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Disease Notes

First Report of *Diplodia corticola* Causing Branch Cankers on Live Oak (*Quercus virginiana*) in Florida

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Numerous cankers on small branches showing dieback were observed on live oak (*Quercus virginiana*) trees in September 2010 in Marion County, FL. Approximately 24 12-year-old landscape trees planted on a farm displayed symptoms. Samples were collected from six of the symptomatic trees and returned to the laboratory for processing. Isolations were made from canker margins after surface sterilization of samples in 2.5% sodium hypochlorite and by plating on potato dextrose agar (PDA). A suspect Botryosphaeriaceae sp. (based on colony morphology) was consistently isolated from the symptomatic branches from all six trees sampled. Fungal colonies consisted of plentiful, white, aerial mycelium that turned dark olive after 5 to 7 days at 23°C with the underside of the cultures turning black (1). Total genomic DNA from three representative Botryosphaeriaceae sp. isolates was extracted and the internal transcribed spacer (ITS1-5.8s-ITS2) region of the rDNA (GenBank Accessions Nos. JF798638, JF798639, and JF798640) using the primers ITS1 and ITS4 (3) and a portion of the β-tubulin gene (Bt), (GenBank Accession Nos. JF798641, JF798642, and JF798641) using the primers Bt2a and Bt2b (2) were amplified, sequenced, and deposited in GenBank. BLASTn searches of the ITS-rDNA sequences resulted in 100% homology (467 of 467, 467 of 467, and 540 of 540, respectively) with ***Diplodia corticola*** isolate CBS 112074 (GenBank Accession No. AY268421). BLASTn searches of the Bt sequences resulted in 99, 98, and 99% (391 of 393, 396 of 400, and 392 of 394, respectively) matches with *D.* ***corticola*** strain UCD2397TX, GenBank Accession No. GU294724. To complete Koch's postulates, nine seedlings of *Q. virginiana*, 0.6 to 0.9 cm in diameter at ground line maintained in a greenhouse, were inoculated with isolate PL949 (GenBank Accession Nos. JF798638 and JF798641) by making a 1.5-cm incision with a single-edge razor blade into the xylem 10 cm above ground line. Inoculations were done by placing mycelial plugs (1 × 0.25 cm) from cultures on PDA in the incision with the mycelium facing the center of the stem. Wounds were sealed by wrapping them with Parafilm. Three negative controls were mock inoculated as previously described except sterile PDA plugs were used. Eight weeks postinoculation, the lengths of the necrotic lesions were measured. Mean lesion length of the inoculated seedlings was 4.12 cm ± SE .45 and ranged between 2.7 and 6.3 cm. The negative control inoculations showed no necrotic lesions. Three of the inoculated seedlings were plated on PDA in an effort to reisolate the inoculated fungus. *D.* ***corticola*** was reisolated from each and all had the same ITS sequence as *D.* ***corticola*** strain CBS 112074. To our knowledge, this is the first report of *D.* ***corticola*** causing cankers on *Q. virginiana* and the first report of the disease occurring in Florida. *D.* ***corticola*** has been reported to cause cankers and dieback in several *Quercus* spp. in Greece, Hungary, Italy, Morocco, Portugal, and Spain and has recently been reported to cause cankers on *Q. chrysolepis* and *Q. agrifolia* in California.

References: (1) A. Alves et al. Mycologia. 96:598, 2004. (2) N. L. Glass and G. C. Donaldson. Appl. Environ. Microbiol. 61:1323, 1995. (3) T. J. White et al. PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, 1990.

ERRATUM: A correction was made to this Disease Note on February 10, 2012. The mean lesion length of the inoculated seedlings was changed to 4.12 cm ± SE .45 and ranged between 2.7 and 6.3 cm.