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NEW DISEASE REPORT

First report of *Phytophthora acerina* and *Phytophthora palmivora* causing root rot, bleeding cankers and dieback of English walnut in Italy

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English walnut (*Juglans regia*) is an important crop, valued for its nuts and timber production worldwide. Italy is a major producer of nuts, although over the last few decades it has gone from being the 3rd largest producer globally to 12th, despite growing demand (Calcagni, 2019). New orchards have been established recently in Italy to meet this demand but this has been accompanied by an increase in reports of new diseases (Gusella et al., 2021; Haegi et al., 2023).

Between 2017 and 2022, a severe decline was observed in two orchards located in the Veneto and Marche regions of Italy. Diseased plants showed leaf chlorosis, shoot blights, stem bleeding cankers, root and collar rot, and sudden death (Figure 1). Field surveys conducted in 2018 (Veneto) and 2022 (Marche) revealed a disease incidence of 36 and 40%, estimated along linear transects of 50 m, respectively.

FIGURE 1 Overview of the main symptoms caused by *Phytophthora* spp. on walnut: (a,b) chlorosis and shoot blight, (c) stem bleeding cankers and (d) inner bark necrotic lesions at the collar.

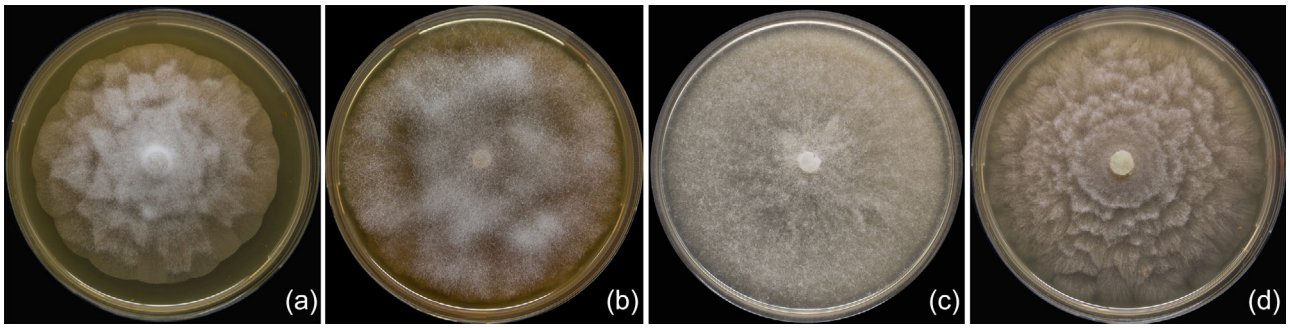

FIGURE 2 Colony morphology of (a) *Phytophthora acerina*, (b) *P. cambivora*, (c) *P. palmivora* and (d) *P. plurivora* after seven days incubation on carrot agar at 20°C in the dark.

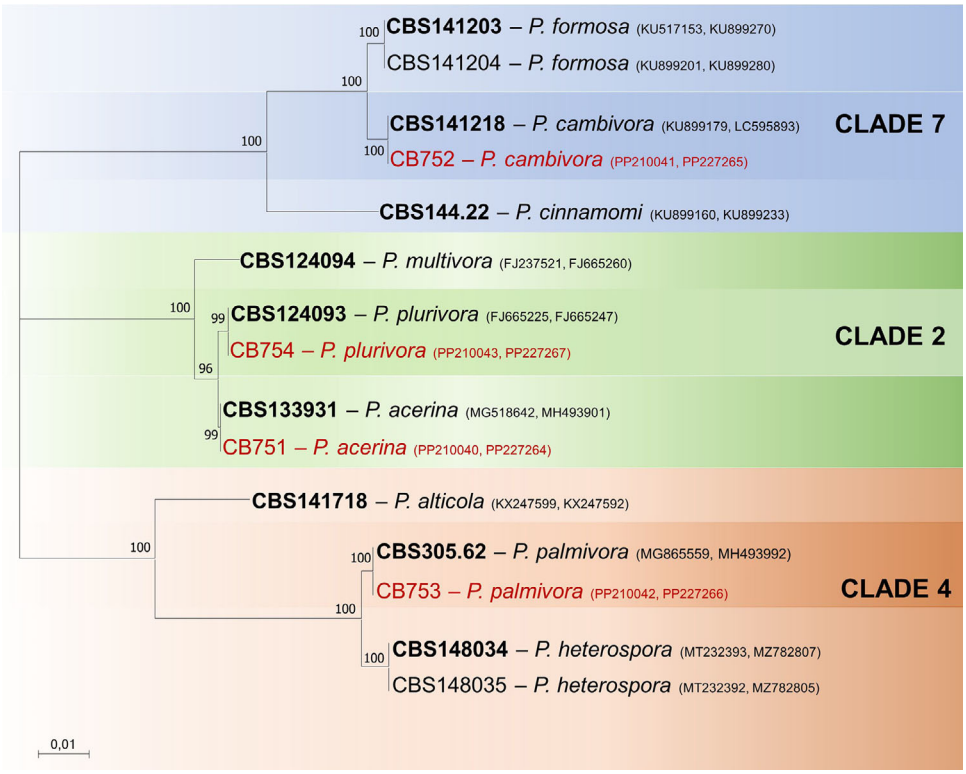

FIGURE 3 Maximum likelihood tree obtained from concatenated ITS and β-tub sequences of *Phytophthora* species. Data are based on the General Time Reversible model. A discrete Gamma distribution was used to model evolutionary rate differences among sites. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Bootstrap support values in percentage (1,000 replicates) are given at the nodes. Ex-type cultures are in bold and isolates obtained in this study in red. GenBank accession numbers are in brackets.

Twenty-six inner bark (stem) and rhizosphere (root and soil) samples were collected from 20 diseased plants, chosen at random, in two monitored plantations. Samples were processed as described by Linaldeddu et al. (2023) and cultured on carrot agar at 20°C in the dark. Based on the colony growth patterns and morphological features of sporangia produced in non-sterile pond water (Erwin & Ribeiro, 1996), the 24 *Phytophthora* isolates isolated were grouped into four species (Figure 2). In particular, *Phytophthora acerina* (11 isolates) was detected in the Marche region, whereas *P. palmivora* (7 isolates) was recovered from the samples collected in Veneto. Two isolates of *P. cambivora* and four isolates of *P. plurivora* were also detected. The morphological identification was confirmed by molecular and phylogenetic analysis based on sequence of the ITS and β-tub regions. Phylogenetic reconstructions were performed with MEGA-X 10.1.8, including all gaps in the analyses and choosing the best model determined automatically by the software (Kumar et al., 2018). Maximum likelihood analysis was performed with a neighbour-joining starting tree generated by the software (Figure 3). The sequences were deposited in

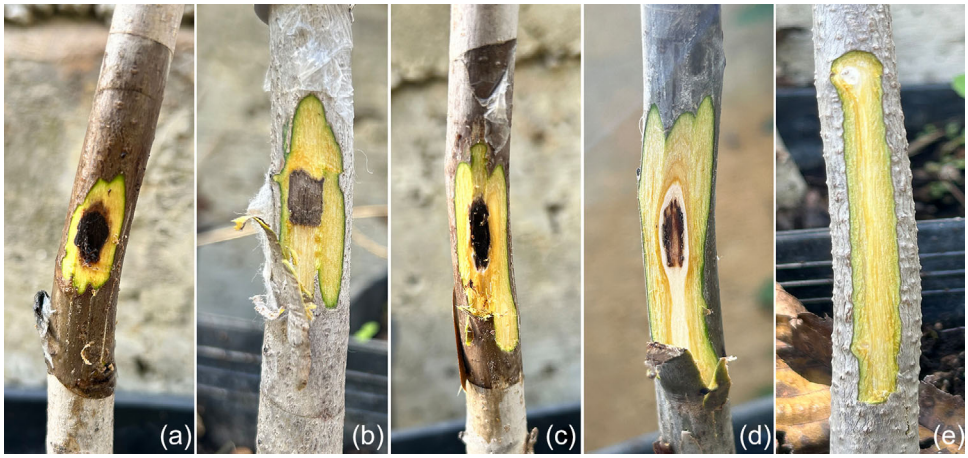

FIGURE 4 Inner bark necrotic lesions recorded on walnut seedlings 30 days after inoculation with (a) *Phytophthora acerina*, (b) *P. cambivora*, (c) *P. palmivora* and (d) *P. plurivora*, and (e) asymptomatic control.

Genbank (*P. acerina*, Accession Nos. PP210040 & PP227264; *P. cambivora*, PP210041 & PP227265; *P. palmivora*, PP210042 & PP227266; *P. plurivora*, PP210043 & PP227267).

The pathogenicity of the four *Phytophthora* species was verified by inoculating one-year-old English walnut seedlings at the collar. The collar area was initially cleaned with ethanol (70%) and then the seedlings were inoculated by removing a piece of outer bark (3 mm Ø) with a sterile scalpel and replacing it with an agar-mycelium plug of the same-size, cut from the margin of a four-day-old colony on potato dextrose agar. Eight plants for each *Phytophthora* species (*P. acerina*, *P. cambivora*, *P. palmivora* and *P. plurivora*) were included in the trial and another eight plants were inoculated with a sterile agar plug as a control. Inoculated plants were watered regularly and maintained in controlled conditions at 22 ±1°C for 30 days. All isolated species proved to be pathogenic on walnut seedlings, causing inner bark necrosis (Figure 4). The most severe symptoms were caused by *P. palmivora* and *P. cambivora*, mean lesion length of 16 ±1 mm and 15 ±3 mm, respectively. Less extensive lesions were recorded for plants inoculated with *P. plurivora* (11 ±2 mm) and *P. acerina* (11 ±1 mm). Control seedlings remained asymptomatic. All *Phytophthora* species were successfully re-isolated from all inoculated seedlings, thus fulfilling Koch's postulates.

To our knowledge, this is the first report of *P. acerina* and *P. palmivora* on English walnut in Italy and worldwide. Given the expansion of walnut orchards in Italy, new planting designs and agronomic practices should take into account the presence of these pathogens. With climate change, it is crucial to monitor the pathways for introduction and dissemination of these pathogens to limit tree decline, mortality and yield reduction.

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REFERENCES

Calcagni, G. (2019) Situazione internazionale, prospettive e potenzialità della nocicoltura. In: *Atti Delle Giornate Tecniche Nazionali SOINoce*, 20–21 September 2019, Bologna, Italy, pp. 10–14.

Erwin, D.C. & Ribeiro, O.K. (1996) *Phytophthora Diseases Worldwide. American Phytopathological Society*. St Paul, USA: The American Phytopathological Society.

Gusella, G., Giambra, S., Conigliaro, G., Burruano, S. & Polizzi, G. (2021) *Botryosphaeriaceae* species causing canker and dieback of English walnut (*Juglans regia*) in Italy. *Forest Pathology*, 51, e12661. https://doi.org/10.1111/efp.12661

Haegi, A., Luongo, L., Garaguso, I., Petrucci, M. & Vitale, S. (2023) First report of *Phytophthora inundata* associated with decline and death of walnut (*Juglans regia*) in Italy. *Plant Disease*, 107, 2267. https://doi.org/10.1094/PDIS-02-22-0362-PDN

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35, 1547–1549. https://doi.org/10.1093/molbev/msy096

Linaldeddu, B.T., Rossetto, G., Maddau, L., Vatrano, T. & Bregant, C. (2023) Diversity and pathogenicity of *Botryosphaeriaceae* and *Phytophthora* species associated with emerging olive diseases in Italy. *Agriculture*, 13, 1575. https://doi.org/10.3390/agriculture13081575

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