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Carole Saurat, Raphaëlle Mouttet, Céline Jeandel, Julien Prost, David Tellez, et al.. First report of thousand cankers disease caused by the fungus *Geosmithia morbida* and its vector *Pityophthorus juglandis* on *Juglans regia* in France. *New Disease Reports*, 2023, 47 (1), <10.1002/ndr2.12151>. <anses-05142507>

HAL Id: anses-05142507

<https://anses.hal.science/anses-05142507v1>

Submitted on 3 Jul 2025

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NEW DISEASE REPORT

First report of thousand cankers disease caused by the fungus *Geosmithia morbida* and its vector *Pityophthorus juglandis* on *Juglans regia* in France

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Funding information

Agence Nationale de la Recherche, Grant/Award Numbers: ANR-11-LABX-0002-01, Laboratory of Excellence-ARBRE

KEYWORDS

quarantine, detection, trapping, emergence, walnut, bark beetles

Thousand cankers disease (TCD) of walnut trees is a disease complex involving the ascomycete fungus *Geosmithia morbida* and its insect vector, the walnut twig beetle *Pityophthorus juglandis*. The disease is named after the large number of cankers that appear on the tree, caused by the wounds made by *P. juglandis* when it feeds and builds galleries under the bark and the parasitic behaviour of the fungus growing in the tissue surrounding the galleries. Several *Juglans* species may be affected. High mortality of walnut trees caused by TCD has been observed in the USA since the mid-1990's, mainly on *Juglans nigra* planted in urban areas. In 2013, TCD was reported for the first time in Italy, in the region of Veneto, on a small number of *Juglans nigra* of different ages (Montecchio & Faccoli, 2014).

As part of a national survey organised by the French plant protection organization, pheromone traps for *P. juglandis* have been installed in some urban areas where *Juglans* spp. are present. In August and September 2022, scolytids were trapped in parks in the cities of Bron and Lyon (region of Auvergne-Rhône-Alpes, 45°43'17.6"N 4°54'00.3"E, and 45°46'33.7"N 4°51'09.0"E respectively), and sent to the laboratory for identification. The morphological characters matched the features of *Pityophthorus juglandis* according to Bright



FIGURE 1 Lateral view of *Pityophthorus juglandis*

(1981) (Fig. 1) and the identity was confirmed by sequence analysis of the mitochondrial cytochrome oxidase I gene. The barcode region was amplified using the primer cocktail from Germain *et al.* (2013). The 658-bp long amplicon was sequenced (GenBank Accession No. OP975730) and BLAST analysis showed 100% identity with *P. juglandis* (KF725084), confirming identification. Following this finding, an

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FIGURE 2 Small brown cankers on the branch and trunk caused by *Geosmithia morbida* (Credit O. Baubet, DRAAF AURA)



FIGURE 4 Brown discolouration of the phloem caused by *Geosmithia morbida*



FIGURE 3 Cracked bark and bleeding symptoms of thousand cankers disease



FIGURE 5 Cultures of *Geosmithia morbida* on malt extract agar medium



active survey was triggered on walnut trees around these traps, looking for symptoms of TCD. Foliar wilt, twig and branch diebacks, small brown bark cankers on the branch and trunk (Fig. 2), sometimes with a cracked appearance (Fig. 3), as well as exit holes were observed on several *Juglans regia* trees of different ages. Removal of the bark around the cankers disclosed a brown discolouration of the phloem and the presence of bark beetle galleries (Fig. 4) where some *P. juglandis* were collected. Sections of branch or bark showing cankers were sampled and sent to the laboratory for analysis. After surface sterilisation with 70% ethanol (v/v), pieces of diseased tissues around the necrotic margins of the cankers were cut and plated onto malt extract agar (MEA) medium supplemented with 100 ppm chloramphenicol, and incubated at 22°C in the dark. After five to seven days, whitish to beige and flat-growing colonies were observed. After subculturing on MEA in the same conditions, the culture was slow growing, lobate, plane, white to yellowish (Fig. 5). All the features observed matched the description of *Geosmithia morbida* in culture made by Kolařík *et al.* (2011), such as the roughened penicillate conidiophores bearing narrowly cylindrical to ellipsoid conidia, 4–5 µm long, sometimes organized in persistent chains. Total DNA was extracted from two cultures and tested positive in real-time PCR with the *G. morbida*-specific assay developed by Rizzo *et al.* (2020). In addition, the rDNA internal transcribed spacer region was amplified for the two cultures using ITS5 and ITS4 primers. The 584-bp long amplicon was sequenced (OP930845 and OP930846) and BLAST analysis showed 100% identity with *G. morbida* (FN434082).

Since this first finding, the presence of both the insect and fungus was also confirmed on *J. nigra* in the same area. An intensive survey is underway to determine the extent of the disease. To our knowledge, this is the second report of *P. juglandis* and *G. morbida* in Europe and the first in France, where eradication measures have been put in place. Their potential spread may endanger the existence of walnut trees and the associated economy in the whole continent.

ACKNOWLEDGEMENTS

The mycology research unit of the ANSES Plant Health Laboratory (LSV) is supported by a grant managed by the French National Research

Agency (ANR) as part of the French government's "Investing for the Future" (PIA) programme (ANR-11-LABX-0002-01, Laboratory of Excellence-ARBRE). We would like to thank Massimo Faccoli (University of Padua) for exchanges on *Pityophthorus juglandis* identity and the situation of thousand cankers disease in Europe.

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How to cite this article: Saurat, C., Mouttet, R., Jeandel, C., Prost, J., Tellez, D. & loos, R. (2023) First report of thousand cankers disease caused by the fungus *Geosmithia morbida* and its vector *Pityophthorus juglandis* on *Juglans regia* in France. *New Disease Reports*, 47, e12151. <https://doi.org/10.1002/ndr2.12151>