May 2009, Volume 93, Number 5
Page 545
DOI: 10.1094/PDIS-93-5-0545C

Disease Notes

First Report of *Lasidiplodia theobromae* and *Cryptovalsa ampelina* Associated with Grapevine Decline from Castilla y León, Spain

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Symptoms of grapevine decline were surveyed. Samples from mature vines exhibiting external symptoms of Eutypa dieback and Esca were collected, as were young plants with and without external symptoms, and fungal isolations were performed. In 2007, 3-year-old grapevines (cv. Tempranillo grafted onto 110R rootstock) with low vigor, reduced foliage, and vascular streaking in the wood were observed. Small pieces of discolored wood were placed onto malt extract agar supplemented with 0.25 g/liter of chloramphenicol, incubated at 25°C, and resulting colonies were transferred to potato dextrose agar (PDA). Isolates were characterized by abundant aerial and fast-growing mycelium covering the plate surface after 3 days, mycelium became dark green. Pycnidia contained thick-walled, aseptate conidia 15 to 35 × 10 to 15 µm. *Lasidiplodia theobromae* was identified based on morphological characteristics (3) and confirmed by banding patterns obtained after the digestion of the 1,200-bp amplicon generated with ITS1 and NL4 primers (2) using restriction endonucleases (2). Single-spore cultures were generated and DNA sequences of the rDNA internal transcribed spacer region, partial sequence of the 5′ end of the β-tubulin gene, and a fragment of the elongation factor further confirmed the identification and revealed genetic similarity with other isolates of *L. theobromae*. A sequence of each fragment was deposited in GenBank with Accession Nos. EU600925, EU597297, and EU597298, respectively. Pathogenicity tests were conducted on four replicate rootstocks (110R) and 15 canes of current-season growth (cv. Tempranillo). Plants were inoculated with an agar plug containing *L. theobromae*; controls were treated with agar only. Grapevines were maintained in a greenhouse at 20 to 25°C. After 3 months, *L. theobromae* was reisolated from internal vascular lesions in 100 and 66% of inoculated rootstocks and canes, respectively. Control plants were asymptomatic and *L. theobromae* was not recovered. Using the same methodology, a fungus identified based on morphological characteristics in culture as *Cryptovalsa ampelina* (1) was isolated from grapevines (cv. Tempranillo) planted in 1987. Cultures in PDA were white to creamy white and cottony with diffuse margins. Colonies covered the 90-mm-diameter petri dish surface in 5 days. Conidia were 20 to 23 × 1 to 1.5 µm, unicellular, hyaline, and filiform. PCR amplifications of the DNA extracts of *C. ampelina* with Camp-1 and Camp-2R primers gave a characteristic DNA fragment of 300 bp (3) and DNA sequences of the ITS4-ITS5 amplicons (GenBank Accession No. EU597296) confirmed the identification. For the first time, the 5′ end of the β-tubulin gene was sequenced and deposited in GenBank (Accession No. EU600926). Pathogenicity tests were conducted as described above for *L. theobromae*. Both pathogens were examined in the same experiment. *C. ampelina* was reisolated from internal brown streaking lesions in 25% of the rootstocks and 33% of the canes. Control plants exhibited no symptoms. *L. theobromae* appeared to be a more aggressive pathogen than *C. ampelina* on grapevine with more internal brown streaking and greater recovery of pathogen from inoculated samples. To our knowledge, this is the first report of *L. theobromae* and *C. ampelina* causing grapevine decline in Castilla y León.

References: (1) J. Luque et al. Phytopathol. Mediterr. 45:S101, 2006. (2) M. T. Martin and R. Cobos. Phytopathol. Mediterr. 46:18, 2007. (3) D. Pavlic et al. Stud. Mycol. 50:313, 2004.