Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Nut Rot Caused by *Neofusicoccum parvum* on Hazelnut (*Corylus avellana*) in Italy

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Hazelnut (*Corylus avellana* L.), which is native to Europe and Western Asia, is a widely distributed and economically important crop in Italy; it is cultivated on 82,104 ha and its production is 110,618 t (ISTAT 2021). A total of 111 black rotted nuts (incidence: 41%) with sunken lesions from Lu and Cuccaro (45°00'21.8"N 8°28'59.6"E), northwestern Italy were collected during the ripening stage of hazelnuts from October to December 2020. Symptomatic half-cut kernels were sterilized in 1% NaClO for 1 min, washed in sterile water twice, and dried on sterile filter paper. The fragments were placed onto potato dextrose agar (PDA) containing streptomycin. After 48 to 72 h of incubation at 25°C, fast-growing white colonies with abundant aerial mycelia were observed. On the opposite side of the plates, colonies were initially white and then turned to dark-grayish olive after one week of incubation. Dark colonies produced globose, hyaline, ellipsoidal, and unicellular conidia ranging from 12.23 to 15 × 5 to 6.71 µm. Morphologically, the causal agent was identified as *Neofusicoccum parvum* (Pennycuik & Samuels) Crous, Slippers & A.J.L. Phillips (Crous et al. 2006; Zhang et al. 2021). The DNA from the isolates HMa-19-2 and Hwb-4b-2 was extracted with the E.Z.N.A. Fungal DNA mini kit (Omega Bio-Tek) according to manufacturer instructions. Molecular identification was confirmed by sequencing the rDNA internal transcribed spacer (ITS) using primers ITS1/ITS4 (White et al. 1990) and the translation elongation factor 1- α (*tef-1 α*) gene using primers EF1-728F/EF1-986R (Carbone and Kohn 1999). The sequences of both

isolates were deposited in GenBank for the ITS (accession nos. MZ848132 and MZ848133) and the *tef-1 α* gene (accession nos. MZ913266 and MZ913267). BLAST analysis showed 99% identity with the ex-type strain of *N. parvum* (CMW9081) for ITS and *tef-1 α* . The maximum likelihood method based on combined sequences of ITS and *tef-1 α* genes was performed, and the isolates of *N. parvum* clustered with the ex-type strain of *N. parvum* (CMW9081). The pathogenicity of both isolates was tested on ripening hazelnuts (BBCH: 85) to evaluate Koch's postulates. Three nuts per isolate and per three replicates were surface disinfected with 1% NaClO. A piece of shell (5 mm in diameter) from nuts was removed with a sterile cork borer, and nuts were inoculated with PDA mycelium plugs of the same diameter cut from 7-day-old PDA colonies (Seddaiu et al. 2021). The control nuts were treated with sterilized PDA plugs. All inoculated nuts produced black lesions with softening pulp. Additionally, abundant white-gray mycelium developed on the inoculation sites. Control nuts showed no symptoms. *N. parvum* was recently reported in Italy on chestnut (Seddaiu et al. 2021) and blueberry (Guarnaccia et al. 2021). The fungal pathogen was also reported as an agent of gray necrosis of hazelnut in Chile (Duran et al. 2020). However, this is the first report of *N. parvum* on hazelnut in Italy. The findings suggest that *N. parvum* could severely affect hazelnut production in Italy. Accurate identification of the pathogen will support growers in managing the disease.

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