Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Colletotrichum nymphaeae* Causing Anthracnose on Almond in Hungary

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Funding: Funding was provided by Hungarian Ministry of Agriculture (AM BGMF / 180-1/2021). Plant Dis. 106:1527, 2022; published online as <https://doi.org/10.1094/PDIS-08-21-1847-PDN>. Accepted for publication 22 October 2021.

Almond (*Prunus dulcis* [Mill.] D. A. Webb) is cultivated in commercial orchards in southwestern Hungary and in numerous backyard orchards predominately in the Buda Hills and central Hungary. In July 2019, anthracnose symptoms and necrotic twigs were observed across almond genotypes in a meadow orchard of Óbuda and in the gene bank collection of the Hungarian University of Agriculture and Life Sciences. Fruits of some genotypes were damaged 100%, but others were less damaged or asymptomatic. Orange, slightly sunken lesions on fruits produced gum. Near the diseased fruits young shoots shriveled, stalks became necrotized, and necrosis developed on twigs. Isolates obtained from orange conidial masses from epicarp, necrotized tissues from twigs and stalks were grown on PDA for 7 days at 25°C in the dark. Upper surfaces of the colonies were white to pale gray, black solid mycelial structures were formed, and the reverse side varied from white to salmon. Acervuli were not formed, but conidia were produced from hyphae. Conidia were unicellular, hyaline, smooth-walled, cylindrical, predominant with both ends rounded or one end acute. Morphometric measurements of conidia (mean $\pm$ SD) were $18.0 \pm 2.2 \times 4.7 \pm 0.6$ μ m ($n = 100$). The isolates were morphologically identified as *Colletotrichum acutatum* sensu lato (Damm et al. 2012). Sexual morphs were not observed. Three monosporic isolates were used for molecular identification. Partial nucleotide sequences were amplified from three loci, internal transcribed spacer (ITS), β -tubulin (*TUB2*), and calmodulin (*CAL*), after White et al. (1990), Glass and Donaldson (1995), and O'Donnell et al. (2000), respectively. The ITS sequences (GenBank accessions MW425388 to MW425390) of the three isolates revealed that they belong to the *C. acutatum* species complex, while BLAST results showed that the *TUB2* sequences (GenBank

accessions MW428285 to MW428287) had 99.3% identity with *C. nymphaeae* strain CBS515.78, and the *CAL* sequences (GenBank accessions MW428288 to MW428290) had 100% identity with *C. nymphaeae* strain FREC138. The phylogenetic tree containing all the valid species of *C. acutatum* species complex confirmed that the isolates clustered to *C. nymphaeae* with high bootstrap support. The fungus was identified as *C. nymphaeae* (Pass.) Aa based on molecular biological evidence. In vivo pathogenicity tests were conducted on 10 healthy fruits and 10 twigs by inserting mycelial agar plugs (5 mm in diameter) onto wounded pericarp and phloem tissues, which were then wrapped in wet cotton and Parafilm. The control treatments received sterile PDA discs. After 15 days, necrotic lesions 12 to 19 mm in diameter developed on fruit and 9 to 18 mm on twigs. Control fruits and twigs were asymptomatic. Koch's postulates were fulfilled with the reisolation of the pathogen from symptomatic tissues. The ITS, *ACT*, and *CAL* sequences of the reisolated *Colletotrichum* were determined and found identical to the original isolates. Anthracnose symptoms are known on almond fruits in several almond growing regions around the world caused by *C. acutatum*, *C. godetiae*, *C. fioriniae*, *C. simmondsii*, and *C. gloeosporioides* (Adaskaveg et al. 1997; de Silva et al. 2021; López-Moral et al. 2020; Shabi et al. 1983). To our knowledge, this is the first report of *Colletotrichum nymphaeae* causing anthracnose of almond anywhere in the world.

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The author(s) declare no conflict of interest.

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Keywords: almond, almond anthracnose, *Colletotrichum acutatum* species complex, *Colletotrichum nymphaeae*, diseases of almond, diseases of nuts, fungal diseases of almond, *Prunus*

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