Disease Note

Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Diaporthe phaseolorum* Causing Stem Canker of Hemp (*Cannabis sativa*)

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Hemp is an annual herbaceous plant that is used for its fiber and oil in a variety of commercial and industrial products. In Florida, it is currently being explored as a new specialty crop. During a field trial from October to January 2019 in Wimauma, FL, a stem canker was observed on up to 60% of 3-month-old plants of 'Eletta Campana', 'Carmagnola Selezionata', and 'Tygra'. Symptoms started on the main stems with light-to-dark brown lesions of different sizes and shapes. Over time, the lesions coalesced into large necrotic areas and bore pycnidia. Isolations were made from diseased stem tissues on general isolation medium (Amiri et al. 2018) after surface disinfection (Marin et al. 2020). The plates were placed in a growth chamber at 25°C under a 12-h/12-h photoperiod. A fungus with white, floccose, aerial mycelium and pycnidia producing alpha- and beta-conidia was consistently isolated. Three single-spore isolates were chosen for identification and pathogenicity tests. Pycnidia on potato dextrose agar were globose to irregular and ranged from 170 to 250 µm long (210 ± 2.5 µm, $n = 50$) and 140 to 220 µm wide (180 ± 2.7 µm, $n = 50$). The alpha-conidia were unicellular, hyaline, ellipsoidal to fusiform, and ranged from 5.3 to 7.7 µm long (6.5 ± 1.6 µm, $n = 50$) and 1.5 to 4.6 µm wide (2.8 ± 1.8 µm, $n = 50$). The beta-conidia were hyaline, elongated, filiform, straight or curved and ranged from 10.2 to 17.7 µm long (16.1 ± 2.2 µm, $n = 50$) and 0.5 to 1.8 µm wide (0.8 ± 0.2 µm, $n = 50$). Perithecia were not observed. Based on morphological features, the fungus was similar to anamorphs of *Diaporthe* spp. (Santos et al. 2011; Udayanga et al. 2015). DNA from the same three isolates was extracted using the FastDNA kit, and the ribosomal internal transcribed spacer (ITS), β -tubulin (TUB), and calmodulin (CAL) regions were amplified following the method of Udayanga et al. (2014) and were Sanger sequenced by Genewiz.

Sequences were deposited in GenBank (accession nos. MT497039 to MT497047 for ITS, TUB, and CAL). BLASTn searches revealed isolates 20-58, 20-59, and 20-60 were 96.34% identical to the epitype isolate *Diaporthe phaseolorum* AR4203 for ITS (KJ590738.1, 527 bp out of 547 bp), 100% for TUB (KJ610893.1, 459 bp out of 459 bp), and 100% for CAL (KJ612135.1, 522 bp out of 522 bp) (Udayanga et al. 2015). Their identity was confirmed by phylogenetic analyses using maximum likelihood and Bayesian inference methods. To complete Koch's postulates, pycnidia of the same three isolates were harvested and crushed in 2-ml Eppendorf tubes containing 0.01% Tween 20. Conidia suspensions were adjusted to 10^6 spores/ml. Three 5-week-old potted plants of Eletta Campana and Carmagnola Selezionata per isolate were inoculated using a 1-ml syringe with a needle by injecting 200 µl of the suspension into the stem. Plants were placed inside clear plastic bags for 48 h and maintained in the greenhouse. Control plants were injected with sterile deionized water and kept under the same conditions. The pathogenicity test was repeated once. Four weeks after inoculation, inoculated plants developed stem cankers, from which the same pathogen was isolated, whereas controls remained healthy. To our knowledge, this is the first report of *D. phaseolorum* causing stem canker on hemp. This pathogen has been reported causing canker on sunflower and *Phaseolus* spp. (Gomzhina and Gannibal 2018; Udayanga et al. 2015; Vrandečić et al. 2009). This discovery may help shape future research into disease epidemiology and management for a crop for which very limited disease information is available at the moment.

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The author(s) declare no conflict of interest.

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