Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Neofusicoccum parvum* Causing Canker on *Castanea sativa* in Spain

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In April 2021, depressed bark with dark-reddish coloration was observed on the stem of a 5-year-old chestnut (*Castanea sativa* Mill.) plant, acquired from a commercial Galician nursery. One tissue sample was collected from the injury of this plant, surface sterilized with 96% ethanol for 30 s, dried on sterilized tissue paper, plated on potato dextrose agar (PDA), and incubated at 25°C. Fungal colonies were consistently isolated and, after 5 days, developed abundant greyish-white aerial mycelium. Two weeks later, pycnidia with fusiform conidia were observed. For molecular identification, internal transcribed spacer (ITS) (ITS1: TCCGTAGGTGAACCTGCGG and ITS4: TCCTCCGCTTATTGATATGC, White et al. 1990), β -tubulin (BT) (BT2a: GGTAACCAAATCGGTGCTGCTTC and BT2b: ACCCTCAGTGTAGT GACCCTTGGC, Glass and Donaldson 1995), and elongation factor (EF) (EF1-728F: CATCGAGAAGTTCGAGAAGG and EF1-1199R: GGGAAG TACCMGTGATCATGT, Walker et al. 2010) were amplified. BLAST analysis revealed that the ITS sequence of isolate LPPAF-971 (accession no. MZ314849) showed 99.63% identity with *Neofusicoccum parvum* isolates ACBA15 (accession no. KX244803) and mywxxq (accession no. MW767713), and 99.4% identity with isolate CMW9081[†] (accession no. AY230943). The BT sequence (accession no. MZ561053) showed 100% identity with isolate GDTCMF1 (accession no. MN022786), and the EF sequence (accession no. MZ561054) showed 98.70% identity with isolate F7 (accession no. MN461166), all

corresponding with *N. parvum*. Pathogenicity tests were carried out on 10 5-year-old chestnut plants on which a 5-mm PDA plug from the edge of an actively growing colony of the fungus was inoculated by a cut in the bark of 1 to 3 branches per plant up to a total of 16 inoculated branches and then wrapped with Parafilm. Five plants inoculated with one plug of PDA without the fungus were used as controls. Plants were placed in a plastic tunnel with a lateral insect net, provided with drip irrigation, and grown under natural conditions. Bark canker symptoms similar to the one observed in the original sample were visible on all inoculated chestnut plants 10 days after inoculation. No symptoms were observed on the controls. The assay was conducted twice. Fungal colonies morphologically identified as *N. parvum* were reisolated from bark cankers on inoculated chestnut plants, fulfilling Koch's postulates. *C. sativa* is a widely distributed multipurpose tree, with important economic, environmental, cultural, and heritage functions (Beccaro et al. 2019), which underlines the importance of this finding with a view to its sanitary control. In addition, *N. parvum*, teleomorph *Botryosphaeria parva* (Pennycook & Samuels) Crous, Slippers & Phillips (Crous et al. 2006), is the causal agent of cankers and die-back in many crops and trees (Phillips et al. 2013) worldwide but, until now, it had not been detected in *C. sativa*. To the best of our knowledge, this is the first report worldwide of *N. parvum* causing disease on chestnut in Spain.

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