Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Neofusicoccum parvum* Associated with Chestnut Nut Rot in Italy

S. Seddaiu,^{1,†} A. Mello,² C. Sechi,¹ A. Cerboneschi,¹ and B. T. Linaldeddu³

¹ Settore Difesa delle Piante Forestali - Servizio della Ricerca per la Sughericoltura e la Selvicoltura, Agris Sardegna, 07029 Tempio Pausania, Italy

² Institute for Sustainable Plant Protection, SS Torino - CNR, 10125 Torino, Italy

³ Dipartimento Territorio e Sistemi Agro-Forestali (TeSAF), Università di Padova, 35020 Legnaro PD, Italy

Plant Dis. 105:3743, 2021; published online as <https://doi.org/10.1094/PDIS-01-21-0072-PDN>. Accepted for publication 26 May 2021.

In autumn 2018, during a study on the pathogens involved in the etiology of chestnut nut rot symptoms observed in three of the main sweet chestnut (*Castanea sativa*) growing areas in Sardinia (site 1: 39°56'55"N, 09°11'45"E; site 2: 39°58'20"N, 09°09'41"E; site 3: 40°52'50"N, 09°08'45"E), *Gnomoniopsis smithogilvyi* was found to be the main causal agent. In addition to *G. smithogilvyi*, 15 out of 450 nuts processed, yielded on potato dextrose agar (PDA, 39 g/liter) at 22°C, white colonies with dense aerial mycelium becoming dark gray after 4 to 7 days. Pycnidia were produced within 4 weeks on half-strength PDA incubated at room temperature under natural daylight. The hyaline, ellipsoid to fusiform, and aseptate conidia measured 13.4 to 19.2 × 4.8 to 7.7 µm (*n* = 50). All morphological characters matched those reported for *Neofusicoccum parvum* by Phillips et al. (2013). Identity of isolates was confirmed by DNA sequence analysis of the internal transcribed spacer region (ITS) and part of the translation elongation factor 1-alpha gene (*tefl-α*). DNA extraction, PCR amplification reactions, and DNA sequencing were carried out according to Linaldeddu et al. (2016). In the phylogenetic analysis based

on combined ITS and *tefl-α* gene sequences, the *N. parvum* isolates clustered within two well-supported subclades. In the first subclade (ML bootstrap = 88%), three isolates clustered together with the ex-type culture of *N. parvum* (CMW9081) while in the second subclade (ML bootstrap = 95%), three isolates clustered together with the ex-type culture of *N. algeriense* (CBS 137504), a species recently synonymized with *N. parvum* by Lopes et al. (2017). Sequences of six representative isolates were deposited in GenBank (MK968559–MK968564 and MT010339–MT010344 for ITS and *tefl-α*, respectively). The pathogenicity of six isolates, belonging to the two haplotypes, was undertaken by inoculating five asymptomatic nuts per isolate. After disinfecting the nut surface with 70% ethanol and removing a piece of shell (5 mm diameter) with a sterile cork borer, the nuts were inoculated with a same-sized agar-mycelium plug cut from the margin of a 5-day-old PDA colony. Ten control nuts were inoculated with sterile PDA plugs applied as described above. Inoculated nuts were kept in a thermostat at 22°C in the dark for 18 days. All nuts inoculated with *N. parvum* showed light brown to dark necrosis of the kernel associated with loss of tissue consistency. The symptoms were congruent with those observed in nature. All *N. parvum* isolates were successfully reisolated from all the inoculated nuts, fulfilling Koch's postulates. No lesions were observed on controls. *N. parvum* is recognized as an emerging plant pathogen worldwide. In particular, several studies report *N. parvum* as a growing threat to agricultural and forest ecosystems in the Mediterranean area (Larignon et al. 2015; Manca et al. 2020). This is the first report of *N. parvum* causing chestnut nut rot in Italy.

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The author(s) declare no conflict of interest.

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Keywords: biological invasion, fungi, forest trees

[†]Indicates the corresponding author.
S. Seddaiu; saseddaiu@agrisricerca.it