

NEW DISEASE REPORT

Lasiodiplodia citricola, a new causal agent of *Acacia* spp. diebackM.B. Costanzo | G. Gusella  | A. Fiorenza | G.R. Leonardi | D. Aiello | G. Polizzi

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KEYWORDS

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Acacia species are woody perennial trees belonging to the family *Mimosaceae* and native to Australia. In summer 2021, unusual necrotic sunken lesions and wood discolouration were observed at the stem level both in the rootstock and in the scion, as well as the graft union on young *A. dealbata* plants grafted on *A. retinodes* in a nursery in Milazzo (north-western Sicily, Italy). In addition, gumming from cracking of the bark and shoot blight were observed (Figure 1). The disease incidence was estimated around 20% on approximately 2,500 plants. Symptoms were different from those previously observed on *A. retinodes* in Sicily (caused by *Cylindrocladium pauciramosum*) (Polizzi & Catara, 2001).

Diseased vascular stem tissue segments were cut and transferred onto potato dextrose agar medium (PDA) and incubated at 25°C in the dark for seven days. Colonies resembling *Botryosphaeriaceae* was consistently isolated (Figure 2). Pycnidia grown on pine needles agar (PNA) were observed using a stereoscopic microscope and harvested. Conidia were hyaline, non-septate, and ellipsoid. The length × width of 50 conidia were 22.52 × 12.54 μm (Figure 3). The internal transcriber spacer region (ITS) was amplified with primers ITS5/ITS4 (White *et al.*, 1990), and primers Bt2a/Bt2b were used for the partial β-tubulin (*tub2*) (Glass & Donaldson, 1995). Resultant sequences were deposited in GenBank with Accession Nos. OM891495-OM891502 for the ITS and OM918761-OM918768 for *tub2*. For comparison, 66 additional sequences were selected and aligned according to recent literature on the *Botryosphaeriaceae* (Zhang *et al.*, 2021). Maximum parsimony analysis of *tub2* region was performed in PAUP v.4.0a (Swofford, 2002). Clade support was assessed by 1,000 bootstrap replicates. *Neodeightonia phoenicum* served as an outgroup. Our isolates clustered within the group of *Lasiodiplodia vaccinii* (65% bootstrap value) (ex-type CGMCC 3.19022), described by Zhao *et al.* (2019), and now synonymised with



FIGURE 1 Symptoms of a. stem canker, b. canker at the graft union, c. gummosis and d. internal necrosis of the stem, observed on trees in a nursery in Milazzo, Italy

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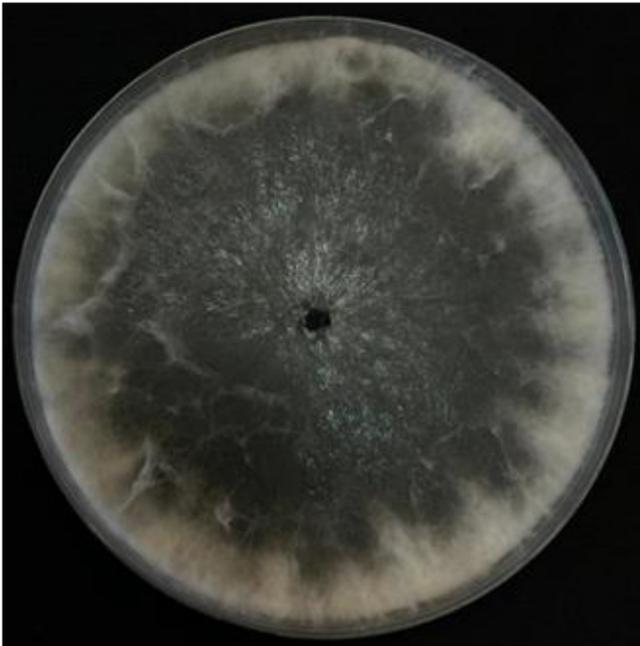


FIGURE 2 Colony of *Lasiodiplodia citricola* grown on potato dextrose agar medium after 7 days.

L. citricola (Zhang *et al.*, 2021). Based on both morphological and molecular results, the organism was identified as *L. citricola* (Figure 4).

Pathogenicity tests were conducted on three one-year-old *A. retinodes* rootstocks and three one-year-old *A. dealbata* grafted pot plants. The inoculum consisted of a mycelial plug from a seven-day-old culture inserted at three different inoculation points on each plant wounded with a cork borer (15 cm from each other) along the stem. Controls were inoculated with sterile PDA. After one week, symptoms of wood discolouration and dieback were observed and then 70% of inoculated plants died (Figure 5). Once symptoms appeared, re-isolations were conducted as described above showing colonies resembling *Lasiodiplodia*, therefore Koch's postulates were fulfilled.

To our knowledge, this is the first report of *L. citricola* causing shoot blight and canker on *A. retinodes* and *A. dealbata* worldwide. High humidity is an important factor for spore dispersion, therefore sprinkler irrigation should be avoided in greenhouses. Moreover, grafting is

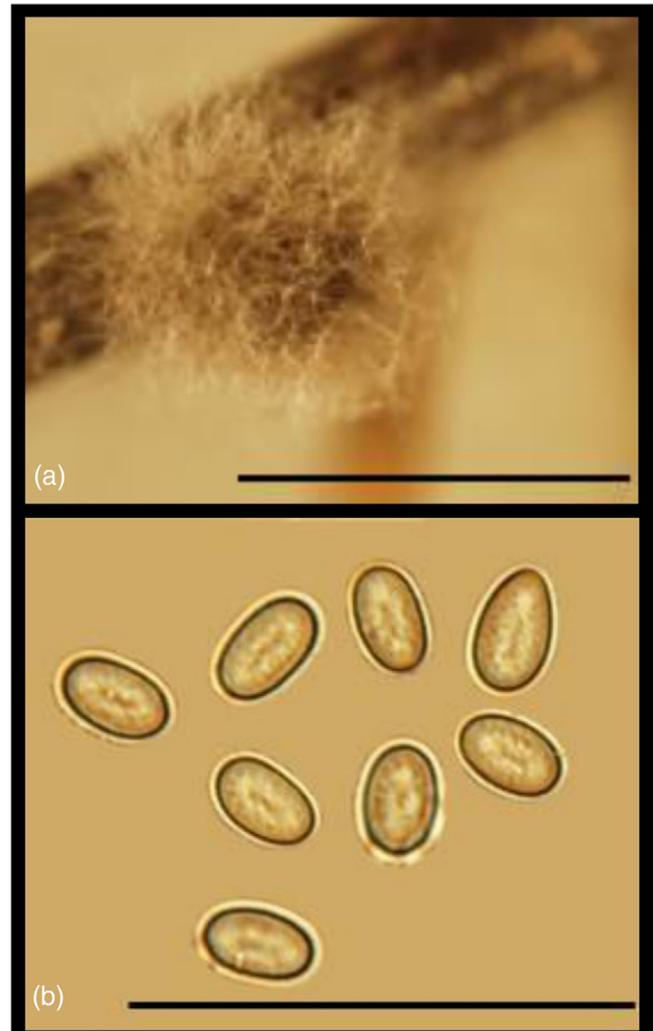


FIGURE 3 Conidioma on pine needle (a) (Bar = 2 mm) and conidia (b) (Bar = 50 μ m)

a crucial step, through which infections can easily occur, especially in the case of *L. citricola*, which has previously been associated with graft failure (Chen *et al.* 2013). Disease management starts with sanitation of the tools and of the propagation material to avoid infection with *Botryosphaeriaceae*.

FIGURE 4 Phylogenetic tree derived from the maximum parsimony analysis of *tub2* gene region. Numbers close to the nodes represent parsimony bootstrap values from 1,000 replicates. The asterisk indicates type material. Isolates in bold are generated in this study. Bar indicates the number of nucleotide changes.



FIGURE 5 Pathogenicity test: a. twig dieback, b. pycnidia at the inoculation point and c. stem of inoculated plant (left), control (right).





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