

NEW DISEASE REPORT

Gnomoniopsis smithogilvyi, the causal agent of chestnut brown rot reported from Portugal

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KEYWORDS

Castanea sativa, decayed nuts, *Gnomoniopsis castaneae*

To ensure fruit quality and promote good storage practices, it is important to identify the fungi associated with chestnut (*Castanea sativa*) nut rots. In 2018, in the Trás-os-Montes region of Portugal, five orchards were selected and 300 fruits were collected from the ground by hand during the harvest period. In the laboratory, the fruits were observed visually and the number of decayed nuts recorded. Morphological symptoms were evaluated in diseased nuts and with the help of a scalpel, five sections (2×2 mm) of an infected cotyledon were cut for fungal isolation. Sections of the nuts were dipped in 70% (v/v) ethanol for 2 minutes and placed in 90 mm diameter Petri dishes containing potato dextrose agar (PDA; 39 g/l) to allow mycelial growth. Plates were incubated at $25 \pm 2^\circ\text{C}$ for seven days in the dark. Single colonies were transferred to new PDA plates to obtain purified isolates for subsequent identification.

Identification of purified isolates was initially based on morphological characteristics and species identification obtained by molecular methods. DNA extraction was performed using the REDExtract-N-Amp™ Plant PCR Kit (Sigma-Aldrich, USA), and the ITS region amplified using the universal primers ITS1 and ITS4 (White *et al.*, 1990). The amplified ITS region was sequenced by Stabvida Laboratories (Caparica, Portugal). Sequences were compared with published sequences in the GenBank databases using BLAST.

Gnomoniopsis smithogilvyi was isolated from chestnut nuts which had cotyledons with a whitish-brown colour (Fig. 1). On PDA, the mycelium of *G. smithogilvyi* was woolly with a greyish colour and colonies developed in concentric circles (Fig. 2). This fungal species was found in 0 to 4.3% of fruits in the studied orchards (Table 1). Isolates Samil4.1, Samil6.1 and Brg-16 were preserved on agar slopes and kept at 4°C

**FIGURE 1** Nuts with symptoms of chestnut brown rot

in the fungal collection of Instituto Politécnico de Bragança. The GenBank Accession Nos. of these sequences are MN956833, MN960397 and MN960408. *G. smithogilvyi* was identified in an industrial processing plant in 2017, 2018 and 2019 and the proportion of infected nuts were 8.0, 5.3 and 5.0% respectively. These values are in line with the study conducted by Driss (2019) on industrial samples from Portugal where it was observed 6.4% of the nuts were affected by *G. smithogilvyi*.

This is the first detection of *G. smithogilvyi* on chestnut nuts in orchards in Portugal. This species has been reported as the main or among the major endophytes of chestnut tree (Visentin *et al.*, 2012) and was isolated from rotten nuts from several countries in Europe and

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TABLE 1 Incidence of *Gnomoniopsis smithogilvyi* isolated from industrial samples (2017-2019) and on nuts from five chestnut orchards in Trás-os-Montes region, Portugal (2018)

Origin	Year	Diseased nuts (%)	<i>Gnomoniopsis smithogilvyi</i> ¹ (%)
Industrial samples	2017	20.7	8.0
	2018	13.3	5.3
	2019	43.3	5.0
Orchards	Parâmio (41° 53' 56.54"N; 6° 52' 55.81"W)	2018	19.3
	Espinhoso (41° 49' 43.53"N; 7° 05' 07.33"W)	2018	16.0
	Sobrado (41° 34' 24.03"N; 7° 27' 53.93"W),	2018	24.8
	Samil (41° 46' 52.14"N; 6° 45' 54.97"W),	2018	23.3
	Espinhosela (41° 52' 16.05"N; 6° 49' 19.12"W)	2018	23.3

¹percentage of total nuts infected with *G. smithogilvyi*

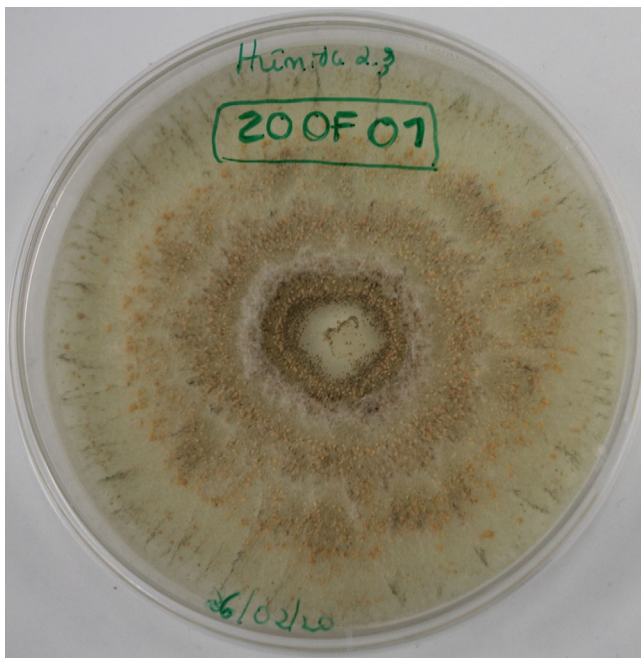


FIGURE 2 *Gnomoniopsis castaneae* grown on PDA

Asia, and in Australia (Lione *et al.*, 2019). The disease symptoms in nuts are a colour alteration and texture degradation and sometimes the kernels are dehydrated (Lione *et al.*, 2019). Several attacks can affect fruit production (Vannini *et al.*, 2017).

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