Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Curvularia pseudobrachyspora* Causing Leaf Spot on Hemp (*Cannabis sativa*) in Florida

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Hemp (*Cannabis sativa* L.) is an emerging crop in Florida, with potential use in a variety of commercial and industrial products, including rope, textiles, bioplastics, and insulation. During a field trial in 2019 (October to December) in Wimauma, FL, leaf spots were observed on up to 70% of 1-month-old hemp plants on several varieties, such as 'Pumma-2', 'Eletta Campana', 'Carmagnola Selezionata', and 'Tygra', with up to 50% leaf damage in the field. Symptoms started on young and old leaves with small yellow spots that eventually turned tan to brown with a yellow halo. Pieces of diseased leaf tissue were surface sterilized with a 10% bleach solution for 90 s, rinsed twice with sterile deionized water, and then placed on general isolation medium (Forcelini et al. 2016). The plates were kept in a growth chamber at 25°C under a 12-h/12-h photoperiod. Fungal colonies with sparse aerial mycelium, fimbriate margins, and pale light gray zones or alternate gray olivaceous-to-brown zones on the surface were consistently isolated and single spored. Four isolates were selected for identification and pathogenicity tests. The conidia measured ($n = 25$) ranged from 20.3 to 31.7 (average = 26.4; SD = 2.8) μm long and from 9.5 to 15.9 (average = 12.9; SD = 1.3) μm wide and were borne in groups from the apex of geniculate conidiophores and mostly curved, ellipsoidal to obovoid, pale brown to brown with two to three distoseptate and middle cells larger and darker than the others. These morphological characteristics were similar to *Curvularia* spp. (Tan et al. 2018). DNA was extracted from the same four isolates using the FastDNA kit (MP Biomedicals, Solon, OH), and the ribosomal internal transcribed spacer (ITS) region, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and translation elongation factor 1- α (EF1- α) genes were amplified following the method by Tan et al. (2018), Sanger sequenced by Genewiz (South Plainfield, NJ), and deposited

in GenBank (accession nos. MT071994 and MT072019 to MT072021; MT085362 to MT085365; and MT085366 to MT085369 for ITS, GAPDH, and EF1- α , respectively). BLASTn searches revealed isolates 19-407, 19-409, and 19-410 were 99.6% identical to *Curvularia pseudobrachyspora* CPC 28808 (99.32% for ITS, 99.58% for GAPDH, and 99.88% for EF1- α), whereas isolate 19-405 had 98.53 to 99.4% similarity to the same species. Phylogenetic analyses using maximum likelihood and Bayesian inference differentiated isolate 19-405 from the other three isolates, suggesting the possibility of a cryptic species. To fulfill Koch's postulates, spores of the same four isolates were harvested in 0.01% Tween 20, and the suspensions were adjusted to 10^5 spores/ml. Four 5-week-old potted plants of 'Puma-3' per isolate were inoculated by spraying the spore suspension to runoff. Plants were kept inside a plastic bag for 72 h and maintained in the greenhouse. Control plants were sprayed with sterile deionized water and kept under the same conditions. The pathogenicity test was repeated once. Two weeks after inoculation, controls remained healthy, whereas all inoculated plants had leaf spots as described above, and the pathogen was reisolated from symptomatic tissue. To our knowledge, this is the first report of *C. pseudobrachyspora* causing leaf spot on hemp. *C. pseudobrachyspora* has been reported causing leaf spot on areca palm (*Areca catechu*) in China (Wang et al. 2019) and on goosegrass (*Eleusine indica*) in Thailand (Marin-Felix et al. 2017). Goosegrass is a common annual weed found throughout Florida (Buker et al. 2002) and might serve as an alternative host in the absence of hemp. More studies are needed to understand the epidemiology of this disease and foster disease management programs in Florida.

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