Detection of *Citrus Psorosis Virus* in Cuba

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ABSTRACT. Psorosis is a very well known citrus disease causing important damage in many countries, in particular in Argentina and Uruguay. Based on reports of symptoms such as bark scaling on trunks and branches observed on orange, mandarin and grapefruit trees, this disease was also present in Cuba. To determine the presence of the virus, 32 samples from trees with psorosis symptoms were collected from the Cítricos Ceiba, Victoria de Girón, Cítricos Sola and Cítricos Ciego enterprises. These samples were analyzed by TAS-ELISA. Four samples, which tested positive for psorosis, were also positive when evaluated by hemi-nested-PCR, electron microscopy and biological indexing on Madame Vinous and Pineapple sweet orange. The four isolates induced symptoms of chlorotic flecking in young leaves of citrus indicator plants and characteristic filamentous viral particles were observed by electron microscopy.

Psorosis is a damaging disease of citrus that is widespread in many parts of the world, including South America and the Mediterranean areas (8). The disease is correlated with the presence of *Citrus psorosis virus* (CPsV), the type member of the *Ophiovirus* genus, with a genome of three single-stranded RNAs (6). In Cuba this disease was recognized in field trees that showed symptoms of bark scaling and internal wood staining of trunks and branches. The disease is limited to old citrus trees grafted with non-certified budwood (5). In this study 32 samples from trees showing some of these symptoms were collected in four enterprises: Cítricos Ceiba (La Habana), Victoria de Girón (Matanzas), Cítricos Ciego (Ciego de Avila) and Cítricos Sola (Camagüey). To confirm the presence of CPsV, samples were analyzed by Triple Assay Sandwich ELISA (TAS-ELISA) using A3221V polyclonal and 13C5 monoclonal antibodies (1, 3). Samples were considered positive when they showed ELISA readings with optical densities (OD) at least three times those of the mean values for the negative controls.

The results indicated that four samples out of the 32 collected were positive by TAS-ELISA (Table 1). These four isolates were from Valencia orange from Cítricos Ceiba. Using primers designed for the

TABLE 1
RESULTS OF ANALYSES OF CUBAN *CITRUS PSOROSIS VIRUS* ISOLATES

Isolate	Symptoms on field trees	Symptoms on indicator plants	TAS-ELISA (OD S/H)[1]	Hemi-nested RT-PCR
Ps 3-1	Bark scaling, flecking and gumming	Flecking	182	+
Ps 5-2	Flecking and scaling on bark and branches	Flecking	226	+
Ps 3-2	Bark scaling and gumming	Flecking	242	+
Ps 3-3	Bark scaling and gumming	Flecking and chlorotic ringspots	238	+

[1]OD S/H: Ratio of sample OD to negative control OD.

CPsV coat protein (2) and hemi-nested reverse-transcription polymerase chain reaction (RT-PCR) analysis, isolates Ps3-1, Ps3-2, Ps3-3 and Ps5-2 yielded the expected fragments for CPsV RNA3. Additionally, the biological characteristics of these isolates were evaluated, with graft inoculation with these isolates inducing variable chlorotic flecking on young leaves after the first month post-inoculation. Isolate Ps3-3 also showed chlorotic ringspots on old leaves, 5 mo post-inoculation. The last symptoms mentioned have been associated with psorosis B (8), but shock reaction, also associated with psorosis B, was not observed. The presence of viral particles in partially purified extracts from Pineapple sweet orange was confirmed by negative staining and electron microscopy. In these preparations, viral particles were detected, having either a closed "O" (Fig. 1) or in some cases a spindle shape, with an oscillation 3-4 µm wide, and showing the same morphology seen in previous reports (4, 7).

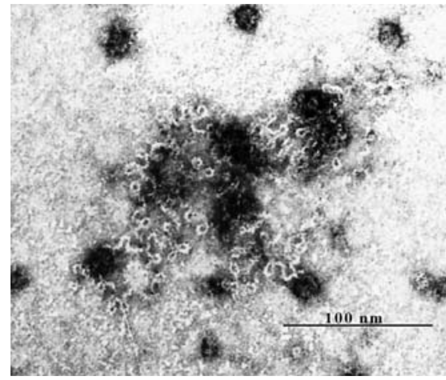

Fig. 1. Flexuous particles observed in partially purified extracts of Pineapple sweet orange inoculated with suspected *Citrus psorosis virus*.

This is the first report of the presence of *Citrus psorosis virus* in Cuba. The cause of the bark scaling symptoms still has to be determined in the case of trees that tested negative for psorosis.

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