Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Colletotrichum fioriniae* and *C. nymphaeae* as Causal Agents of Anthracnose on Walnut in Italy

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English walnut (*Juglans regia* L.) is a species grown for either high quality wood or fruit production. In Italy walnut cultivation occupies an area of about 4,600 ha (<http://www.fao.org/faostat>). In 2019 to 2020, walnut fruits (cv. Lara) with anthracnose symptoms were collected from walnut orchards in the Province of Venice (Northern Italy). Affected fruits showed necrotic and circular lesions with acervuli in the center, causing the complete mummification of the fruit as described by Da Lio et al. (2018). Orange conidial masses appeared under wet conditions. The fungus was isolated from necrotic tissues, and conidial masses were put on potato dextrose agar (PDA) medium. Plates were incubated at 25°C for 5 to 7 days. The colonies were white to pink on the upper side and pink with black spots on the reverse. Acervuli formed and produced conidial masses on PDA after 6 days. Culture and conidial morphology were in concordance with published descriptions of *C. acutatum* sensu lato (Damm et al. 2012). To confirm the identity, internal transcribed spacers (ITS), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), actin (ACT), and beta-tubulin (TUB2) genes were amplified and sequenced using the primer pairs ITS1/ITS4 (White et al. 1990), GDF1/GDR1 (Guerber et al. 2003), ACT512F/ACT783R, and BT2Fd/BT4R (Da Lio et al. 2018). The isolates belonged to two different species of *Colletotrichum acutatum* complex: *C. fioriniae* (Marcelino & Gouli) and *C. nymphaeae* (Pass). Sequences of two representative isolates, *C. fioriniae* CREADC-F2317 and *C. nymphaeae* CREADC-F2372, were deposited in GenBank with accession numbers MZ153170 and MZ191794 (ITS), MZ203522 and MZ224013 (GAPDH), MZ203521 and MZ224012 (ACT), and MZ203523 and MZ224014 (TUB2). For all the genes, isolates had a 100% similarity to multiple *C. fioriniae* and *C. nymphaeae* accessions, respectively. Maximum likelihood trees based on

concatenated sequences of the four genes were constructed using MEGA 6.0 (Tamura et al. 2013). The phylogenetic analysis grouped the isolates in the *C. fioriniae* and *C. nymphaeae* clusters, respectively. The two isolates CREADC-F2317 and CREADC-F2372 were used to confirm pathogenicity on walnut fruits. Fruits of cultivar Lara were surface disinfected by dipping in 3% NaOCl for 1 min, rinsed in sterile distilled water, and arranged in sterile humid chambers. Fruits were wounded with a sterile needle and then inoculated with 20 µl of 10⁶ conidia/ml suspension of each isolate (one wound per fruit). Fruit treated with sterile distilled water served as a control. Inoculations were conducted on three fruits per replicate and three replicates per treatment arranged in a complete randomized block design. After 7 days of incubation at 25 ± 1°C, all the inoculated fruits showed typical anthracnose symptoms and lesions with cream to salmon pink acervuli, whereas the controls remained healthy. The species *C. nymphaeae* and *C. fioriniae* were reisolated from the rotted fruit. Pathogenicity tests were repeated twice with the same results. The morphology of the reisolated fungi was consistent with the inoculated one, fulfilling Koch's postulates. The species *C. fioriniae* and *C. nymphaeae* have been described affecting numerous species worldwide (Damm et al. 2012). *C. fioriniae* and *C. nymphaeae* have been previously reported to cause severe anthracnose on walnut: *C. fioriniae* in France (Da Lio et al. 2018) and Hungary (Varjas et al. 2019), and *C. nymphaeae* in France (Da Lio et al. 2018) and Brazil (Savian et al. 2019). To our knowledge, this is the first report of *C. fioriniae* and *C. nymphaeae* as causal agents of walnut anthracnose in Italy.

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