PLMVd was detected in naturally infected peach, plum apricot, pear, wild pear and quince plants as well as in an Italian peach calico infected plant.

Sensitive detection and strain discrimination of plum pox virus using Rt - Real Time Pcr - Fret Method

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A new method of Plum pox virus detection and strain identification / discrimination, based on real time PCR with fluorescence resonance energy transfer (FRET) probes, has been developed. One 'universal' donor probe as well as D and M (Rec) specific acceptor probes labelled with different fluorophores are utilised for one-tube detection and typing of amplicons during the reaction. Two different channels are used for simultaneous detection of D and M (Rec) type isolates. Post-reaction melting curve analysis provides further data and allows also positive identification of EA type isolates, based on different melting curve profiles. The method is fast, sensitive and provides a way of quantification of PPV types in mixed infections.

Application of scanning electron microscopy for diagnosis of phytoplasmas in single and mixed infections in papaya

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Virus and virus-like diseases are a major threat to papaya production in Mexico, where it is a leading commercial crop in the States of Michoacan, Oaxaca, Jalisco, Nayarit, Yucatán, Veracruz and Chiapas. Additionally, the State of Baja California Sur is the main producer of organically grown papaya destined for USA market. Infection with papaya ringspot virus (PRSV), papaya mosaic virus (PMV) and phytoplasma has been reported from different Mexican states. Some symptoms of yellow type diseases such as mosaics, stunting, bunchy top and leaf chlorosis, necrosis and malformations are somewhat similar in appearance, but provoked by distinct pathogens. A set of complex diagnostic procedures are needed for correct and precise diagnosis of pathogens so that timely and correct control measures can be taken.

Using scanning electron microscopy (SEM) technique phytoplasmas were detected in the phloem tissues of field and greenhouse-indexed papaya plants from Baja California Sur. The 32 regional varieties as well as cv. Maradol with numerous symptoms of dieback, mosaics, bunchy top and yellow crinkle were analyzed. The pathogen was detected in stems, leafstalks, roots, axillary leaflets, leaf veins and in germinated seeds within the fruit. No viral infection was revealed by test-plants and molecular techniques. Analysis by a SEM technique of papaya samples from Veracruz and Irapuato, both field-grown and mechanically infected with PRSV and PMV in various combinations, revealed phytoplasmas in phloem of most of the tested samples. In some cases, along with phytoplasma, rod-shaped bacteria were also distinguished. Further investigations would determine phylogenetic relations between phytoplasmas from samples collected from different states and provide more information about mixed infections and disease epidemiology.

Oral Session IV

New viruses identified in fig trees exhibiting fig mosaic disease

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Though fig mosaic disease has been known for decades but the causal agent has been elusive. Here we present data on the incidence of at least four new viruses isolated from fig trees exhibiting mosaic

symptoms. One of the viruses is closely related to the recently identified European mountain ash ringspot-associated virus. The second is a member of the genus *Badnavirus* and is related most closely to *Citrus yellow mosaic virus*. The other two viruses belong to the family Closteroviridae. Detection protocols have been developed for each of the four viruses and a limited survey of fig mosaic trees carried out. The first agent was present in all samples while the rest were found in a subset of the samples. The incidence of the European mountain ash ringspot-associated virus-like agent in all tested material and its morphology that is identical to the fig mosaic agent indicates that the virus is indeed the causal agent of the disease. The provisional name Fig mosaic associated virus has been given to the virus. Transmission trials are under way to identify vectors of the latter three new viruses and better understand their involvement in disease development.

Fig latent virus 1, a new putative member of the family flexiviridae

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A virus with filamentous particles ca. 700 nm long, denoted Fig latent virus 1 (FLV-1) is widespread in Apulian (southern Italy) fig orchards, in trees showing or not symptomts of mosaic disease. FLV-1 was consistently identified in different batches of symptomless seedlings, which prompted its naming. It was transmitted by sap inoculation to a very restricted range of herbaceuos hosts without inducing apparent symptoms and was transmitted through fig seeds to a very high percentage (80 to 100%). FLV-1 was succesfully purified from root tissues of infected figs. A virus-specific antiserum raised in rabbits, proved useful for its detection in fig leaf dips by immune electron microscopy. Bundles of filamentous particles were observed in the cytoplasm of parenchyma cells of infected fig trees and seedlings. The viral genome is a single stranded positive sense RNA about 8,000 nt in size, 6,500 of which have been sequenced starting from the polyadenylated 3' terminus. Genomic RNA consists of four open reading frames encoding, in the order from the 5' to the 3' end, the replicationassociated protein (ORF1), a 42 kDa putative movement protein (ORF2), the 46 kDa coat protein (ORF3), and a 12 kDa protein with nucleic acid binding properties. The genome structure and organization resembles that of members of genus *Trichovirus*, family *Flexiviridae* and, indeed, FLMaV-1 clusters with trichiviruses in phylogenetic trees constructed with the coat protein and the movement protein sequences. However, a distinct difference with all members of the genus rests with the size of the coat protein subunits (46 versus 22-27 kDa) and the presence of ORF4, which is not represented in all trichoviruses.

Keywords: *Ficus carica*, *Flexiviridae*, FLV-1, RACE-PCR, sequencing

Molecular characterisation of viruses from kiwifruit

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In 2003 Apple stem grooving virus was discovered in Actinidia accessions from China, being held in quarantine in Auckland. Subsequent examination of kiwifruit germplasm from the same source has detected several additional viruses, including a ~300 nm rigid rod related to Ribgrass mosaic virus (*Tobamovirus*) and a 700-750 nm flexuous virus related to Citrus leaf blotch virus (*Flexiviridae*). Currently these viruses have not been reported from commercial kiwifruit crops in New Zealand or elsewhere. The biological properties of the viruses from kiwifruit and their phylogenetic relationships with similar viruses from other plants will be described, and the possible implications for the international movement of Actinidia germplasm will be discussed.

Towards the elucidation of the taxonomic position of Prunus-infecting viral agents belonging to the Foveavirus genus and their relationship with Apple stem pitting virus

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Surveys in Greece, Italy and Iran on pome and stone fruit trees identified viral agents closely related to one another and belonging to the Foveavirus genus. In the 289 bp RdRp fragment generated by PDO RT-PCR (Foissac et al., 2005), the amino acid genetic distance between *Apple stem pitting virus* (ASPV) and *Apricot latent virus* (ALV) is around 12.5%. Divergence values in the same range were observed between the uncharacterized agents and ASPV or ALV, impeding their taxonomic assignment. Complete genomic sequences were determined for one of these agents (apricot isolate A18) and for isolates of ALV (PAS LA2 isolate) and of the closely related *Peach sooty ringspot virus* (PSRSV, Caserta 12 and SB12452 isolates). The preliminary data obtained from the comparisons of the sequences showed two different situations depending on the genomic regions considered. In the region comprising the RdRp gene and the two first genes of the triple gene block (ORF2 and ORF3), there is a close relationship between the *Prunus*-infecting *Foveaviruses* and ASPV, with a genetic divergence at the amino acid level below the species demarcation criteria for the Foveavirus genus. However, in the region comprising the ORF4 and the CP gene, a much higher divergence level is observed. In particular, the region of the first 211 amino acids of the CP seemed to be highly variable (up to 74% of divergence between ASPV and ALV-like sequences). Interestingly, the C-terminal part of the CP is on the contrary very conserved (between 97.3% and 88.6% of identity). These findings suggest the existence of recombination in the evolutionary history of these agents or, conversely, the adaptation of ASPV-related viruses to a new type of host, such as *Prunus*. (Foissac et al., 2005. Phytopathology, 95,617-625)