Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Colletotrichum godetiae* Causing Anthracnose and Twig Blight on Persian Walnut in Hungary

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Persian walnut (*Juglans regia* L.) fruit with preharvest anthracnose symptoms, necrotic fruit stalks, and twigs with necrotic buds, and apical necrosis were collected in a Hungarian orchard next to Nágocs in September 2018. Disease incidence was approximately 15% on a Hungarian-bred walnut cultivar, 'Milotai 10'. Similar symptoms were found on Persian walnut in other locations (e.g., Milota, Érd, Sarród, and Kocs). Acervuli were observed on necrotic lesions on fruit and twigs with pale orange conidial masses. Conidia were hyaline, unicellular, and fusiform. Morphometric measurements of conidia showed mean length $\pm$ SD $\times$ width $\pm$ SD = $15.9 \pm 1.7 \times 4.5 \pm 0.4$ μ m, with a length/width ratio 1:0.3 ($n = 100$). The fungus was isolated from conidial masses on potato dextrose agar (PDA) medium amended with chloramphenicol (25 mg/liter). A total of 12 isolates were obtained as pure cultures by single-spore isolations and incubated at 23°C in the dark for 10 days. The colonies were white to gray or grayish-orange on the upper side and with black spots on the reverse side. The isolates showed morphological characteristics of *Colletotrichum acutatum* in sensu lato (Jayawardena et al. 2016). Molecular analyses were conducted to identify the exact species. The internal transcribed spacer (ITS) region, actin (ACT), and calmodulin (CAL) partial genes were amplified by ITS1F/ITS4R, ACT512F/ACT783R, and CAL1/CAL2 primers (Carbone and Kohn 1999; O'Donnell et al. 2000; White et al. 1990). The sequences of ITS region (GenBank accession nos. MK367398 to 99, MK367401 to 02) showed 100% identity with *Colletotrichum godetiae* sequence. ACT gene sequences (GenBank accession nos. MK415991 to 92, MK415994 to 95) showed 100% identity with the deposited *C. godetiae* type strains from walnut. The obtained sequences of CAL gene (GenBank accession nos. MK415998 to 99,

MK416001 to 02) were the same and showed 100% identity with other *C. godetiae* sequences from other host plants. The fungus was identified as *C. godetiae* Neerg. Pathogenicity tests were accomplished in the field and under laboratory conditions (25°C on thermostat) on 10 green walnut fruit and 10 walnut twigs each (cv. Milotai 10). Tests were conducted on living trees, collected fruit, and 2-year-old twigs by inserting mycelial agar plugs (5 mm in diameter) onto wounded pericarp tissues, which were then wrapped with wet cotton and Parafilm. Wounded tissues on five fruit and five 2-year-old twigs were treated with noncolonized PDA plugs as noninoculated controls. After 14 days, necrotic lesions 9 to 17 mm in diameter developed on fruit on living trees. Necrosis (length of 12 to 17 mm and width of 7 to 12 mm) was measured on phloem of walnut twigs, and almost two times larger in cambium. No necrosis developed around control wounds. Koch's postulates were fulfilled with the reisolation of the pathogen from symptomatic tissues, and isolates were identical morphologically and by sequence analysis of ITS region, ACT, and CAL partial genes to the original isolates. Damm et al. (2012) described two *C. godetiae* strains associated with walnut, one isolated in Austria and another one of unknown origin. An epidemic event of walnut anthracnose caused by *Colletotrichum* species, mainly *C. godetiae*, was reported in France (Da Lio et al. 2018). The pathogen was isolated from nuts, buds, insects, and stems. To our knowledge, this is the first report of anthracnose of walnut fruit caused by *C. godetiae* in Hungary. Anthracnose caused by *C. godetiae*, and previously reported *C. fioriniae* (Varjas et al. 2019), is becoming an increasing preharvest problem on Persian walnut in Hungary.

References:

- Carbone, I., and Kohn, L. M. 1999. Mycologia 91:553.
- Da Lio, D. et al. 2018. Sci. Rep. 8:10765.
- Damm, U., et al. 2012. Stud. Mycol. 73:37.
- Jayawardena, R. S., et al. 2016. Micosphere 7:1194.
- O'Donnell, K., et al. 2000. Mycoscience 41:61.
- Varjas, V., et al. 2019. Plant Dis. 103:2964.
- White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.

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