Evidence for Association of *Citrus psorosis virus* with Symptomatic Trees and an *Olpidium*-like Fungus in Texas

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ABSTRACT. Citrus psorosis disease is associated with *Citrus psorosis virus* (CPsV), the type member of the *Ophiovirus* genus. In past years, observations from Argentina, Texas and elsewhere suggested that there may be a vector of CPsV since virus-free trees became infected in the field. Recently, there has been an increase in psorosis incidence in Texas, and surveys in certain orchards suggest possible tree-to-tree transmission. Other ophioviruses have been shown to be transmitted by *Olpidium brassicae*, and therefore we have been studying the possibility of a similar mode of transmission for CPsV. Examination of roots of infected and healthy trees showed the presence of an *Olpidium*-like fungus. Biological indexing, ELISA and RT-PCR were used to confirm the presence of CPsV in the diseased trees. Using RT-PCR, we detected CPsV in the roots of infected trees, and in zoospores released from sporangia in infected roots, but not from healthy controls.

The widespread occurrence of citrus psorosis disease in Texas in the late 1940s (2) was the reason for establishing a voluntary virus-free budwood program (8). The possibility that there was also a natural vector of the disease was based on observations in California (6) and Texas (7, 9) and some experimental evidence from Argentina (1). Citrus psorosis is caused by *Citrus psorosis virus* (CPsV), the type member of the genus *Ophiovirus*. Recent evidence showing that another member of this genus, *Mirafiori lettuce virus* is transmitted by the root-infecting fungus *Olpidium brassicae* (4), and the circumstantial evidence that another ophiovirus might be transmitted by a soil factor (5), prompted an investigation to determine whether CPsV transmission was associated with *Olpidium*. Spatial and spatio-temporal analyses of data from surveys over a period of three years in four citrus orchards in Texas containing psorosis-infected trees were consistent with disease spread via vectors (3).

Biological indexing of 13 grapefruit trees on Madam Vinous seedlings was positive for psorosis. Young leaves from grapefruit trees with bark scaling symptoms ranging from 'typical psorosis' to 'psorosis-like' (small area of scaling) were collected for DAS-ELISA and analyzed with a CPsV Agritest kit (Valenzano, Italy), and assays were conducted as per manufacturer's instructions. Leaves which displayed ringspot symptoms gave a 95.8% positive result by DAS-ELISA. Leaves from trees with typical psorosis symptoms gave 66.7% positives, but only 5.4% of the samples from trees with a few scattered areas of scaling and none of the 'psorosis-like' trees gave positive results. Reverse-transcription polymerase chain reaction (RT-PCR) assays using ophiovirus primers OP-1 and OP-2 as described by Vaira *et al*. (10) were conducted on leaf samples from nine grapefruit trees which were positive for ELISA, and from three healthy trees. Eight of the nine symptomatic trees gave a specific amplification product of 136 bp, while none of the healthy samples yielded this product (Fig. 1). Two rounds of PCR were found to be necessary for clear amplification products to be seen on the gels.

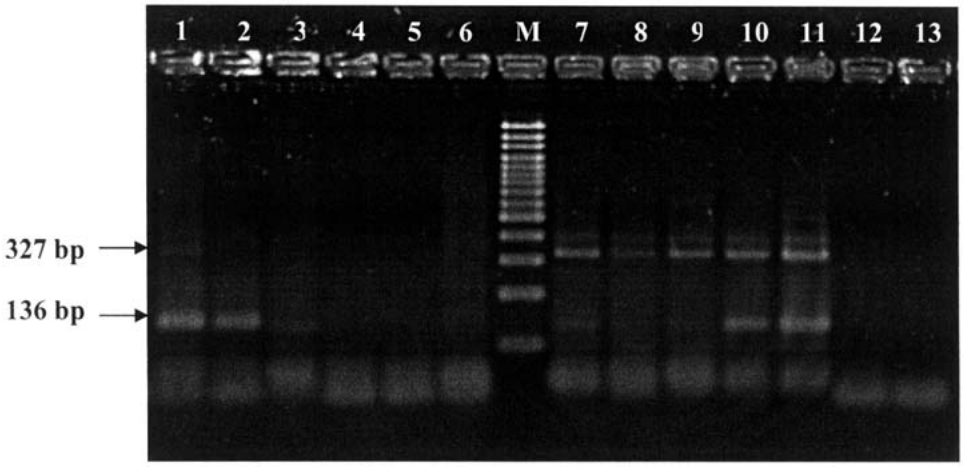

Fig. 1. Agarose gel electrophoresis of products from RT-PCR analysis for *Citrus psorosis virus* in symptomatic trees. Arrows indicated sizes of products. l. M: 100bp ladder. Lanes 1-4 & 7-11: leaves of psorosis-infected grapefruit trees. Lanes 5, 6 & 12: leaves of healthy grapefruit trees. Lane 13 is a blank sample.

Feeder roots from psorosis-infected trees were examined by light microscopy. Sporangia resembling *Olpidium* were observed in high numbers. Roots from healthy trees were observed to have far fewer sporangia. The fungus was baited onto onion skin pieces and after an incubation period of 24-36 h, sporangia were clearly visible (Fig. 2).

To determine whether CPsV was present in citrus roots and in fungal tissue, RT-PCR using primers OP-1 and OP-2 (as above) was conducted on feeder roots from infected and healthy trees, and on zoospores collected from infected and healthy trees. For zoospore collection, two methods were used. In the first, washed roots were air-dried for 2 weeks, and then pulverized in a mortar in distilled water. The fine particles were removed with sieves, and the spores which passed through the

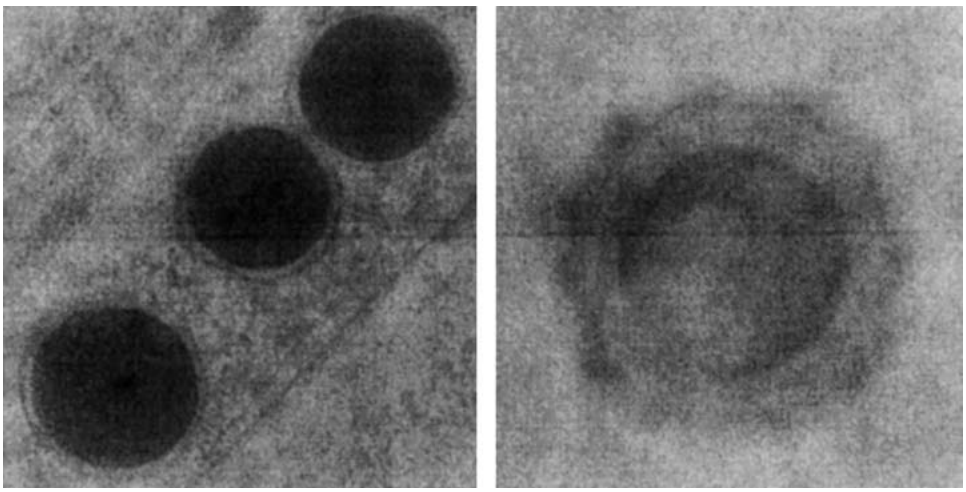

Fig. 2. Resting sporangia of an *Olpidium*-like fungus found in the onion skin pieces incubated with sporangia-containing citrus roots.

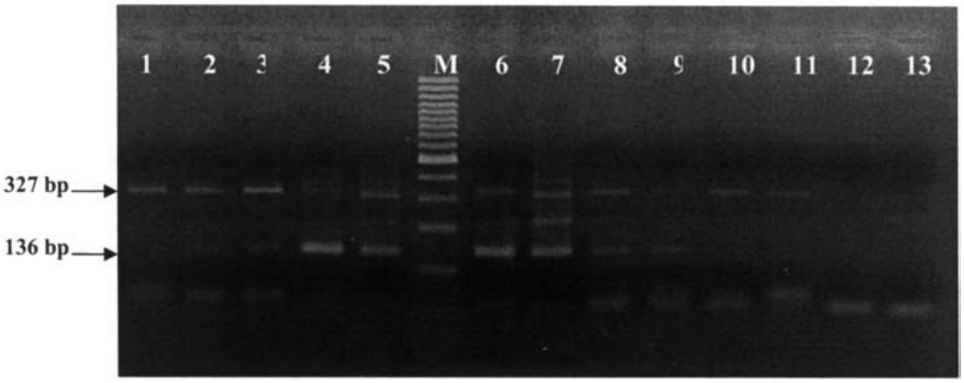

Fig. 3. Agarose electrophoresis of RT-PCR products amplified from samples analyzed for *Citrus psorosis virus*. Arrows indicate sizes of expected *Citrus psorosis virus* PCR products (136 bp) and an unexpected product (327 bp). M: 100bp ladder. Lanes 1, 10 & 11: leaves of seedlings raised in soil with psorosis-infected sour orange roots. Lanes 2-7: zoospores collected from the roots of psorosis infected trees. Lane 8: leaves of infected grapefruit tree. Lane 9: roots of infected trees. Lane 12: zoospores collected from the roots of healthy tree. Lane 13 is a blank sample.

325-mesh sieve were centrifuged at 2,500 rpm for 5 min to remove plant debris. The supernatant was centrifuged at 9,000 rpm for 10 min to collect the zoospores. For the second method, dried roots were soaked in distilled water for 12-16 hr, and zoospores were collected by centrifugation as described above.

An amplification product of 136 bp was observed from infected leaves, roots and zoospores from infected roots (Fig. 3). The two zoospore collection methods produced identical results. Healthy controls did not contain any amplification products. The intensity of the amplification product from roots appeared less than from leaves or zoospores. A second amplification product of 327 bp was frequently observed from infected plant and zoospore tissue. Both amplification products were cloned, sequenced, and a GenBank BLAST search conducted. The 136 bp product had 90% identity with RNA1 of CPsV (Accession AY224663), while the 327 bp product had 80% and 85% identity with *Sorghum bicolor* (Accession AB084898) and *Allium cepa* (Accession AF465822) aldehyde dehydrogenases respectively.

To study CPsV transmission by *Olpidium*, Madam Vinous sweet orange seedlings were planted in soil containing infected roots. Other seedlings growing in liquid medium were inoculated with zoospores from infected roots, and then planted in potting mixture after six weeks. After three months, none of the plants in either treatment had developed leaf flecking symptoms, and DAS-ELISA and PCR tests were negative. The results of this study showed that an *Olpidium*-like fungus is associated with citrus roots, and that CPsV could be detected in zoospores from infected roots. Further work is required to confirm that this fungus is able to transmit the virus to healthy citrus.

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