



First report of nut rot caused by *Gnomoniopsis castaneae* on *Castanea sativa* in Greece

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In October 2015, a fruit rot disease was observed in Pieria (Northern Greece) causing losses ranging from 20 to 30%. Internal symptoms of diseased fruits, collected from European chestnut (*Castanea sativa* Mill.) orchards, consisted of a brown discoloration beginning from the margin of the endosperm, which lost its consistency resulting in a white, chalky or sponge-like appearance. Nuts were surface disinfected in 1% sodium hypochlorite for 10 min. The outer shell was removed with a sterile scalpel and pieces of infected tissue were plated onto malt extract agar (MEA) and incubated in the dark at 25 °C for one week. Single spore cultures gave rise to white colonies which turned to greyish brown with concentric rings of orange and creamy drops which contained one-celled, hyaline, ovoid to oblong conidia measuring 5.2–7.7 × 2.0–3.8 µm (average 6.8 × 2.7 µm). Abundant acervuli produced on all cultures were dark brown to black in colour, subglobose to globose measuring 125–325 × 118–276 µm (average 208 × 185 µm). Symptoms as well as morphological characteristics of the fungus tally with those of *Gnomoniopsis castaneae* G. Tamietti (Visentin et al. 2012). The ITS1–5.8S-ITS2 region was amplified with primers ITS4/ITS5 while partial amplification of beta-tubulin (Bt) and elongation factor 1-alpha (EF1) was performed with Bt2a/Bt2b and EF1-728F/EF1-1199R primers, respectively. The amplicons were sequenced [GenBank Accession Nos. MH107826–MH107830 (ITS), MH213477–213481 (Bt), MH213482–213486 (EF1)] and BLAST search revealed 99–100% identity with the ex-type sequences of *G. castaneae* isolates (HM142946 for ITS, KR072532 for Bt and JQ791198 for EF1). Wounded nuts

were inoculated with MEA plugs with actively growing fungal mycelia and inoculation points covered with Parafilm. Three weeks post-inoculation, fruits showed symptoms of brown rot, while controls inoculated with MEA disks remained symptomless. *G. castaneae* was consistently reisolated from inoculated nuts. *G. castaneae* is a nut pathogen and also behaves as endophyte and saprobe of *C. sativa* (Visentin et al. 2012). The two species, described independently, *Gnomoniopsis smithogilvyi* L.A. Shuttleworth, E.C.Y. Liew & D.I. Guest and *G. castaneae*, have been proven to be the same species (Shuttleworth et al. 2015). Brown rot caused by *G. castaneae* has been reported on *C. sativa* nuts in Australia, France, Italy, New Zealand and Switzerland (Farr and Rossman 2018). However, to our knowledge this is the first report of this pathogen as causal agent of nut rot on *C. sativa* in Greece.

References

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