

Disease Notes (continued)

First Report of Pear blister canker viroid, Peach latent mosaic viroid, and Hop stunt viroid Infecting Fruit Trees in Tunisia. I. Fekih Hassen, J. Kummert, and S. Marbot, 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.* 88:1164, 2004; published on-line as D-2004-0802-01N, 2004. Accepted for publication 7 July 2004.

Viroids of fruit trees are plant pathogens distributed worldwide and can cause severe losses and economic damage to crops. A survey of fruit trees was carried out in 17 orchards in the northern and Sahel regions of Tunisia. Samples were collected in field trees of peach (*Prunus persica* L.), pear (*Pyrus communis* L.), and almond (*Prunus dulcis* Mill.) that showed symptoms potentially caused by viroids (leaf mosaic in peach, blister canker in pear, and necrotic leaves in almond). The investigation was conducted during May, September, and December 2003 to screen for the presence of *Pear blister canker viroid* (PBCVd) on pear, *Peach latent mosaic viroid* (PLMVd) on peach, and *Hop stunt viroid* (HSVd) on the three plant species in naturally infected field trees. The detection method was based on one-tube reverse transcription-polymerase chain reaction (RT-PCR) assays using a Titan kit (Roche Diagnostics, Penzberg, Germany). DNA amplification was obtained by using previously reported primer pairs for PLMVd and HSVd (1,4). For PBCVd, forward primer 5' GTCTGAAGCCTGGGCGCTGG 3' and reverse primer 5' CCTTCGT CGACGACGAGCCGAG 3' were designed using an available sequence (3). Positive controls included isolate D168 of PLMVd (obtained from Dr. B. Pradier, Station de Quarantaine des Ligneux, Lempdes, France) and propagated in GF 305 rootstock and HSVd (provided by Dr. R. Flores, Instituto de Biología Molecular y celular de Plantas, Valencia, Spain) propagated in cucumber. The method described by Grasseau et al. (2), with some modifications, was used to prepare the samples for RT-PCR. RT-PCR analysis of nucleic acid preparations from leaves and bark of peach, pear, and almond showed that PLMVd occurred in the northern and Sahel regions of Tunisia. Of 37 peach trees tested, 12 were found infected with PLMVd. Two pear trees among 73 tested were infected with PBCVd. HSVd was detected in 2 of 11 almond, 1 of 37 peach, and 7 of 72 pear trees tested. One pear tree infected with HSVd was also infected with PBCVd. Symptoms observed in fruit trees were not consistently associated with the presence of viroids. Nucleotide sequence analyses of cloned amplification products obtained using the PBCVd, PLMVd, and HSVd primers confirmed a size of 315, 330, and 300 nt, respectively, and revealed a sequence similar to sequence variants from other isolates previously characterized for each viroid. PBCVd was 99% identical with

the P47A isolate variant 9 (GenBank Accession No. Y18043); PLMVd shared 85 to 96% identity with the PC-C32 Italian isolate of PLMVd from peach (GenBank Accession No. AJ550905), and HSVd shared 99 to 100% identity with the HSVd from dapple plum fruit (GenBank Accession No. AY460202). To our knowledge, our investigation reports for the first time, the occurrence of PLMVd, PBCVd, and HSVd infecting fruit trees in Tunisia, stressing the need for a certification program to aid in prevention and spread of fruit tree viroids in this country.

References: (1) N. Astruc. *Eur. J. Plant Pathol.* 102:837, 1996. (2) N. Grasseau et al. *Infos-Citil* (Centre Technique Interprofessionnel des Fruits et Légumes). 143:26, 1998. (3) C. Hernandez et al. *J. Gen. Virol* 73:2503, 1992. (4) S. Loreti et al. *EPPD Bull.* 29:433, 1999.

First Report of *Botrytis cinerea* on Pansy Flowers in Buenos Aires. M. C. Rivera, Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453 (1417), Buenos Aires, Argentina; and S. E. Lopez, Facultad Ciencias Exactas y Naturales, Ciudad Universitaria (1428), Buenos Aires, Argentina. *Plant Dis.* 88:1164, 2004; published on-line as D-2004-0812-01N, 2004. Accepted for publication 14 July 2004.

Pansy (*Viola × wittrockiana*) is an ornamental annual plant produced as a potted plant in greenhouses around Buenos Aires, Argentina. Flower rot with signs of gray mold was observed on pansy cv. Crown during the autumn of 2003. Diseased tissues were surface sterilized by immersion in 2% NaOCl for 1 min, placed on 2% potato dextrose agar (PDA), and incubated at 22°C. Fungal mycelia were initially white and became gray after 72 h. After 4 days, colonies were 4 cm in diameter and sporulated profusely. Black sclerotia developed after 7 days. Mycelia were septate with dark branched conidiophores bearing unicellular, ellipsoid, hyaline conidia that measured 8 to 12 × 6 to 8 μm in botryose heads. These characteristics agree with *Botrytis cinerea* Pers.:Fr. (1). Pathogenicity tests were performed by spraying 10 healthy pansy plants during bloom with 3 ml of a conidial suspension (10⁶ conidia per ml) per plant. Controls were treated with sterilized distilled water only. Plants were covered with plastic bags for 2 days and incubated at 18 to 22°C. The flowers developed water-soaked lesions between 4 and 6 days after inoculation. Fifty percent of the flowers were pendulous because flower blight reached the peduncle. The pathogen was reisolated from diseased flowers after superficial sterilization with 2% NaOCl and isolated on PDA. Gray mold has a rapid development during bloom, and the pathogen was able to enter undamaged flower tissues. No disease symptoms were observed on leaves. This report adds pansy as a new host of *B. cinerea* to a previous list of ornamentals grown in Argentina where gray mold was observed.

Reference: (1) M. V. Ellis and J. M. Waller. *Sclerotinia fuckeliana* (conidial state: *Botrytis cinerea*). No. 431 in: *Descriptions of Pathogenic Fungi and Bacteria*, CMI, Kew, Surrey, UK, 1974.