perceived biosafety risks associated with pathogen-derived resistance. We applied 'Intracellular Antibody Capture Technology' (IACT) for the development of resistance to *Cucumber mosaic virus* (CMV), one of the plant viruses with the widest host range, including both herbaceous and woody plants. Applying the IACT method, a single-chain variable antibody fragment (scFv) library was used against three proteins derived from the RNA-dependent RNA polymerase (RdRp) of CMV subgroup I strain I17F with the aim of blocking their function and thus preventing viral infection in planta. The proteins analyzed were - 'Full-length' (839 aa) consisting of the complete 2a gene, 'Motifs' (132 aa) covering conserved motifs (IV-VII) and 'GDD' (22 aa) centered on the GDD conserved motif (VII) complex of CMV. The scFv library (4 x 104 colonies with 95% diversity) was screened for positive interactions by using a yeast two-hybrid system. Of the three RdRp proteins tested the 'Full-length' and 'Motifs' proteins interacted with 96 and 25 library prey constructs, while the 'GDD' protein caused transactivation and was discarded from further analyses. Those scFvs that have tested positive in back-transformations will now be analysed for interaction with the 2a viral protein in vivo using a pPVX expression vector, and mammalian system. After which stably tobacco

transformed with positively interacting scFvs will be produced and screened for the degree and breadth of resistance. This work provides a model system for the development of resistance to other plant viruses.

Keywords: yeast two-hybrid system, scFv library, viral proteins, screening, transformation

Agro-Ecological Incidence Of Pepper Veinal Mottle Virus, Genus Potyvirus, Family Potyviridae, On Cultivated Pepper (Capsicum AnnuumL.) in Nigeria.

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The agro-ecological distribution of *Pepper veinal mottle virus* (PVMV) (Family Potyviridae, genus Potyvirus) and its disease incidence and severity were observed on cultivated pepper between year 2002 to 2005 in six agro-ecological zones in Nigeria, comprising the major pepper producing areas of the humid forest, derived savanna, southern Guinea savanna, mid-altitude, northern Guinea savanna and Sudan savanna the virus was isolated and its physical properties determined. PVMV was confirmed to be present in cultivated pepper fields showing characteristics PVMV disease symptoms in the six agro-ecological zones surveyed but with significant difference in disease incidence and severity within the agroecological zones. Out of the three thousand suspected viral infected pepper leaves sample collected from the fields in the six agro-ecological zones, 88% were confirmed to be PVMV positive through the serological test while 12% were found to be negative. The incidences of PVMV diseases were observed to be high in the derived savanna and the humid forest compared with the other agro-ecological zones. The percentage PVMV disease incidence ranged between 39.14% with 34.48% severity in the Sudan savanna to 50.12% incidence and 43.85% severity in the derived savanna zone.

Towards generation of an infectious full- length cDNA clone of Apple stem pitting virus

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Our aim is the generation of an infectious full - length cDNA ASPV clone which will be used in studies on disease development and symptom expression. Two strategies for the generation of an infectious full - length clone of ASPV are being followed. Initially a ligation strategy has been developed by subdividing the genome of the ASPV isolate PA 66 into six fragments. During amplification and digestion of the fragments high sequence variability occurred, which required determination of the whole genome. The sequence of the new genotype PB 66 has only 80% sequence identity with the original isolate PA 66. The ligation strategy has been modified and the sequence was subdivided into three fragments, which will be ligated into the Plasmid 1657 containing the 35S promoter. The second strategy is based on a full - length PCR of the ASPV genome to circumvent the variability of the virus RNA. It is based on the higher conservation of the 3' and 5' ends of the sequence of RNA viruses, because of their importance for virus infectivity. To amplify the 9,3kb PCR fragment it was necessary to adapt the PCR protocol and to test different polymerases. The obtained full - length fragment will also be inserted into the Plasmid 1657.

Molecular Characterization and full length Genome Sequencing of Citrus Yellow Mosaic virus associated with Rangpurlime cultivar

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Citrus yellow mosaic virus (CMBV) is a badna virus reported from India. It causes graft transmissible severe mosaic disease in Rangpurlime. There are three viral diseases viz. Tristeza, Indian citrus ringspot. The genome of the virus associated with sweet orange has been sequenced and characterized in USA in 2001 (Huang and Hartung, 2001). As this was the only report available on genome characterization of CMBV, the study was undertaken to sequence and characterize full genome of CMBV associated with Rangpurlime from Tirupathi (Andhra Pradesh). The full length genome of *Citrus yellow mosaic virus* associated with Rangpur lime consists of 7522 nucleotides with (G+C) content 43.75% and comprises of

six ORFs. ORF3b is the largest ORF and consists of putative cysteine-rich region (CX2CX11CX2CX4CX2C) and cysteine-rich, zinc finger-like RNA binding domain (CXCX2CX4HX4C). ORF3b also contains domains homologous to those of aspartic protease, reverse transcriptase and RNase H which are highly conserved among all plant pararetroviruses. ORF3A contains domain homologous to those of movement protein. The intergenic region of 724 nucleotides between ORF6 and ORF1 consists of putative promoter elements. ORF1 has the plant cytosolic t-RNA methionine binding site and the numbering of nucleotides of CMBV genome starts from here. Genome of CMBVRL consists of unique EcoRV, XbaI and NcoI restriction sites. The total molecular weight of genome is 4564.6 kDa.

Assessment of molecular diversity in the polymerase gene of several Citrus tristeza virus isolates in northern and southern Iran

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Citrus tristeza virus (CTV) contains a monopartite, single stranded RNA genome organized into 12 open reading frames (ORFs). CTV isolates show a considerable degree of sequence diversity in the 5'-half of the genome. This virus is widely distributed in all citrus growing regions of northern and southern Iran. In this study genetic diversity of 16 Iranian CTV isolates based on sequence of the 5'- terminal part was determind using two overlapping primer pairs from ORF1. Total RNA was extracted from Fars (6 isolates), Boushehr (4 isolates) and Mazandaran (6 isolates), reverse-transcribed and amplified. CN487/CN489 primer pair spanning nucleotides 697 to 1105 of the CTV genome produced the expected 409 bp amplicon for all isolates. A phylogenetic analysis based on the nucleotide sequences of the CN487/CN489 amplified product and published sequences clustered the Iranian CTV isolates into two groups. While on the basis of the CN488/CN491 amplified product, these isolates formed four groups. The results showed that while various isolates fall into different clusters, each cluster includes both Fars, Boushehr and Mazandaran isolates. The results of multiple alignment of the nucleotide sequences of these 16 isolates showed a higher genetic variability between nt. 1082 and 1484 than between nt. 697 and 1105 of the CTV genome. Furthermore, based on the sequences corresponding to nucleotides 1082 to 1484, the Iranian CTV isolates showed most similarity to those from California (SY568, 98%) and Japan (NuagA, 98%). However the percentage identity on the basis of the other genomic region (nt. 697-1105) was 91-98% to the Japanese isolate (NuagA). Noticeably, the close relationship of the Iranian CTV isolates to the Californian and Japanese isolates, have already been shown in several studies that have been conducted on the CP gene analysis. This may reply the origin of the Iranian CTV isolates that possibly have been derived from the Californian and Japanese isolates, through importation of infected planting material in 1964 and 1969 respectively.

An international effort to study the diversity of Plum pox virus.

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The understanding of the geographic dynamics and the genetic evolution of *Plum pox virus* (PPV) populations is crucial for an efficient management and control of the disease. Intensive research of the PPV variability in the last years has allowed the identification of 2 new groups, i.e. Rec and W, showing that present knowledge of the PPV genetic diversity may still be completed by further discoveries. Moreover, occurrence of several divergent isolates, found within common strains, extends our vision of the intra-group variability and has the potential to limit the accuracy or efficiency of typing methods. An international effort supported by the European FP7 SharCo project has therefore been initiated with the aim to provide a realistic view on the current diversity of PPV worldwide, by the large-scale analysis of complete and/or partial (P3-6K1, CP) genome sequences for a large number of natural field isolates. The obtained sequence data accompanied by standardized information (country/region of origin, natural host, symptoms….) for the analysed isolates will be integrated in a webqueryable reference database. A

centralised lyophilised collection of PPV isolates is being simultaneously developed, together with a living collection of epidemiologically or molecularly interesting isolates.

Preliminary results on resistance to PPV-M in *Prunus persica* (L.) Batsch

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Plum Pox virus (PPV) is the causal agent of Sharka disease, one of the most dangerous disease of the stone fruits and in particular for peach. PPV is present in several areas in the world including North America and Asia. In Europe, where Sharka was firstly reported and where is still spreading, PPV is infecting both fruit trees and wild plant species. In these cases, the application of quarantine measures is time consuming, expensive and not effective, also when virus-free material is employed for the plantation of new orchards. A fast spread of the strain PPV-M recently occurred in several areas in Europe ad in particular in Italy, where the disease is causing severe losses on peach crop, threatening the nursery industry as well. Where Sharka is endemic the only sustainable strategy is the employment of resistant varieties. In this work the results of the assays conducted in the winter of 2007-2008 on 20 peach [*Prunus persica* (L.) Batsch] accessions inoculated with PPV-M strain in greenhouses and periodically checked for symptom expression, are presented. Serological and molecular tests (RT-PCR) were individually carried out on all the plants in order to verify the presence of the virus. 'GF305' seedlings were the healthy control. Moreover, 'GF305' plants, graft-inoculated with PPV-M inoculum, were also included as positive control. Four peach accessions were found asymptomatic and virus free while the remaining accessions were found tolerant either (2) or susceptible (14). Putatively *Myzus persicae* resistant 'PI 914559' and 'S 6699' peach accessions were found healthy after 24 hours of exposure to PPV-M infected *Myzus persica*, while the control 'GF305' seedlings was found positive to ELISA test just after one hour of exposure to the infected aphids.

Reaction of PPV infected scions of European plum genotypes grafted onto rootstocks with hypersensitivity resistance to PPV

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Via latently infected plant material, *Plum pox virus* (PPV) spreads over short and long distances throughout countries where PPV host plants are cultivated. At present, the only way to circumvent this way of virus dissemination in *Prunus domestica* is the use of genotypes with hypersensitivity resistance to PPV. It is well known, that scions of genotypes with a high hypersensitivity index grafted onto PPV infected rootstocks die off after a short period of growth. Vice versa, PPV infected buds grafted onto branches of hypersensitive trees are rejected by the hypersensitive genotype. So the use of hypersensitive genotypes could prevent the spread of PPV infected trees from the nursery. The present study deals with the use of genotypes with hypersensitivity resistance as rootstocks for genotypes sensitive to PPV. As rootstocks interspecific hybrids between *P. domestica* and *P. spinosa* and between *P. domestica* and *P. cerasifera*, respectively, were used. In spring 2008, each 200 plants of four rootstocks with hypersensitivity resistance were grafted with buds taken from trees of PPV infected plum genotypes. Most of the shoots growing from the grafted buds stopped growth after a short period and died off. The length of the scion shoot prior to their death varied between 0.3 and 50 cm. Some buds were rejected by the rootstock so that they did not start growing. 53 out of 800 plants grew until the autumn and were kept under dormancy conditions for three months. In spring 2009, 51 % of the remaining plants died off, some after a short growing period and others without shooting. 25 out of 26 plants showed no PPV symptoms, PPV could not be detected by DAS-ELISA. There was only one plant still growing in April 2009 which was proofed to be infected by PPV. The results indicate that the use of hypersensitive rootstocks might be a powerful strategy to avoid the short and long distance transport of PPV via infected plant material.

The inheritance of the hypersensitive resistance of European Plum (*Prunus domestica* L.) against the Plum pox virus

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According to our present knowledge, the use of the hypersensitivity resistance is the most promising mechanism to control the economic effect of Sharka disease in European plum (*Prunus domestica* L.). The inheritance of the resistance trait was investigated on progenies of crossing combinations with at least one hypersensitive cultivar. 47 different crossing combinations with about 1000 seedlings have been proved for sharka resistance. These combinations resulted from crossings between hypersensitive and sensitive genotypes, hypersensitive and hypersensitive ones and between hypersensitive genotypes and quantitatively resistant ones. The progenies were tested for resistance by using a double grafting method with a virus infected interstem in the green house. The percentage of hypersensitive seedlings in crossing combinations with two hypersensitive parents is significantly higher than in combinations with only one hypersensitive parent. In crossing combinations with one or two hypersensitive parents the percentage of seedlings in hypersensitive classes 1 and 2 is equal. The results of the inheritance of hypersensitive resistance obtained so far confirm former results and help for a better understanding of the inheritance of the resistant trait. The selected genotypes provide a base for further selection and breeding of PPV resistant European plum cultivars.

The spatial distribution of Plum pox virus (PPV) in the leaves of European plum cultivars with different degrees of PPV resistance

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There are two components of virus resistance in plants: (1) The reduction of the virus titer within the plant tissue and (2) the inhibition of the systemic spread of the virus within the plant. Both or only one of these components can get effective in resistant cultivars. In order to determine the degree of PPV resistance in 250 cultivars and selections of Europen plum (*Prunus domestica*), the spread of PPV in the leaf blades was observed. Cultivars to be tested were chip budded on myrobalane seedling rootstocks infected with PPV-D isolate. Plants were grown in an insectproof greenhouse. In June, leaves were taken, immediately put into fixations soluton, embedded into resin and cut into 5 µm sections. PPV was localised within the leaf tissue using the Immunogold Silver Staining Method applied after sectioning. Observations were made with the light microscope. Different patterns of PPV spread within the leaf blade of plum cultivars could be described. Along with the determination of the virus titer within the leaves the determination of the spatial distribution of PPV could provide additional information for the reliable characterisation of the PPV resistance of PPV host genotypes.

The investigation of Plum-Pox-Virus infections in some peach and apricot cultivars and rootstock

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The stone fruit tree species can be infected by a lot of viruses which are not visible symptoms, such as *Plum-Pox virus* has became one of the most frequently and the most grave disease in Europe for all species of *Prunus genus*. That is way, to introduce in cultivation as well as to obtain of peach and apricot cultivars with tolerance and genetic resistance to *Plum-Pox-virus* have reprezented one of the important obiective of the research programme at the Research Station for Fruit Growing *Constanta*. This paper presents the data regarding the susceptibility of some peach and apricot cultivars and rootstock confronted by the natural infection with local *Plum-Pox virus* strains. The material taken into study was represented by the peach and apricot cultivars and rootstock with constitute the national collection from Research Station for Fruit Growing *Constanta*. The *Plum-Pox-virus* detection has been made using biological and serological method. In the biologic test were used GF 305 seedlings for peach and Luizet cultivar for apricot. The observations pursued to detect typical symptoms of the *Plum-Pox virus* attack, both on indicator leaves. It is remarquable that on the one hand there was large differences of manifestations of the *Plum-pox virus* symptoms among the species (peach and apricot). An the other hand, there was hight variability regarding the manifestation intensity of the attack among the cultivars and hibrids.

Susceptibility of different prunus rootstocks to natural Plum pox virus (PPV-D) infection in Spain

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The use of less susceptible rootstocks to natural *Plum pox virus* (PPV) infection is one of possible strategies to reduce viral incidence in nursery blocks. The main Prunus rootstocks used in the Spanish stone fruit industry were evaluated during two consecutive vegetative periods (2006-2007) in two different areas in Valencia (Spain) with different PPV inoculum pressure: Llíria (high) and Carlet (low). The tested rootstock species were: Nemaguard, *Prunus marianna* GF8-1, Adesoto 101, Cadaman, Myrobolan 29C and Garnem. The Llíria plot was analyzed in three occasions (Fall 2006, Spring 2007 and Spring 2008), the Carlet plot was analyzed in two occasions (Fall 2006 and 2007) by DASI-ELISA (5BIVIA, Durviz, kit). The virus incidence in Llíria was 59,4% in Spring 2008. The most susceptible

rootstocks were: Adesoto 101 (96,9%) and Mariana GF8-1 (96,1%) and none plant of *Cadaman* and Garnem rootstocks were infected. In Carlet, virus incidence was lower (0,3% in Fall 2007) and only Adesoto 101 (1,4%) and Nemaguard (0,6 %) rootstocks were infected. Aphid species were monitored by Moericke yellow water traps sited in both localities from May 2006 until October 2007. May resulted the month with more abundant caught aphids. Cumulative numbers of aphid species were similar in both plots: 5,575 (Llíria) and 5,265 (Carlet). Aphid species landing on the crop were estimated by the sticky shoot method during a complete year. The main PPV-vector aphid species that landed on the grown rootstocks in Llíria and Carlet were *Aphis spiraecola* (56.4% and 56.8%) and *A. gossypii* (4.1% and 12.7%), respectively.

Evaluation of transgenic *Prunus domestica* L., clone C5 resistance to Plum pox virus

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Plum pox virus (PPV) is one of the most devastating diseases of *Prunus* species. Since few sources of resistance to PPV have been identified, transgene-based resistance offers a complementary approach to developing PPV-resistant stone fruit cultivars. C5, a transgenic clone of *Prunus domestica* L., containing the PPV coat protein (CP) gene, has been described as highly resistant to PPV in greenhouse tests, displaying characteristics typical of post-transcriptional gene silencing (PTGS). Moreover, C5 trees exposed to natural aphid vectors in the field remained uninfected after 4 years while susceptible transgenic and untransformed trees developed severe symptoms within the first year. In our study, a high and permanent infection pressure of PPV-Rec was provided by bud grafting of inoculum in the field trial of clone C5 conducted in the Czech Republic, in which PPV-infected and healthy control trees were used. Moreover, trees with combined inoculations by PPV, ACLSV and PDV were also used in the trial. The presence of the viruses throughout the tree tissues, the relative titre of the viruses and symptoms on C5 trees have been monitored over the years. The resistance stability of C5 clones under permanent infection pressure is discussed.

Evaluation of different peach genotypes for resistance to PPV-M

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A total of 122 peach seedlings, obtained in different cross breeding events using *Prunus* genotypes considered tolerant or resistant to *Plum pox virus* (PPV), were evaluated for the response to PPV–M infection. Tested selections were grafted onto GF305 peach seedlings and successively infected with a PPV-M isolate. For each selection three plants were inoculated and an healthy one was maintained as negative control. Symptoms evaluation was performed according to a detailed scale as reported by Decroocq et al., (2005). The response of infected plants to the challenge of PPV inoculum was evaluated either on the selections or the GF305. Different diagnostic approaches (ELISA, RT-PCR and TaqMan real time RT-PCR) were used for virus detection. Plant response was classified into five groups as suggested by Faggioli et al., 1999, according to the symptoms and the virus detection. After two years of trials a percentage of 82.8% (101/122) of the thesis resulted sensitive, showing symptoms, with different severity, both on the selection and GF305; the 4.1% (5/122) did not show symptoms on the selection, but gave a heavy symptomatology on the rootstock, whereas no symptoms were observed in the remaining thesis (16/122). Among the 21 asymptomatic selections, 15 resulted negative when assayed both in ELISA and RT-PCR, but all of them were positive when assayed with the more sensitive TaqMan real time RT-PCR (Olmos et al., 2005), that revealed the presence of a very low virus titer. On the basis of these results, 7 selections from 'Maria Aurelia' x SD45 F1 hybrid (*Prunus persica* x *Prunus davidiana*) and 8 selections obtained from different cross breeding between commercial peach cultivars and weeping peaches (S2678) could be considered 'highly tolerant' or resistant and must be submitted to further investigations.

Molecular characterization of some new Canadian isolates of Plum pox virus

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Plum pox virus (PPV) was first detected in Canada in 2000. Since then an intensive survey program has be undertaken to determine the distribution of the virus with the goal of eradicating the virus from Canada. A number of Canadian isolates have been characterized and only isolates of PPV strain D have been found in commercial orchards. This research was conducted in part to: a) determine the relationship of PPV D isolates found in commercial orchards with PPV D isolates found in homeowner or residential properties; and b) analyze unusual isolates to confirm strain and/or determine identity. A total of 5 homeowner isolates were obtained for analysis. Four isolates were confirmed as strain D isolates, and formed 3 distinct clades. One of these isolates (H- 0170) grouped with the Canadian Subgroup II, 2 isolates (H-4688, H-4880) grouped with the Canadian Subgroup I, and the fourth isolate (H-4782) formed a separate and distinct clade. The fifth homeowner isolate was strain typed as a member of the strain PPV Rec.

Key words: *Pepper veinal mottle virus*, Incidence, Severity, Pepper, Agroecological Zones.

Biolistic transfection of plants by infectious cDNA clones of Plum pox virus

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Plant biolistic transfection by two *Plum pox virus* (PPV) infectious cDNA clones (strains PPV-M and PPV-D) using the gene gun apparatus PDS 1000-He (Biorad) was optimized. *Nicotiana benthamiana* plants were germinated by five on Petri dishes (diameter 6 cm) with MS growth medium. In the age of four weeks (5 – 6 leaf stage, total leaf surface about 1.5 cm2 per plant) the plants were subjected to biolistic transfection and three days later they were transplanted into common soil substrate. The plant survival after transplantation was about 70 %, the transfection efficiency was over 80 % (compared to 6 % efficiency reached by mechanical plant inoculation). The plants showed typical PPV symptoms two weeks post transfection (leaf distortions and mosaic). The virus presence was confirmed by immunoblotting, RT-PCR, as well as by successful transmission by sap to healthy plants and subsequent virus purification. The cotransfection of *N. benthamiana* plants by PPV-M and PPV-D led to mixed infection with prevalent PPV-D.

In vivo thermotherapy and in vitro chemotherapy of plums, apricots and peaches artificially infected with PPV-D and PPV-M strains.

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Plum cultivars Čačanská lepotica and Švestka domácí, apricot cvs. Leskora and Velkopavlovická, peach cvs. Redhaven and Earliglo artificially infected with PPV-D and PPV-M were treated by in vivo thermotherapy at 37°C. A successful treatment was recorded in cases of plum cv. Čačanská lepotica and apricot cvs. Leskora and Velkopavlovická. Plum cv. Čačanská lepotica and apricot cv. Velkopavlovická were PPV-D free, apricot cv. Leskora was PPV-M free seven and nine months after finishing the in vivo thermotherapy. However, both of the peach cultivars remained PPV infected after the treatment. Furthermore, five peach trees died during the treatment. In vitro cultures of plum cv. Bluefree and apricot cv. Hanita infected with *Plum pox virus* (PPV) were used for the virus elimination by chemotherapy. Low ribavirin concentrations of 5 and 10 mg.l-1 in MS medium were applied in the treatment. PPV was completely eliminated by ribavirin in concentration of 5 mg.l- 1 in plum cv. Bluefree within twenty weeks, and in apricot cv. Hanita in twelve weeks of the application. The presence of PPV was not proved by RT-PCR. Clones of plum cv. Bluefree and apricot cv. Hanita were re-tested by RT-PCR one year after the termination of the ribavirin treatment and negative results confirmed the elimination of PPV. PPV free clones rooted in modified MS medium by Paunovic (2007) during six weeks.

Distribution of Plum pox virus strain in natural sources in the Czech Republic.

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In the Czech Republic, the distribution of *Plum pox virus* (PPV) has been monitored for the last 15 years. Also, individual strains of PPV have been monitored since the end of the 20th century. PPV-M was typed in natural sources of plum, myrobalan and blackthorn from 1999 to 2004. PPV-M was detected in 5, 88% of investigated plum trees; 7, 41% of myrobalan trees and 4, 0% of blackthorn shrubs, respectively. Distribution of PPV-D, PPV-M and PPV-Rec was investigated in 2005-2008. 52-94 samples of plum, myrobalan and blackthorn were tested in individual years. PPV was detected by DAS-ELISA with specific polyclonal antibodies; PPV-M by DASI-ELISA with specific monoclonal antibodies; PPV-D, PPV-M and PPV-Rec were detected by RT-PCR. The presence of PPV-D varied from 94, 7% to 100%, the presence of PPV-M from 0, 0% to 3, 2% and the presence of PPV-REC from 0, 0% to 2, 1% during 2005-2008. More than 95% of natural sources of PPV were infected with PPV-D and less than 2, 5% of natural sources of PPV were infected with PPV-M or PPV-Rec. The presence of PPV-C and PPV-ElAmar was not proved in plum, myrobalan and blackthorn trees infected with PPV.

Typing and distribution of Plum pox virus isolates in Romania

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Plum pox or Sharka, caused by *Plum pox virus* (PPV) is considered as the most destructive disease of plum. Although PPV is widespread in all plum growing areas from Romania and causes serious yield losses, little is known about the variability of its isolates at country level. For this reason, a large-scale study was performed with the aim to get a picture of the prevalence and distribution of PPV strains in plum. During three years surveys, 200 PPV isolates collected from 23 different plum orchards from Transylvania, Moldavia and Muntenia areas were investigated. DAS-ELISA and IC/-RT-PCR were used for PPV detection. PPV strains were serologically determined by TAS-ELISA using PPVD and PPV-M specific monoclonal antibodies. Molecular strain typing was done by RTPCR targeting three genomic regions corresponding to (Cter)CP, (Cter)NIb/(Nter)CP and CI. RFLP analysis was used to distinguish D and M strains, based on RsaI polymorphism located in (Cter)CP. All PCR products targeting (Cter)CP and 13 PCR products spanning the (Cter)NIb/(Nter)CP were sequenced. The typing of PPV isolates revealed that PPV-D is the prevalent strain in all the three areas. The higher incidence of PPV-D was noticed in Moldova (84%) and the higher rate of PPV-Rec was recorded in Transylvania (18%). The mixed infections (D+Rec) was more frequent in Muntenia (24 %).Overall results provided that in Romania the predominant strain is PPV-D (73%), follow with a much lower frequency by PPV-Rec (14%). Mixed infections (PPV-D+PPV-Rec), which might generate additional variation by recombination, are also frequent (13%).

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Cloning and sequencing of a mild naturally induced PPV isolate

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A mild PPV isolate (PPV-B2) was naturally induced after low temperature treatment of *Nicotiana benthamiana* plants infected with the local isolate PPV-DGR. Compared to the mother isolate, PPV-B2 is not aphid transmissible, replicates less efficiently in *N. benthamiana* and *N. clevelandii* and causes no symptoms on the last mentioned experimental host. Both isolates were cloned, sequenced and 14 amino acid substitutions were determined between them as follows: two in P1, two in HC-Pro, two in P3, one in

CI, two in 6K2, four in NIa and one in NIb. Three nucleotide substitutions were observed in the untranslated 5' and 3' terminal regions. In addition, PPV-D-GR, which does not succeed in invading *Prunus* spp. host plants, differed from the reference PPV-D (X16415) isolate with 32 substitutions scattered among P1, HC-Pro, P3, NIa/VPg and CP proteins. The biological impact of the above substitutions is under investigation.

Assessment of the genetic structure of Plum pox virus (PPV) in Serbia

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Numerous studies have reported the widespread occurrence of the main strains of PPV (PPV-M, -D and -Rec) in Central and Eastern Europe these last years (Matic et al., 2006, Dallot et al., 2008, Gadiou et al., 2008, Zagrai et al., 2008). In Serbia, Sharka disease is known to occur since the mid-1930's and the coexistence of all three PPV-M, -D and –Rec strains has been reported (Jevremovic et al., 2008). Moreover, it has been hypothesised that PPV-Rec could originate from ex-Yugoslavia (Glasa et al., 2005). The objective of this present study was to assess the genetic structure of the PPV populations in this country and to identify potential factors determining such a structure. 185 peach, apricot and plum trees were sampled in 53 orchards located in 27 distinct sites during a large survey undertaken in the main stone fruit growing regions of the country in 2005 and 2006. All samples were used directly for PPV diagnostic, strain typing and sequencing. PPV diagnostic and strain typing was performed by IC-RT-PCR using classical already published primers targeting the CIP, CterNIb-NterCP and CterCP coding regions. The genetic diversity of the PPV populations was assessed by sequencing a 427 bp PCR fragment located in the CterNib-Nter CP coding region for 67 isolates originating from the three main Prunus species and from the different sampling sites. Moreover, 19 isolates were further sequenced on both entire CP and partial P3-6K1 coding regions. The prevalence, host and geographical distributions of the three PPV strains were evaluated and evidence of genetic differentiation within each PPV strain was further investigated. The results of these analyses will be presented.

Preliminary studies on the use of the Cascade Rolling Circle Amplification technique for Plum pox virus detection

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Isothermal techniques for the amplification of nucleic acids have emerged in the last years. In contrast to the Polymerase chain reaction (PCR), the most prevalent method to amplify DNA in vitro, the reactions can be run at constant temperatures. Specificity and sensitivity are at least as high as by using PCR and the methods are less time consuming. Therefore, the isothermal amplification of nucleic acids provides also a powerful tool for the detection of *Plum pox virus* (PPV), the causal agent of the Sharka disease. The cascade rolling circle amplification (CRCA), first described by Thomas et al. (1999), is based on the rolling circle mechanism a lot of viruses use to replicate their genome multiplicatively. It is advanced by the amplification of the released strand to achieve exponential accumulation of DNA. Circular Probes, also called Padlock probes (PLP), which arise from the ligation of the terminal region of DNA probes upon side by side hybridization to the target serve as template (Nilsson et al. 1994). For detecting PPV by CRCA the RNA was extracted and reverse transcribed to cDNA using a PPV specific primer. Several PLPs varying in length and sequence of the complementary region to the cDNA were designed and tested. Furthermore, different pairs of primers for the subsequent amplification were developed. For specific ligation Ampligase and T4 DNA Ligase were tested. In CRCA, two polymerases with strong strand displacement activity were compared: Phi29 DNA Polymerase and Bst DNA Polymerase. These enzymes differ in the optimal reaction temperature. Ligation as well as amplification do occur, but there is high background amplification also in negative and no template controls. Discrimination is possible after restriction digestion is carried out. As proven by sequencing of reaction products non-specific

signals are a result of primer polymerization. Current work focuses on the reduction of the background amplification and improvement of the sensitivity.

Sampling and analysis of symptomless plants for Plum pox virus detection in nurseries

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Conventional sampling and extract preparation methods for ELISA must be modified to reduce contamination risks when highly sensitive molecular amplification methods are used. The modifications include: hand collection of samples, special disruption procedures, preparation of extracts into plastic bags, composite samples, etc. Different methods are proposed for large scale testing by both serological and molecular methods as well as the results of comparisons between visual inspections and laboratory analysis, are discussed. In symptomless plants, 3-4 mature leaves should be hand collected from the basal part of one-year-old shoots of nursery plants. The basal part of the leaves including the peduncle should be used for extract preparation. For many years PPV positive detection was associated to the presence of symptoms: visual inspection of symptoms and DASI-ELISA analysis for PPV were coincidental in 82% of the nursery plants, however in 8% of symptomless plants PPV was detected. PPV detection in composite samples (using 3 leaves or 3 dormant buds/plant) was higher by real-time PCR than by ELISA, especially during dormant period. PPV detection using 5 complete spurs or dards per mother plant in winter showed 6.1% and 11.8% post-test probability of disease on trees that tested negative by spot real-time RT-PCR and DASI-ELISA analysis, respectively. The analysis of 4,224 *Prunus* nursery plants by spot real-time RT-PCR and DASIELISA (using 5B-IVIA) showed 93.6% of coincidental results. All this data should contribute to improve protocols for PPV detection in nurseries.

Survey on plum pox virus in Norway

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In 1998 *Plum pox virus* (PPV) was detected for the first time in Norway. Virus symptoms were observed on several trees in a collection of plum cultivars at Njøs Research Station in the Sogn og Fjordane County in West Norway. The Norwegian Food Safety Authority and the Norwegian Crop Research Institute immediately started surveying other variety collections around the country, nuclear stock material and orchards in all important plum-growing areas. Since 1998 we have surveyed the main part of the commercial plum orchards in Norway. About 75 000 individual trees have been tested. About 1 % of the trees have been found infected by PPV. Only the PPV-D strain has been found. It is suspected that the main infection source was infected plums or apricots imported to Njøs around 1970 or earlier. In most plum orchards in Norway, the spread of PPV by aphids is relatively slow. Therefore, we expect to be able to eradicate PPV from commercial plum orchards in the near future.

The eradication work is continuing.

The presence of Peach latent mosaic viroid (PLMVd) in Greece

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Peach latent mosaic viroid (PLMVd) is the causal agent of the economically important peach latent mosaic disease, which is responsible for reduction of tree vigor and fruit quality. In Greece, PLMVd has been reported for the first time in pear and wild pear, in 2001 and in 2004 in apricot. On the other hand, in Greece, there is no information on the presence of this viroid in its main host, peach and its germplasm. In this study the presence of PLMVd in peach and peach germplasm (nursery stock) was examined thoroughly, as well as in other *Prunus* species, and in pome fruit species. Leaf samples were collected from orchards in Pella and Imathia prefectures of Macedonia, and in Magnesia and Argolida prefectures of Thessaly and Peloponnesus, respectively. The presence of the viroid was ascertained by RT-PCR assays, slot-blot hybridization, nucleotide sequencing and RT-LAMP assays. RT-LAMP assays were used for fist time internationally for the detection of PLMVd. Peach (48/53), plum (11/24) apricot (4/15) and cherry (2/15) tree samples were found infected with PLMVd. It was interesting to see that more than 50% (108/214) of peach germplasm examined (nursery stock) was found infected. The viroid was also detected in pear, wild pear and quince samples. Our positive results were obtained by using three different assays as well as nucleotide sequence analysis and were performed in three different labs in three countries.

Pospiviroidae viroids in naturally infected stone and pome fruits in Greece

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Viroid research on pome and stone fruit trees in Greece is important, as it seems that such viroids are wide spread in the country and may cause serious diseases. Our research dealt with three Pospiviroidae species infecting pome and stone fruits, namely *Apple scar skin viroid* (ASSVd), *Hop stunt viroid* (HSVd) and *Pear blister canker viroid* (PBCVd). Tissue-print hybridization, reverse transcription-polymerase chain reaction (RT-PCR), cloning and sequencing techniques were successfully used for the detection and identification of these viroids on a large number of pome and stone fruit tree samples from various areas of Greece (Peloponnessos, Macedonia, Thessaly, Attica and Crete). The 47 complete viroid sequences obtained (25 ASSVd, 11 PBCVd and 11 HSVd) were submitted to the GenBank. Our results showed the presence of ASSVd in apple, pear, wild apple (*Malus sylvestris*),wild pear (*Pyrus amygdaliformis*) and sweet cherry; HSVd in apricot, peach, sweet cherry, apple and wild apple; and PBCVd in pear, wild pear, quince, apple and wild apple. This research confirmed previous findings of infection of Hellenic apple, pear and wild pear with ASSVd (Kyriakopoulou, P.E., and Hadidi, A.1998, Boubourakas et al. 2006), and pear, wild pear and quince with PBCVd (Kyriakopoulou et al., 2001, Boubourakas et al., 2006). Our findings also revealed for the first time the natural and mixed infection of apple and wild apple with ASSVd, HSVd and PBCVd, of apple and pear with ASSVd and PBCVd, and of apple with ASSVd and HSVd, as well as the natural infection of Hellenic sweet cherry and peach with HSVd. Finally, to our knowledge, this is the first published report of detecting HSVd in infected apple and wild apple and ASSVd in sweet cherry.

Detection by Tissue Printing Hybridization of Pome Fruit Viroids in the Mediterranean Basin: Incidence and Biodiversity

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Available data on the incidence and biodiversity of *Pome fruit viroids* in Mediterranean basin are limited. Before starting a research survey to fill this gap, a tissue-printing hydridization (TPH) method to detect *Apple scar skin viroid* (ASSVd), *Pear blister canker viroid* (PBCVd) and *Apple dimple fruit viroid* (ADFVd) has been developed, validated and used in large-scale indexing of *Pome fruit viroids* in Bosnia and Herzegovina, Malta, Lebanon and Turkey. A total of about 1,200 trees have been tested. Positive results obtained by TPH were confirmed by at least one additional detection method (RT-PCR and/or Northernblot hybridization). PBCVd was detected in 18%, 12% and 8% of the tested pear trees in Bosnia and Herzegovina, Malta and Turkey, respectively, showing a wider diffusion of this viroid than expected. Interestingly, in all these countries several ancient native cultivars tested positive to PBCVd infection. In contrast, ASSVd was never detected and ADFVd was only detected in symptomatic trees (cv. Starking Delicious) in Lebanon, confirming a restricted spread of these viroids in Mediterranean basin. Full-length cDNA clones of PBCVd and ADFVd of the different geographic origin were molecularly characterized, with several new polymorphic positions in the genome of both viroids being identified. In the frame of this research, PBCVd in Bosnia and Herzegovina (1), Malta (2) and Turkey (unpublished), and ADFVd in Lebanon (4) were first recorded, indicating that TPH is a useful technique for exploring *Pome fruit viroid* spread.

First report and molecular analysis of Apple scar skin viroid in sweet cherry

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Apple scar skin viroid (ASSVd) is a serious pathogen of pome fruits. Recently, it has been reported in Chinese apricot and Chinese peach (Zhao and Niu 2006, 2008). In the context of our research on fruit tree viroids in Greece, ASSVd was initially detected in a sweet cherry tree cv *Tragana Edessis* (Rupka) from Florina (Macedonia) by RT-PCR and this finding was confirmed by direct sequencing. This tree is located at the edge of a newly established apple orchard, along with other sweet cherry and wild cherry (*Prunus avium*) trees. In order to verify this interesting finding, we examined 4 sweet cherry trees, 2 wild cherry trees and their neighbouring apple trees for ASSVd in the orchard. The examination was done by imprint hybridization using an ASSVd-specific DIG-labelled probe at stringent hybridization conditions and by RT-PCR using two different ASSVd specific primer pairs. We obtained ASSVd-positive results for all 6 cherry trees. No ASSVd was detected in the apple trees of the orchard. Purified ASSVdpositive RT-PCR products from the cherries were directly sequenced or cloned into the pGEM-T vector and then sequenced. ASSVd sequences were obtained from 5 trees. These sequences are 327-340 nucleotides long and share 97-99% identity with ASSVd isolates from Indian (Asian) apples. These results are similar to our data for other ASSVd variants from pome fruit trees in Greece. The cherry ASSVd sequences differ from the prototype isolate of ASSVd (Hashimoto and Koganezawa 1987) at 18-29 sites. There are 15 nucleotide changes common to all Hellenic ASSVd variants, i.e. from pome fruit trees and sweet cherry around Greece. There are no cherry-specific nucleotide changes in the ASSVd sequences obtained. To our knowledge, this is the first published report of detecting ASSVd in naturally infected cherry, including its molecular analysis.

Screening for fruit tree viroids in Lithuania

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Viroids are the smallest plant pathogens known so far, consisting of a small circular RNA which does not code for proteins and is infectious and able to replicate on a variety of host plant species. Viroids are classified into two families, the *Avsunviroidae*, that replicate in the chloroplasts of the infected cells and the Pospiviroidae, that replicate in the nucleus. *Avsunviroidae* family members invade mostly woody plant species. The members of Pospiviroidae invade mostly herbaceous plant species and some fruit trees of *Prunus* and *Malus genera*. A large number of fruit trees in the Lithuanian orchards were also found to be infected by the phytoplasmas. This work was aimed at testing fruit trees for the presence viroid infection. This is the first study aimed at searching for viroid infection in the fruit trees in Lithuania. Mature fruit trees of *Prunus* and *Malus genera* were first screened for viroid infection based on exposure of visual symptoms in 2007. The apple, cherry and apricot trees were tested in the Southern, Central, and Northern parts of Lithuania. Leaf samples from trees exposing decline, defoliation, leaf and branch proliferation, leaf wilting and smaller size, bark damage and changes in leaf color were analyzed using R(eturn) PAGE and RT-PCR assays. The obtained results showed that phytoplasmas are much more common in plants exposing decline and abnormal morphological traits in the trees of *Prunus* and *Malus* species than viroids.

Molecular characterization of hellenic variants of Apple scar skin viroid and Pear blister canker viroid in pome fruit trees

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Apple scar skin viroid (ASSVd) and *Pear blister canker viroid* (PBCVd) are members of the genus *Apscaviroid* (family Pospiviroidae). In order to study the nucleotide sequence and secondary structure of Hellenic isolates of these viroids, a large number of naturally infected fruit trees were initially tested with imprint hybridization. Total RNA extracts from hybridization-positive samples were reverse-transcribed and amplified by polymerase chain reaction using 2 different specific primer pairs for each viroid. Purified RT-PCR products were directly sequenced or cloned into the pGEM-T vector and then sequenced. ASSVd variants from 2 apples, 3 wild apples (*Malus sylvestris*) and 2 pears are 330 nucleotides long. They differ from the prototype isolate of ASSVd at 3-14 sites, and 3 nucleotide changes are identical among all Hellenic variants. Most of the changes occur in the variable region of ASSVd (nucleotides 100-180). Hellenic ASSVd variants share significant homology (97-99%) with ASSVd isolates from Asian apples. Three variants, deriving from different hosts and areas, are identical to each other (wild apple and apple from Pella [Macedonia] and pear from Achaia [Peloponnessos]). PBCVd variants from 4 apples, 1 wild apple, 3 pears and 1 quince are 314-315 nucleotides long. There are 6-35 nucleotide changes between all Hellenic variants and the prototype PBCVd isolate. Nineteen changes are identical among the majority of the Hellenic variants, regardless of origin, and 28-35 changes occur in apple and wild apple PBCVd sequences. These differences are not located in a specific region of PBCVd. In addition, most Hellenic PBCVd variants are 91-98% homologous to Australian PBCVd isolates from pear, quince and nashi pear (*Pyrus pyrifolia*) and Bosnian pear isolates. The ASSVd and PBCVd Hellenic sequences can form rod-like secondary structures. This is the first detailed molecular study of ASSVd and PBCVd in Hellenic pome fruit orchards and wild pome fruit trees.

Identification of Peach latent mosaic viroid and hop stunt viroid in different peach cultivars showing dapple fruit, fruit yellow mosaic and cracked sutures symptoms

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From the early 1990s, a fruit peach syndrome characterized mainly by small discoloured spots (dapple fruit) and/or yellow areas on skin (yellow mosaic), cracked suture and deformations occurs in most commercial orchards in the Emilia Romagna region (central-north part of Italy). In the past, *Peach latent mosaic viroid* (PLMVd) and occasionally *Hop stunt viroid* (HSVd) have been detected in trees with symptomatic fruits. In order to ascertain the presence and the frequency of these two viroids, fruit samples have been collected from five different cultivars: 'Royal Glory', 'Crimson Lady', 'Grenat', 'Diamond Princess' and 'Laura'. Dapple fruit symptoms affected all cultivars, whereas 'Grenat' samples also showed evident yellow mosaic and fruit deformation and 'Royal Glory' severe cracked sutures. The results obtained showed a large diffusion of the two viroids, mainly in mixed infection; more specifically PLMVd was found in 100% of 'Royal Glory', 'Diamond Princess' and 'Laura' samples, in 90% of 'Grenat' samples and in 70% of 'Crimson Lady' samples; HSVd affected 100% of 'Crimson Lady' and 'Laura' samples, 90% of 'Royal Glory' samples and 30% of 'Grenat' samples, whereas it was not found in the 'Diamond Princess' samples. The role that the viroids could play in the expression of the symptoms in the fruit peach samples has been complicated by the high number of samples infected by both viroids (60%); in any case, PLMVd was confirmed to be strictly associated with the yellow mosaic, cracked suture and fruit deformation symptoms. The aetiological origin of the dapple fruit disease, however, seem to be more complicated, since in the 'Diamond Princess' only PLMVd has been found to be associated with the symptoms, whereas in all other cultivars the presence of HSVd in high percentages could have influenced the symptoms expression.

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Real-Time Reverse Transcription PCR assay for Peach latent mosaic viroid detection

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Prunus spp. is affected by a couple of viroids, *Peach latent mosaic viroid* (PLMVd) and *Hop stunt viroid* (HSVd). In this study we focused on PLMVd, which is classified in the *Avsunviroidae* family and is widely distributed in peach germoplasm of Europe, Asia, Australia, North and South America. Peach latent mosaic disease is economically important because it affects fruit quality, reduces the lifespan of the trees, and causes peach trees to be more susceptible to other biotic or abiotic stresses. Reverse Transcription Real Time PCR for PLMVd detection was carried out. In our case, TaqMan probe was chosen due to its high specifity of reaction. Primers and the probe were designed by software Beacon Designer 7.0 and the reaction was carried out by Real-Time machine Opticon 2 (Software Opticon Monitor 3). PCR products were used as standarts in number of 108 – 10 16 copies. The method assay is accurate and reliably detects PLMVd in bark, leaf, fruit, flower blossom and pollen grain tissues of trees during the whole growing season. Furthermore, it is well adapted for the routine detection of PLMVd, because it eliminates any risk of contamination and it obviates the need for post- PCR processing steps. This system may replace the commonly used diagnostic techniques to detect this viroid.

Assessment of susceptibility to European stone fruit yellows phytoplasma of new plum variety and of five rootstock/plum variety combinations

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During 2003-2008 a research was carried out to assess the plum susceptibility to infection by European stone fruit yellows (ESFY, '*Candidatus* Phytoplasma prunorum') phytoplasmas. Two different trials were carried out to verify susceptibility of new variety/cultivar compared to some currently available rootstock/scion combinations. Half of the new variety belonged to Japanese and the other half to European plum groups respectively and were evaluated in more than one plot each with four plants grafted into Myrabolan 29C. The orchard was located in a ESFY severely infected area of Northern Italy: yearly monitoring by visual inspection and PCR/RFLP identification of phytoplasmas allowed to verify an increasing phytoplasma presence in both symptomatic and asymptomatic plants. After five years 8 selections from Japanese plums showed ESFY symptoms or pathogen presence in 50% of the plants, and 9 selections showed 20% of infection. Only 9 selections showed absence of both symptoms and pathogen, although European selections/cultivars were not symptomatic plants belonging to 6 cultivars were positive to phytoplasma presence. In a parallel experiment 5 rootstocks were grafted with 3 Japanese variety: the majority of the combinations showed phytoplasma symptoms and presence starting from the first year after plantation; however two of the rootstocks appeared to induce a delay in symptoms appearance indicating that rootstock could probably induce some resistance to ESFY in Japanese plum but only for a some years after plantation.

Molecular identification of phytoplasmas associated with diseases of *Prunus* sp. at the Canadian Clonal Genbank

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Surveys for phytoplasma were conducted at the Canadian Clonal Genebank during 2008. Symptoms of leaf reddening and shot holes followed by tissue necrosis, were observed in *Prunus* sp., including apricot, peach and nectarine. Total DNA was extracted from leaf samples collected and used as a template in a nested polymerase chain reaction (PCR) with phytoplasma universal primers P1/P7-R16F2N/R16R2 that target the conserved 16S ribosomal RNA (rRNA). PCR products were subjected to RFLP analysis for partial characterization. Nested PCR products were purified, cloned and sequenced. Ribosomal sequences were compared to those of reference in GenBank and phylogeny was carried out to determine the relationships among phytoplasmas identified.

Detection and distribution of European stone fruit yellows in apricot cv. Bergeron and epidemiological studies in the province of Trento (Italy)

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The aim was to investigate the field performance of "Bergeron" on "Wavit" in 4 experimental fields in the province of Trento (Italy), where European Stone Fruit Yellows (ESFY) caused by "*Candidatus* Phytoplasma prunorum" has been constantly spreading since 2000. This included visual inspections for typical symptoms (early bud-break during dormancy and premature leaf-roll) and a highly sensitive real-time PCR assay; 25% of the propagation material was checked with this method and found to be healthy, before planting in 2005. The epidemiology of the disease was also studied by focusing: the presence of the vector Cacopsylla pruni (Scopoli) on conifers; the detection of "Ca. Phytoplasma prunorum" in psyllid eggs and the trasmission efficiency at different stages. This was done by exposing apricot trees in 2 locations, during 2 periods from January to July, to the overlapping presence in the orchards of the

reimmigrants and the new generation of *C. pruni*. The results obtained demonstrated that "Bergeron"seems to be highly susceptible to ESFY: typical bud-break was rarely observed, but up to 20-30% of the plants showed premature leaf-roll, fruit deformation and dieback. As regards the vector: *C. pruni* was caught only once on Picea abies during winter; "Ca.Phytoplasma prunorum" was found in 4 eggs samples from 4 locations; the preliminary results on the exposed trees confirmed that the reimmigrants could be the most efficient vectors at least on apricot. This research was supported by the Provincia Autonoma di Trento.

PCR/RFLP based method for molecular characterization of *'Candidatus* Phytoplasma prunorum' strains using aceF gene

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New molecular typing tools for phytoplasmas belonging to the 16SrX phytoplasma group have been recently developed based on non-ribosomal genes aceF, pnp, imp, and SecY. In the present work we chose to perform a PCR-RFLP method based on aceF. In fact, this genetic marker showed high variability among strains of 16SrX group; moreover it allowed to differentiate French hypovirulent '*Candidatus* Phytoplasma (Ca. P.) prunorum' strains from the virulent ones. Stone fruit samples were mostly collected in North-east Italy; few samples from Turkey and Bosnia & Herzegovina were also included in the work to explore variability. French hypovirulent and virulent strains were used as reference strains. Part of Italian samples was not field collected and they became infected by *Cacopsylla pruni* in controlled conditions. Sequencing of aceF gene was performed on part of the samples tested, and based on the alignment few restriction enzymes were selected for 'Ca. P. prunorum' strain differentiation. Nested-PCR was performed using previously developed primers on all samples, and RFLP analyses were carried out with HaeIII and Tsp509I enzymes. HaeIII enzyme permitted to split some of the Turkish and Bosnian samples from the others, including all the Italian and French samples. On the other hand, Tsp509I enzyme allowed differentiation within the Italian and French strains. Combining the results obtained with the two restriction enzymes it was possible to distinguish among the 'Ca. P. prunorum' strains investigated in this study, 4 different RFLP subgroup (indicated with AceFA, -B, -C and –D). In North-east Italy, where a large number of samples were processed, we can affirm that the strains belonging to AceF-A and B subgroups were the predominant and the mixed infection of the two strains was also quite common. Distinction between Italian hypovirulent and virulent strains is still under investigation.

Establishment of a quantitative real-time PCR assay for the specific quantification of *Candidatus* Phytoplasma prunorum in plants and insects

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A real-time PCR assay for the quantification of *Candidatus* Phytoplasma prunorum has been established which combines the specificity of detection with a low cost method of quantitative PCR. The assay uses the specific primers ECA1/ECA2 with a SYBR Green I protocol. A gene fragment of Ca. P. prunorum with the target of the primers has been cloned and is used as standard for quantification by the standard curve method. The assay has been successfully applied to measure the concentration of Ca. P. prunorum in insects as well as in different kinds of plant samples.

16SrI-B Phytoplasma Infections in Plum and in Sour Cherry in Lithuania

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Recently, in the late summer of 2008, phytoplasma-related diseases of plum (*Prunus domestica* L.) and sour cherry (*Cerasus vulgaris* Mill., syn. *Prunus cerasus* L.) were detected in Lithuania. The plum exhibited symptoms of witches'-broom, shoot proliferation and abnormally small leaves . The diseased cherry developed symptoms related with unusual unseasonal time of flowering, some proliferation, dropping of leaves, and general decline of the tree. Molecular investigation by polymerase chain reaction, restriction fragment length polymorphism and sequence analysis of 16S rDNA, revealed phytoplasmas belonging to group 16SrI ('*Candidatus* Phytoplasma asteris' group), subgroup B. The insect-vector is unknown. The most important phytoplasmas in stone fruit plants worldwide are related to 16Sr-X, 16SrV, 16SrI, and 16SrIII phytoplasma groups. Previously obtained data and the results of this investigation indicate that the 16SrI-B subgroup of phytoplasmas is the most prevalent phytoplasma infection in both grass and woody plants in Lithuania. The subgroup 16SrI-B phytoplasma was identified in plums for the first time.

Evaluation of the susceptibility of pear and plum-trees varieties and rootstocks to Candidatus Phytoplasma prunorum by means of realtime PCR

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The diseases produced for phytoplasmas are difficult to control due to the fact that there are no available direct measures of control. One of the means to avoid the damages produced by these diseases is to have resistant or tolerant plant material. For the diseases produced for phytoplasmas has been observed that the minor expression of symptoms is owed often to the lack of reinfections and therefore to a minor concentration of the population of phytoplasmas. This fact is associated also in the difficulty of detecting the phytoplasma in the tolerant varieties though they are infected. For the *Prunus* species important differences of susceptibility to European stone fruit yellows (ESFY) phytoplasma have been mentioned, the apricot, Japanese plum and the peach trees are more susceptible than the *Prunus cerasifera* (Myrabolan) and that the *Prunus domestica* genotypes. Likewise in the monthly detection of the *Candidatus* Phytplasma pyri in infected trees of two varieties of pear (cv Blanquilla and cv Bartlett), the phytoplasma was detected with only PCR in trees of the variety Bartlett, whereas in the variety Blanquilla the accomplishment of the nested PCR was necessary. This was also related to the presence of symptoms that were much more evident in the variety Bartlett. The purpose of this work is to apply the real-time PCR to quantify the phytoplasma concentration in pear and plum trees previously infected by Ca. P. pyri and Ca. P. prunorum respectively. A selection of different pear and plum varieties and rootstocks were analyzed in order to establish the relation between the presence of symptoms and the estimated phytoplasma concentration. Samples were amplified both by nested-PCR with universal and specific primers and by Real time PCR.

Individuation of *Candidatus* Phytoplasma prunorum type a and type b in *Cacopsilla pruni* individuals

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Recent investigations on molecular characterization of the '*Candidatus* Phytoplasma prunorum' (16SrX-B subgroup), causal agent of the European Stone Fruit Yellows (ESFY) syndrome, on the non ribosomal tuf gene allowed to individuate two groups of isolates, named 'type a' and 'type b', with a distinct geographical distribution in Italian stone fruit growing areas (Ferretti et al., 2007, 2008). Considering the role of *Cacopsylla pruni* (Scopoli) in the epidemiological cycle of the disease, the presence of the two groups of isolates has been investigated also in infected individuals of the psillid, sampled in different Italian areas. Both types has been identified in *C. pruni* specimens captured on apricot, plum and wild *Prunus* species.

Phytoplasma manipulates psyllid vector behaviour by altering host plant odour

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Apple proliferation is one of the major plant diseases affecting apple growing in Europe. This disease is caused by *Candidatus* Phytoplasma mali (Bacteria: Mollicutes), which colonizes the phloem of infected trees. The main symptoms of this disease are dwarf sized fruits and the proliferation of axillary buds (witches brooms). The phytoplasma is vectored by the jumping plant louse *Cacopsylla picta* (Hemiptera: Psyllidae). Complex multitrophic interactions between *Malus domestica*, *C. picta*, and *Ca*. P. mali were investigated in laboratory and field. Results from Y-tube olfactometer trials showed that immature adults of *C. picta* differentiated between the odour of healthy and infected apple trees and preferred the odour of infected trees. GC-MS analysis of the headspaces collected from healthy and infected apple trees revealed the induction of the sesquiterpene ß-caryophyllene in infected trees. Y-tube olfactometer trials revealed ß-caryophyllene to be an attractant for *C. picta*. In conclusion, the pathogen manipulated its vector indirectly by inducing an allomone in the plant, which increased its attractiveness. Finally, the use of this compound for trapping the vector was proved in field experiment.

Detection and identification of phytoplasmas in pear trees in the Czech Republic

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Survey on the occurrence of phytoplasma diseases in pear trees was conducted in the Czech Republic over the years 2006 to 2008. A total of 160 pear trees with symptoms of premature leaf reddening, yellowing, dieback of branches and also asymptomatic trees were tested for the presence of phytoplasmas. Nested PCR procedures were employed with different sets of primers to amplify phytoplasma 16Sr DNA plus spacer region. Among the plants tested, 36 trees were positive in nested PCR analyses. However, by RFLP analyses of 16Sr DNAs (R16F2n/R2 nested PCR products) it was possible to identify phytoplasmas in 28 of the studied trees. '*Candidatus* Phytoplasma pyri' (ribosomal subgroup 16SrX-C, pear decline - PD), '*Candidatus* Phytoplasma asteris' (16SrI-C, clover phyllody – CPh), and '*Candidatus* Phytoplasma mali' (16SrX-A, apple proliferation – AP) were identified in 11, 7 and 2 pear trees, respectively. A mixed infection of PD with CPh (3 trees), PD with '*Candidatus* Phytoplasma solani' (16SrXII-A, stolbur – STOL) (1 tree), PD, AP and CPh (1 tree), AP with CPh (2 trees) and AP together with phytoplasmas belonging to ribosomal group 16SrI were identified after digestion with MseI, TruI, SspI, RsaI and HhaI endonucleases. Sequence analysis of selected samples and its comparison with the data available in the GenBank confirmed the phytoplasma presence and its

previous identification by RFLP. The identification of stolbur and apple proliferation represents the first report of such an occurrence of these phytoplasmas in pear trees.

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Seasonal variations of pear decline

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Pear decline disease (*Candidatus* Phytoplasma pyri, PD) was monitored in pear trees cv Deveci in two orchards in Bursa, Turkey. PD infections on twenty pear trees in each orchard were previously determined by molecularly. Fluctuations of PD were determined throughout a year monthly. The tests were performed from roots, shoots, leaf midribs, fruits and flowers of the trees depend on the season. Samples were analyzed with PCR using P1/P7 and fU5/rU3 universal primer pairs. Nested PCR products were digested with RsaI restriction enzyme and digested products revealed the same profile of PD control. RFLP results were supported by sequencing of three selected PD isolates. The results revealed that the detection rate of PD had different averages according to the sampling tissue and the period. The flower tissues were sampled in March and the infection rate of PD was 75%. The fruit tissues only sampled in September and the infection rate of PD was 100%. Root, shoot and leaf samples were collected longer period of the year, but the rate of PD was comparatively less than flower and fruits. The infection rate in roots, shoots and leaves was found as 18, 19 and 10%, respectively. The present result have revealed that the best period to detect PD infection in pear trees was between November to March for root samples. April, October, November and December were the best time for leaves and PD could be detected in shoot samples whole year around except July and August.

Effect of pear decline for Turkish pear production

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Pear decline is an important threat for Turkish pear production. In this study, we attempt to compare several pomological characteristics, total phenolic content and total antioxidant capacities in *Candidatus* Phytoplasma pyri infected and noninfected 'Deveci' pear from Bursa, Turkey. The samples were taken in October 2008 on harvesting maturity from four infected and noninfected trees based on pear decline symptoms. Presence of *Candidatus* P. pyri was later confirmed by nested PCR tests. The result indicated that infection significantly reduced fruit size, width, length; and increased pH, color values of a, b and hue. Abortive and healthy seed numbers and weights, soluble solids and acidity did not change significantly. Similarly, the infection did not affect the flesh color. To investigate a possible differential response on skin and flesh of fruits, total phenolic (TP) and total antioxidant capacity (TAC) skin and flesh tissues analyzed separately. The results indicated that, as expected, infected skin tissue had higher total phenolic and total antioxidant capacity for both methods analyzed (TEAC and FRAP). Skin TP content increased from 806 to 923 µg gallic acid equivalent (GAE)/g fresh weight (fw) while flesh TP content increased from 195 to 249 µg GAE/g fw. TAC also found to be enhanced on infected fruits. On average, noninfected trees had 32.4 and 28.3 µmol TE/g fw for TEAC and FRAP, respectively. Infection increased these averages to 35.4 and 32.3 µmol TE/g fw tabulating 18 and 12% increase in flesh tissue. Similarly, the TEAC and FRAP averages increased from 4.0 to 5.8 and 3.3 to 4.9 µmol TE/g fw, respectively.

Identification of phytoplasmas associated with apple trees showing shoots proliferation and leaves deformation

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Five apple trees showing proliferation of shoots and deformation of leaves were tested to detect the possible presence of phytoplasmas. Total DNA was extracted from shoots using DNeasy Plant Mini Kit (Qiagen GmbH) and was subjected to a nested PCR for amplification of phytoplasma 16S ribosomal (r) DNA. Universal primers P1/P7 followed by universal primers R16F2n/R16R2 as well as specific primers: R16(I)F1/R16(I)R1 for Aster yellows group, AY (16SrI) and fAT/rAS for Apple proliferation group, AP (16SrX) were used in a nested PCR. All phytoplasma rDNA fragments were amplified by nested PCR with R16F2n/R16R2. Using fAT/rAS AP-specific primers in nested PCR, products of expected size were obtained for DNA fragments extracted from four apple trees but not from 'Evelina' tree. Phytoplasma rDNA fragment isolated from this apple tree was amplified by nested PCR with R16(I)F1/R16(I)R1 AY-specific primers but not with fAT/rAS AP-specific primers. Restriction fragment length polymorphism (RFLP) of R16F2n/R16R2 primed nested PCR products was performed using endonucleases RsaI, BfaI, AluI, HpaII to identify phytoplasmas. Restriction profile of nested PCR products from four apple trees was identical to each other, to the control AP-infected apple and patterns published previously and indicated that they were infected by a phytoplasma classified in 16SrX group as the species *Candidatus* Phytoplasma mali. Samples from 'Evelina' apple trees produced a restriction profile identical to AY-infected strawberry – now classify as *Candidatus* Phytoplasma asteris. Nested PCR products primed with R16F2n/R16R2 were purified using QIAquick® Gel Extraction Kit (Qiagen, GmbH), sequenced and analyzed using Lasergene (DNASTAR) computer program. Sequence analysis of 16Sr DNA fragments of phytoplasmas isolated from apple trees confirmed the PCR-RFLP results.

Diagnostics of fruit tree phytoplasmas - Importance of latent infections

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In the period 2000-2008 more than 1300 fruit trees from different regions of Slovenia were tested for the quarantine phytoplasma: Apple proliferation (AP), Pear decline (PD), and European stone fruit yellows (ESFY). The majority of the samples were collected within systematic official surveys, conducted in order to assess the presence of these phytoplasma diseases in Slovenia in production and mother plant orchards. Samples were taken from trees with and without expressed symptoms. DNA was extracted from the roots (latent infection) or shoots (trees with symptoms). The presence of phytoplasmas were checked using nested PCR, RFLP and real time PCR test (Hren et al., 2007). AP, PD and ESFY were confirmed as being present in several areas in Slovenia where fruit trees are cultivated. AP was found not only in apple, but also in stone fruit trees such as cherry, apricot and plum (Mehle et al., 2007). We observed that especially young trees didn't show typical symptoms (Lešnik et al., 2007) and some infected fruit trees were symptomless. Some latent infections were detected only by using high sensitive diagnostic methods, such as real time PCR, but one year later the infection was confirmed also by using less sensitive methods. Infected trees without symptoms represent a hidden source of infection, so an early detection of infected trees and their removal is almost equally important as intensive vector control, especially in newly established plantations in fruit tree growing areas with high disease occurrence.

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Differential host DNA methylation might be the cause of phytoplasma elimination upon the treatment with auxins

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Phytoplasmas from the class Mollicutes are wall-less, pleomorphic endocellular plant pathogenic bacteria that live in the phloem of their plant hosts. Phytoplasmas have reduced genomes of 530 – 1130 kb. Therefore, they are highly dependent on the intake of the nutrients form their hosts, cannot be grown on artificial media and cannot be inoculated mechanically on healthy plants to produce infection. It has been shown that a transfer of in vitro grown phytoplasma-infected *Catharanthus roseus* plantlets from medium supplemented with cytokinine, 6- benzylaminopurine (BA) to the one supplemented with auxins, indole-3-acetic acid (IAA) or indole-3-butyric acid (IBA) can induce remission of symptoms and even permanent elimination of '*Candidatus* Phytoplasma asteris' reference strain HYDB. To elucidate the possible mechanism of phytoplasma elimination from *C. roseus* shoots caused by IBA-treatment, we measured and compared endogenous auxins' levels and general methylation levels in healthy periwinkles, periwinkles infected with different '*Candidatus* Phytoplasma' species and phytoplasma-recovered periwinkles. Healthy shoots did not respond, or responded very weakly to exogenously added auxin, maintaining their phenotype, nominal levels of methylation and hormone concentrations. Phytoplasma-infection caused a change in endogenous levels of auxins in infected periwinkle shoots infected with different '*Candidatus* Phytoplasma' species, but general methylation levels were not statistically different from healthy plants except in the case of 'Ca. P. asteris', which was the phytoplasma strain eliminated from tissues when periwinkles were transferred to IBA containing medium. Therefore, low level of host genome methylation caused by 'Ca. P. asteris' infection of periwinkles, statistically elevated after IBA-treatment, might be the cause of this phytoplasma elimination.

Current status of European stone fruit yellows phytoplasma in Bosnia and Herzegovina

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Stone fruits from commercial as well as abandoned orchards were evaluated for ESFY presence during 2004-2007 years, monitoring western and southern districts of Bosnia and Herzegovina. In first survey conducted in period of 2004 till 2005 ESFY were identified on peach and apricot plants in both surveyed districts. During 2007 new survey were done and samples were taken from symptomatic and symptomless plants of peach (*Prunus persica*), apricot (Prunus armeniaca), plum (*Prunus domestica*), Japanese plum (*Prunus salicina*), myrobolan (*Prunus cerasifera*) and cherry (*Prunus avium*). Samples were analyzed using real-time PCR and nested PCR approaches. Concerning previous results, presence of ESFY phytoplasma was additionally identified in Japanese plum and myrobolan as two new phytoplasma hosts in Bosnia and Herzegovina.

Almond witches - broom phytoplasma (*Candidatus* Phytoplasma phoenicium) a real threat to almond, peach and nectarine

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Almond witches'- broom phytoplasma ('*Candidatus* Phytoplasma phoenicium') was recently reported in association with a devastating disease that killed over a hundred thousand almond (*Prunus dulcis*) trees in Lebanon and in Iran. This phytoplasma belongs to the pigeon pea witches' broom group (16SrIX) which was reported for the first time on stone fruits. The vector has not yet been identified, even though some preliminary data were gathered at our laboratory. However, more recently over a hundred peach and nectarine trees showed symptom of early flowering, rosetting and fruit abortion followed by shoot proliferation, chlorotic smaller leaves that gave the tree a bushy appearance. Most infected trees did not set any fruits. DNA sequencing demonstrated that this isolate is very closely related to 'Ca. Phytoplasma phoenicium'. In one field, the workers stated that in 2007 they observed only a limited number of trees with such symptoms, but that in 2008 approximately 90 trees were diseased. Farmers responded rapidly by eradicating symptomatic trees and in 2009 only few cases were observed and quickly eradicated. The rapid spread of Almond witches' broom over large geographical areas in Lebanon and Iran and the susceptibility of peaches and nectarines to this disease calls for immediate and coordinated regional and international action to establish a stricter local and international quarantine measures in order to eradicate or prevent the further spread of this emergent devastating stone fruit disease.

Results of patch- grafting of tissue infected by *'Candidatus* phytoplasma pyri' on pear and by *'Candidatus* phytoplasma prunorum' on apricot

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Because the pear varieties 'Conference', 'Comice' and 'William', grafted in open field on different rootstocks resulted susceptible to *'Candidatus* phytoplasma pyri', transmitted by *Cacopsylla pyri*, and, in the same orchard, sixty-eight apricot varieties, all grafted on Prunus cerasifera, resulted differently susceptible to *'Candidatus* phytoplasma prunorum' , we planned a work program with the aim to discover a source of immunity among seven new combination of pear varieties/rootstock and six new combination apricot varieties/rootstock exposed to the contact with the associated phytoplasma micro-organisms, by patch grafting of infected tissues on their phloem. We used also the pear combination 'Comice' on *Pyrus* communis and the apricot combination 'Palummella' on *Prunus* cerasifera already resulted, in open field, both susceptible to the associated phytoplasma, transmitted by the specific vectors. For these experiments 776 health young plants, cultivated in pots, under insect proof greenhouses, were utilized. The molecular analyses, using PCR/RFLP technology, to verify pathogen presence were carried out on leaves collected in each vegetative season since 2003 to 2008. The new pear combination resulted susceptible to 'Ca. phytoplasma pyri' is 'William' on *Pyrus betulaefolia* but the evidence that either the pear combination 'Comice' on *Pyrus* communis or the apricot combination 'Palummella' on *Prunus cerasifera* resulted in the past susceptible were resulted, in these experiments, negative to molecular test does not permit us to declare immune all the other new combinations tested. So we can only conclude that this kind of grafting is not efficient in transmitting these two pathogen to young plants, cultivated in pots. We will continue to test other kinds of grafting techniques on these young plants in pots to understand if the different reaction to these two pathogens can be studied or not under green-house conditions.

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Evaluation of different detection methods of virus and phytoplasmas for a pear and apple certification program

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The virus *Apple stem grooving virus* (ASGV), *Apple stem pitting virus* (ASPV) and *Apple chlorotic leaf spot virus* (ACLSV), and the phytoplasmas *Candidatus* Phytoplasma pyri and Ca.P.mali cause significant diseases in Malus and *Pyrus* species. The sensibility of the molecular detection methods of these virus and phytoplasmas has been reported for years, nevertheless it is still recommended to confirm their absence by biological indexing. In this work the presence of this virus by means of RT-PCR and of Ca. P. pyri and Ca.P.mali by PCR is indicated, in trees obtained from certified plant material. Likewise, the detection of these viruses has been done in positive controls of different transmissible diseases like, Ring mosaic, Red mottle, and Russet wart. In all of them ASPV, ASGV and/or ACLSV have been identified, indicating that these are responsible for the disease on their own or in synergism with other viruses or pathogenic organisms. Different methods of extraction, primers and seasons have been tested. The best primers for the detection of ASPV and ASGV by RT-PCR are those used by Massart et al. (2008). With those primers it is possible to detect the two viruses simultaneously both in purified RNA or in crude extracts and also in the extractions done from phloem in winter and from young outbreaks in spring. For phytoplasma detection the best results were obtained with PCR using purified DNA and fO1/rO1 primers. The results obtained with other non ribosomal primers and with the PCR-dot blot method are also presented. The detection of ASPV, ASGV and ACLSV by RT-PCR and of the phytoplasmas Ca.P.Pyri and Ca.P.mali by PCR can be used year-round for the specific detection of these pathogens directly in their woody hosts.

Molecular detection of pear decline phytoplasma in pear trees and their biochemical responses

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This study was carried out to determine the presence of phytoplasma diseases in pear fruit trees and their biochemical responses in the experimental research garden of Ankara University. Phytoplasmas were detected and characterized by PCR-RFLP analysis. PCR analysis was performed using the primer pairs of P1/P7 and fO1/rO1 to identify the phytoplasmas involved in the diseases. The infected trees, with symptoms or without symptoms, were found positive with the pear decline phytoplasma. Their biochemical responses such as PPO (Polyphenol oxidase), MDA (malondialdehyde), chlorophyll, carotenoids and anthocyanin contents were varied among the cultivars (Duchesse d'Angouleme, Beurre Hardy, Abbe Fetel, Coscia and Deveci) tested. However, no significant differences were observed in proline contents between the cultivars regardless of the presence of symptoms. The cultivars Duchesse d'Angouleme and Beurre Hardy were found more resistant to the effect of disease. The results suggest that the PD phytoplasma, detected in all infected trees, plays a crucial role on the biochemical metabolisms of the diseased plants. Therefore, the resistance of pear trees infected with PD phytoplasma could be easily checked by using biochemical markers.

Key words: Pear, phytoplasma, PCR-RFLP, proline, PPO, MDA.

Variation among *Apple chlorotic leaf spot virus* isolates affecting fruit tree cultivars and ornamental rosaceous hosts

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Apple chlorotic leaf spot virus (ACLSV), the type member of the genus *Trichovirus*, is among the pathogens targeted in certification programs aimed at producing virus-tested propagation material. ACLSV exists worldwide in numerous strains (isolates) in a wide range of rosaceous hosts. ACLSV isolates from pome and stone fruit cultivars have been studied extensively. In the present study, RT-PCR performed with primers targeting the ACLSV coat protein region, was applied for isolates from natural,

poorly studied, ornamental hosts - dwarf flowering almond (*Prunus glandulosa*), medlar (*Mespilus germanica*) and hawthorn (*Crataegus* spp.). Alignment of the nucleotide sequences of virus isolates derived from these hosts showed sequence variability among clones of isolate Dfa1114 from dwarf flowering almond, Med from medlar and Haw from hawthorn but not Dfa1292. These results support previous suggestions that a mixture of ACLSV variants could be present in one tree. Most of the isolates studied by us have the combination of amino acid pairs described recently as crucial for infectivity (Yaegashi et al. 2007). Most isolates show the pairs also observed in isolate B6 but Haw shows the A4-like amino acids. However, variants Haw.6 and Dfa1114.5 have additional modifications at some of these positions. The effectiveness of these modifications needs to be further studied. A phylogenetic tree prepared with the above isolates and a large number of ACLSV sequences available in Genbank demonstrated that none of the isolates included in this study are closely related to previously analyzed isolates. The two Dfa isolates are related despite significant divergence (90% homology). Both isolates belong to a poorly resolved cluster, which contains mainly *Maloidae* isolates, among which is B6. Isolate Haw belongs to another, more resolved group, which contains, among others, the A4 isolate and an isolate from Hawthorn characterized in Greece (N. Katis, personal communication).

Application of recombinant antibodie fragments for supression of a viral disease caused by Tomato yellow leaf curl virus

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Tomato yellow leaf curl virus (TYLCV) is a complex of geminivirus species prevalent in the tropics and sub-tropics, which causes severe diseases in economically important crops such as tomato. Conventional strategies for disease management have shown little success and new approaches based on genetic engineering need to be considered. We generated two single-chain variable fragment antibodies (scFv-ScRep1 and scFv-ScRep2) that bound strongly to continuous epitopes within the TYLCV replication-associated protein (Rep). The TYLCV-Ir C1 gene (encoding Rep) was expressed as glutathione-Stransferase (GST) and maltose-binding protein (MBP) fusions. Purified MBP-Rep was used to immunize mice allowing the construction of a scFv phage display library. Two specific recombinant antibody fragments (scFv-ScRep1 and scFv- ScRep2) were obtained through panning of naïve and pre-immunized phage display libraries. Immunoassays showed that scFv-ScRep1 recognized an Nterminal epitope of Rep, whereas scFv-ScRep2 recognized a more central epitope. Coding regions of these scFv fragments were sub-cloned into plant expression vector and expressed in plant, both as stand-alone and as N-terminal GFP fusions. Initial results indicated that both scFvs and both fusions accumulated to detectable level in the cytosol and nucleus of plant cells. Transgenic plants challenged with TYLCV-Ir showed that the scFv-ScRep1 but more so the fusion proteins were able to suppress TYLCV-Ir replication. These results show that expression of a scFv-ScRep1-GFP fusion protein can attenuate viral DNA replication and prevent the development of disease symptoms. The present article describes the first successful application of recombinant antibodymediated resistance approach against a plant DNA virus.