

Disease Notes (continued)

Peach latent mosaic viroid Detected for the First Time on Almond Trees in Tunisia. I. Fekih Hassen, S. Roussel, and J. Kummert, Unité de phytopathologie, Faculté Universitaire des Sciences Agronomiques, Passage des déportés, 2, 5030 Gembloux, Belgium; H. Fakhfakh and M. Marrakchi, Laboratory of Molecular Genetic, Immunology and Biotechnology, Faculty of Sciences of Tunis, 2092 Elmanar Tunis, Tunisia; and M. H. Jijakli, Unité de phytopathologie, Faculté Universitaire des Sciences Agronomiques, Passage des déportés, 2, 5030 Gembloux, Belgium. Plant Dis. 89:1244, 2005; published on-line as DOI: 10.1094/PD-89-1244A. Accepted for publication 19 August 2005.

Almond (*Prunus dulcis* Mill) is an important crop in countries of the Mediterranean area. Until now, among viroids, only *Hop stunt viroid* (HSVd) is known to infect cultivated almond trees (2). In 2004, a survey of almond trees was carried out in orchards in different regions of Tunisia, a major producing and exporting country of almond. Symptoms such as mosaic and necrotic lesions, potentially caused by the *Peach latent mosaic viroid* (PLMVd), were observed on leaves of cultivated almond trees. Since PLMVd was recently detected in peach and pear trees in Tunisia (4), the presence of this viroid in almond trees was studied. The detection method on the basis of one-tube reverse transcription-polymerase chain reaction (RT-PCR) assays was previously described and validated for the detection of this viroid in fruit trees (4). Amplification products were obtained by using previously reported primer pairs of PLMVd (1). Positive controls included RNA preparations of twigs of PLMVd-infected GF 305 peach seedlings. These materials, provided by B. Pradier (Station de Quarantaine des Ligneux, Lempdes, France), were positive as revealed by chip budding on peach seedling indicator plants grown under greenhouse conditions. RT-PCR analysis of nucleic acid preparations from leaves of almond showed specific amplification products with the expected size of 337 bp for two almond trees among 17 trees tested. Nucleotide sequence analyses of cloned amplification products obtained with the PLMVd primers confirmed a size of 337 bp and revealed a sequence similar to sequences from other PLMVd isolates previously characterized. The sequences shared 94 to 98% identity with the reference isolates of PLMVd from peach (EMBL Accession No. M83545, AF170511, AF170514, and AY685181). The two infected almond trees are proximal to each other and peach trees infected with PLMVd. This suggests that one tree may have served as a source of inoculum for the other through agronomic practices such as pruning or the aphid *Myzus persicae* (3). Alternatively, PLMVd may have originated in an unknown host and was then transmitted to almond trees. Our investigation shows that almond is a new host for PLMVd.

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First Report of *Bipolaris sacchari* Causing Leaf Spot on *Lygodium japonicum* and *L. microphyllum* in Florida. M. L. Elliott, University of Florida, Department of Plant Pathology, Fort Lauderdale Research and Education Center, Fort Lauderdale 33314; and M. B. Rayamajhi, USDA/ARS, Invasive Plant Research Laboratory, Fort Lauderdale, FL 33314. Plant Dis. 89:1244, 2005; published on-line as DOI: 10.1094/PD-89-1244B. Accepted for publication 12 August 2005.

Lygodium japonicum (Thunb.) Sw. (Japanese climbing fern) and *L. microphyllum* (Cav.) R.Br. (Old World climbing fern) are invasive, noxious weeds in Florida. During 2001, *L. japonicum* sporelings were collected from natural sites in Hamilton, Highland, and Madison counties and transported to Broward County (Fort Lauderdale) for research use. During February 2002, leaf spots were observed on pinnules (leaflets) of these containerized plants growing in a shadehouse. A fungus with *Bipolaris*-like spores was isolated from affected pinnules and purified and stored for future evaluation. In early 2005, the fungus was grown on 1.5% water agar, with sterile, wheat straw pieces embedded in the agar surface, at 26°C and a 12-h photoperiod using cool-white fluorescent and Verilum full-spectrum bulbs. Conidia were 80.5 ± 14.5 µm (range 53.2 to 123.4 µm) × 15.1 ± 1.6 µm (range 12.1 to 19.4 µm), pale brown, slightly curved, narrowly ellipsoid, without a protuberant hilum, distoseptate (8 ± 1, range 6 to 10), and germinated from both polar cells. Conidiophores were septate and smooth. Conidiogenous nodes were smooth. On the basis of these characteristics, the fungus was identified as *Bipolaris sacchari* (E. Butler) Shoem. (1,2). Pathogenicity toward *L. japonicum* and *L. microphyllum*

was determined using conidia produced on potato dextrose agar at 26°C and a 12-h photoperiod. A 1 × 10⁶ conidia ml⁻¹ suspension was sprayed until runoff on healthy plants grown in 450-ml containers. Control plants were sprayed with sterile water. There were four replicate plants per treatment. Plants were covered with plastic bags to maintain high humidity and placed in a growth chamber with a 12-h photoperiod at 28°C (light cycle) and 22°C (dark cycle). Bags were removed after 72 h, and small (1 to 2 mm), water-soaked spots were evident throughout the plant canopy on both *Lygodium* spp. Plants were incubated for three more weeks under the same photoperiod and temperatures with ~70% relative humidity (light cycle) and ~45% relative humidity (dark cycle), and then evaluated for disease. At least 50% of *L. microphyllum* pinnules and 25% of *L. japonicum* pinnules on each inoculated plant had small (1 to 2 mm), brown leaf spots or larger (approximately 5 mm) necrotic spots. *B. sacchari* was reisolated from both types of spots from both *Lygodium* spp.; there was no evidence of fungal sporulation on the plants. No symptoms were apparent on control plants. To our knowledge, this is the first report of *B. sacchari* on a non-Poaceae host in Florida.

References: (1) J. L. Alcorn. Ann. Rev. Phytopathol. 26:37, 1988. (2) A. Sivanesan. Graminicolous Species of *Bipolaris*, *Curvularia*, *Drechslera*, *Exserohilum* and Their Teleomorphs. CAB International, Wallingford, Oxon, UK, 1987.

Natural Occurrence of Viruses in *Lycopersicon* spp. in Ecuador. S. Soler, C. López, and F. Nuez, Centro de Conservación y Mejora de la Agrobiodiversidad Valenciana (COMAV), Camino de Vera 14, 46022 Valencia, Spain. Plant Dis. 89:1244, 2005; published on-line as DOI: 10.1094/PD-89-1244C. Accepted for publication 26 July 2005.

The Andean region is home of important genetic diversity for the genus *Lycopersicon*. A survey of three asymptomatic populations of *L. hirsutum*, 17 of *L. parviflorum*, 188 of *L. pimpinellifolium*, and four cultivated populations of *L. esculentum* was made in nine departments of Ecuador. Samples were analyzed serologically for *Tomato spotted wilt virus* (TSWV), *Tomato mosaic virus* (ToMV), *Tobacco mosaic virus* (TMV), *Cucumber mosaic virus* (CMV), *Potato virus Y* (PVY), *Potato virus X* (PVX), *Groundnut ringspot virus* (GRSV), *Tomato chlorosis spot virus* (TCSV), and *Pepino mosaic virus* (PepMV). Samples positive as determined using double-antibody sandwich enzyme-linked immunosorbent assay (absorbance values three times higher than negative controls) were analyzed using reverse transcription-polymerase chain reaction (RT-PCR) with virus-specific primers. *L. pimpinellifolium* was the only species of the four found to be infected with viruses. In the department of Manabí, ToMV was detected in 15 of 16 plants from one population, but only a single plant was infected with PepMV. In this department, PepMV was also detected in a single-plant population that corresponded to a volunteer plant found in the wild and TSWV was detected in another plant. In Esmeraldas and Guayas, two single-plant populations were found infected with PepMV and CMV, respectively. TMV, PVY, PVX, GRSV, and TCSV were not detected in this survey. Specific primers were selected for ToMV (To1/To2, genome coordinates 3498-3518/4902-4922, AJ417701), PepMV (Pe1/Pe2 genome coordinates 5030-5050/5913-5935, AJ606359), CMV (Cm1/Cm2 genome coordinates 541-561/1756-1779, D00356), and TSWV (Ts1/Ts2 genome coordinates 4078-4101/4738-4769, AF208498). Amplicons of the expected size were obtained using RT-PCR and then cloned and sequenced. DNA fragments of ToMV, PepMV, and TSWV showed identities greater than 99% with respective sequences in the GenBank database. The highest identity of the CMV DNA fragment was 92% with an isolate from Indonesia (AB042292). The occurrence of viruses such as CMV, ToMV, and TSWV in coastal Ecuador was not surprising. However, infected plants were not found among the samples collected in the departments of Azuay, Carchí, El Oro, Imbabura, Loja, and Pichincha in eastern Ecuador. *L. chilense*, *L. chmielewskii*, *L. parviflorum*, and *L. peruvianum* were previously reported as natural hosts of PepMV in central and southern Peru (2), and the virus was also detected in *L. esculentum* in Chile (1). Our results show that PepMV now occurs in wild *L. pimpinellifolium* populations along the Pacific coast of the South American continent and that it must have efficient means of transmission, although no specific vectors have as yet been identified for this virus. To our knowledge, this is the first report of PepMV in Ecuador and *L. pimpinellifolium* as a natural host of PepMV.

References: (1) M. Muñoz et al. Fitopatología 37:67, 2002. (2) S. Soler et al. J. Phytopathol. 150:49, 2002.