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NEW DISEASE REPORT

First report of *Neofusicoccum parvum* causing canker and dieback on *Alnus glutinosa* in Portugal

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In March 2022, sunken cankers were observed on the stem and branches of young and mature *Alnus glutinosa* trees in central Portugal (Canelas, 40°42'17''N; 8°33'59''W). Cankers were characterised by necrotic lesions on the outer and inner bark that progressively girdled the circumference of stem and branches causing extensive canopy dieback (Figure 1). The incidence of the disease was estimated at 70% (linear plot of 50 m long). To isolate the causal agent, 20 samples including twigs, branches and stems were collected from seven diseased trees chosen at random along the transect. Isolations were performed from ten chips (5 mm^2) of inner bark and xylem tissues cut aseptically from the margin of the necrotic lesions and transferred onto Petri dishes containing potato dextrose agar (PDA).



FIGURE 1 Diseased *Alnus glutinosa* (a), sunken cankers on twigs and branches (b,c), inner bark necrotic lesions (d).

Isolations yielded twenty fungal colonies showing a greyish-white aerial mycelium typical of *Botryosphaeriaceae* species. Based on colony appearance and morphology of conidia (hyaline, fusiform, aseptate and thin-walled) produced after 15 days on sterile *Fraxinus ornus* twigs on PDA at 25°C, isolates were identified as *Neofusicoccum parvum* (Figure 2). The identity was confirmed by sequencing the internal transcribed spacer region of two representative isolates, using the universal primers ITS1 and ITS4. DNA extraction, PCR amplification and

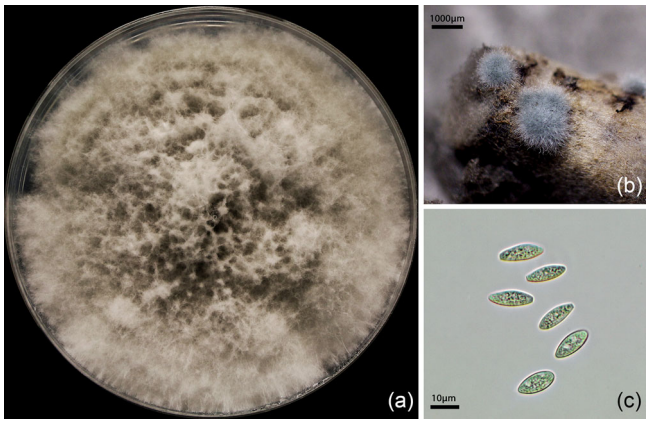


FIGURE 2 Colony morphology of *Neofusicoccum parvum* after seven days on potato dextrose agar at 25°C in the dark (a), pycnidia formed on sterile *Fraxinus ornus* twigs on potato dextrose agar (b), and hyaline and aseptate conidia (c).

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FIGURE 3 Symptoms observed on common alder seedlings 30 days after inoculation with *Neofusicoccum parvum*: wilting (a), outer bark discoloration (b), and inner bark necrotic lesions (c); asymptomatic control seedlings (d-f).

sequencing were performed according to Linaldeddu *et al.* (2020). A BLASTn search showed 100% identity with sequence AY236943 of the ex-type cultures of *N. parvum* (CMW9081). The ITS sequence of a representative isolate (CBP109) was deposited in GenBank (Accession No. OQ547268).

Pathogenicity tests were performed by inoculating an agar mycelium plug (3 mm diameter) cut from the margin of an active colony (isolate CBP109) growing on PDA onto the wounded stem of two-year-old common alder seedlings using the methodology reported by Linaldeddu *et al.* (2020). Ten seedlings were inoculated with the fungus and ten, used as controls, with a sterile PDA plug. Seedlings were kept in controlled conditions at 21°C and watered regularly for 30 days. At the end of the experimental period, all seedlings showed necrotic lesions similar to those observed in the field; six out of ten seedlings also showed wilting symptoms (Figure 3). Average lesion size was 38 ±14 mm (mean ±SD). Control plants remained asymptomatic. *Neofusicoccum parvum* was successfully re-isolated from all inoculated seedlings, thus fulfilling Koch's postulates.

This study represents the first report of *N. parvum* affecting *Alnus glutinosa* in Portugal and in Europe. *Neofusicoccum parvum* is a plurivorous pathogen reported on about 300 plant hosts (Phillips *et al.*, 2013; Batista *et al.*, 2021). In Portugal *N. parvum* has been reported in both forest and urban environments and its pathogenicity confirmed on different hosts such as pine and oak trees (Lopes *et al.*, 2016; Batista *et al.*, 2020). The occurrence of this invasive species on declining *Alnus glutinosa* trees and the recent discovery of several *Phytophthora* species in the same sites (Bregant *et al.*, 2023), could explain the rapid devastation of extensive alder ecosystems observed recently in Portugal.

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