New Disease Reports

First report of fungal complex causing grey necrosis of hazelnut in Chile

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During the productive stage of *Corylus avellana* (hazelnut) in La Araucanía, Chile (October-December 2018-19) we observed symptoms consisting of a brown-greyish spot occurring at the bottom of the nut and enlarging upwards to the apex, and affecting around 30% of plants, mainly cv. Barcelona (Figs. 1-2). In severe infections the bracts were also affected. Symptoms were consistent with grey necrosis reported in hazelnut in Italy, and caused by a fungal complex formed mainly by *Alternaria* spp., *Diaporthe* spp.and *Fusarium* spp. (Belisario *et al*., 2009).

To isolate causal microorganisms from infected plants (35 plants, cv. Barcelona), small pieces of kernels and bracts were cut from the margin between diseased and healthy tissues and were observed by variable pressure scanning electron microscopy (Fig. 3). They were disinfected by immersion in 80% ethanol for 5 mins, followed by sodium hypochlorite (40 g/l) for 20 s, and rinsed 3 times with sterile distilled water. They were then placed onto potato dextrose agar (PDA) media (4 g potato extract, 20 g dextrose and 15 g agar per litre of sterile distilled water). Samples were maintained in the dark for five days. Thirty-five fungi isolated from infected tissues were transferred to PDA.

Molecular identification was based on the sequencing of the ribosomal internal transcribed spacer 2 region (ITS2) amplified using primers fITS9 (5′-GAACGCAGCRAAIIGYG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (Duran *et al*., 2017). PCR products were purified and sequenced by Macrogen Inc. (Korea). BLAST analysis limited to type material showed 99.5-100% identities with five species: *Fusarium sporotrichioides* (GenBank Accession Nos. MF629821 and MF629827), *Alternaria alternata* (MF629825), *Diaporthe* sp. (MF629820 and MF629822) and its anamorph *Phomopsis* sp. (MF629824), and *Neofusicoccum* sp. (MF629823). Isolates were deposited in the Chilean Culture Collection of Type Strains with the codes: CCCT19.169 and CCCT19.170 (*F. sporotrichioides*); CCCT19.171 (*A. alternata*); CCCT19.172 and CCCT19.167 (*Diaporthe* sp.); CCCT19.173 (*Phomopsis* sp.); and CCCT19.166 (*Neofusicoccum* sp.).

To test Koch's postulates, pathogenicity tests were performed on healthy hazelnut plants (one year old) in laboratory conditions at 25°C with natural daylight. Each fungus was first grown on PDA for seven days at 25°C. A 5 mm diameter disc was cut from the leading edge of the colony and placed separately, as well as together with other isolates, on individual, intact plant stems and wrapped in plastic film. For control plants, PDA without fungal growth was used. After one month disease symptoms were visible, while no symptoms appeared on the controls (Fig. 4). Fungi were consistently re-isolated from the plants onto PDA. We noted that *Diaporthe* sp. showed the highest disease incidence in the early stages. This was in contrast to Battilani *et al*. (2018) who found a greater incidence in ripened nuts. This could be attributed to a variation in parameters such as rainfall which may affect dissemination and infection levels of *Diaporthe* sp. (Battilani *et al*., 2018). *Alternaria* sp. showed the highest disease incidence in the latter stages, which was in accordance with Belisario & Santori (2009) who defined this fungus as a secondary coloniser.

To our knowledge, this is the first report of grey necrosis on hazelnut from Chile and control strategies are urgently required due to high economic losses reported in Europe (i.e. 30% in Italy). In addition *F. sporotrichioides* and *A. alternata* are highly active mycromycetes and are associated with mycotoxin production which is a human health risk (Lugauskas & Stakènienè, 2002).

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Figure 1

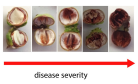

Figure 2

Figure 3

Figure 4

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