Diseases Caused by Fungi and Fungus-Like Organisms

Colletotrichum fioriniae Infecting Invasive Japanese Hop (*Humulus scandens*) in the United States

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Funding: Funding was provided by Agricultural Research Service (Project 8044-22000-047-00D). Plant Dis. 106:330, 2022; published online as <https://doi.org/10.1094/PDIS-06-21-1191-PDN>. Accepted for publication 14 July 2021.

Japanese hop (*Humulus scandens*) is a nonnative, invasive plant that colonizes disturbed riparian areas throughout the eastern United States and Canada, forming dense, monocultural stands that displace native plant communities due to a high reproductive rate, rapid growth, climbing vines, and dense shading (Balogh and Dancza 2008). It is capable of serving as a reservoir for agronomically important plant pathogens, such as the *Tomato spotted wilt virus* and powdery mildew species that infect commercial hemp and hop fields (Weldon et al. 2020; Yoon et al. 2018). In the spring of 2016, diseased populations of *H. scandens* were observed along the Monocacy River in Frederick County, Maryland, with severe chlorotic and necrotic leaf lesions. Symptomatic leaves were surface sterilized and placed in moist chambers at 25°C for sporulation. Sporulating acervuli, lacking setae, developed on irregular, tan necrotic leaf lesions following 7 to 12 days in a moist chamber. Conidia were hyaline, aseptate, smooth-walled, fusiform to cylindrical with both ends acute. Conidia measured ($n = 100$) (length $\times$ width; average $[\pm \text{std. err.}]$, range): 12.42 (± 0.10) μm , 8.41 to 14.48 μm ; $\times$ 3.91 (± 0.03) μm , 3.03 to 4.91 μm . Monoconidial fungal cultures were obtained by transferring conidia with a sterile glass needle to acidified potato dextrose agar and incubating at 25°C for 2 to 3 days. Based on phenotypic characteristics and conidial morphology and size, the pathogen appeared to belong to the *Colletotrichum acutatum* complex (Damm et al. 2012). Therefore, six loci (ITS, *GAPDH*, *CHS1*, *HIS3*, *ACT*, and *TUB2*) were amplified and sequenced from the representative isolate, 16-008, for species characterization (GenBank accessions MW023070 to MW023075) (Damm et al. 2012). For the ITS region and *ACT*, *GAPDH*, and *CHS1* loci, isolate 16-008 was 100%

identical to *C. fioriniae* and shared 99% similarity to *TUB2* and *HIS3* for multiple accessions of *C. fioriniae* in GenBank. Gene sequences were aligned, trimmed, concatenated, and analyzed against 32 reference strains within the *C. acutatum* complex (Damm et al. 2012). Concatenated loci were used to generate a maximum likelihood phylogeny using W-IQ-TREE (Trifinopoulos et al. 2016). Results from the phylogenetic analysis demonstrated that isolate 16-008 was most genetically similar to *C. fioriniae* with a bootstrap support of 100%. Based on phenotypic and sequence analyses, isolate 16-008 was identified as *C. fioriniae*. *H. scandens* seedlings from Maryland ($n = 3$) were inoculated with a conidial suspension (10^7 conidia/ml) with 0.125% Tween 20 and applied with an atomizer until runoff. Inoculated plants were placed in a dew chamber at 25°C for 2 days. Experimental plants were distributed in a mist tent at 25°C with 14 h of light and monitored for 2 weeks. Negative control plants ($n = 2$) were sprayed with a sterile 0.125% Tween 20 water solution. All inoculated plants were symptomatic by 12 days postinoculation. No symptoms were observed on the mock-inoculated plants. Symptoms were identical to diseased field samples. Inoculations were repeated with the same results. *C. fioriniae* was reisolated and confirmed from excised leaf lesions via ITS and *ACT* sequencing. To our knowledge, this is the first report of *C. fioriniae* naturally infecting *H. scandens* within the United States (Farr and Rossman 2020). Future studies will evaluate the host range of this isolate due to the species' broad host range and the weed's extensive distribution.

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The author(s) declare no conflict of interest.

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Keywords: invasive plant, *Colletotrichum fioriniae*, *Humulus japonicus*

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