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New or Unusual Disease Reports

First report of *Fusarium clavum* and Fusarium venenatum causing leaf spots on *Magnolia grandiflora*

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Summary. *Magnolia grandiflora* is an important ornamental tree in urban areas. In 2025, leaf spot symptoms were observed on *M. grandiflora* leaves in Viterbo, Italy. The causal agents were isolated and identified through morphological characterization and multilocus phylogenetic analysis of EF1-α, RPB1 and RPB2 gene sequences, and were identified as *Fusarium venenatum* and *F. clavum*. Pathogenicity tests on healthy *M. grandiflora* leaves produced characteristic brown leaf spots within 3 d post-inoculation. Re-isolations of the inoculated fungi confirmed Koch's postulates. This study demonstrated that *F. venenatum* and *F. clavum* caused the leaf spot symptoms on Magnolia, and is the first report of this disease in Italy. These results provide the basis for developing targeted disease management strategies for emerging diseases caused by these fungi in urban areas.

Keywords. Emerging disease, *Nectriaceae*, urban trees.

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INTRODUCTION

Magnolia (*Magnolia grandiflor*a L.) is a popular ornamental species that is widely planted in both public and private green areas for its evergreen foliage, large and fragrant flowers, and high aesthetic value. Beyond its ornamental significance, *M. grandiflora* contributes to the ecological balance of urban ecosystems, providing shade, improving air quality and supporting urban biodiversity (Sjöman *et al.,* 2018; Novak *et al*., 2023). Among the approx. 200 species within *Magnolia*, *M. grandiflora* is particularly valued for its notable tolerance to drought conditions (Vastag *et al*., 2020). Nonetheless, like other urban trees species, magnolias planted in cities are frequently subjected to multiple stress factors, including soil compaction, air pollution and restricted rooting space (Balraju *et al*., 2023; Vettraino *et al*., 2025a, b). Such conditions can weaken tree vitality and increase their susceptibility to biotic agents.

Global warming and changes in temperature and humidity can influence the development, survival and spread of pests and pathogens, as well as the spread and survival of natural enemies, competitors and vectors. Consequently, abiotic stress, together with the impacts of climate change and high risks of introducing infected plant material from nurseries into urban environments, creates a complex and potentially dangerous scenario (Garrett *et al*., 2021; Antonelli *et al*., 2023; Raum *et al*., 2023;Antonelli *et al*., 2024; Lahlali *et al*., 2024;). In recent years, foliar diseases have become increasingly prevalent in urban trees, posing dual threats by compromising their aesthetic values and physiological functions, including photosynthesis, nutrient transport and overall plant health (Knox *et al*., 2012; Esmaeilzadeh *et al*., 2020; Hu *et al*., 2023; Huang *et al*., 2025;).

Leaf diseases of magnolia plants commonly manifest as brown spots that impair the visual quality of the foliage, and substantially reduce photosynthetic performance, with negative consequences for tree growth, vigour and resilience.

During routine monitoring of urban trees in April 2025, brown leaf spots were observed on two mature magnolia trees located in Viterbo (Central Italy). This study aimed to determine the etiology of this disease, to provide knowledge as the basis for effective disease management.

MATERIALS AND METHODS

Field observation and fungi identification

In April 2025, leaves of *M. grandiflora* exhibiting leaf spot symptoms were collected in Viterbo, Italy (42.4186°N; 12.1042°E). About 10% of the leaves exhibited brown- spots distributed on the leaf surface (Figure 1).

A total of ten leaves showing brown spots symptoms were collected, transported to the laboratory in sterile plastic bags, and processed within 24 h of collection. Tissue samples were sterilized according to Vettraino *et al.* (2021), with procedural adjustments. Specifically, the leaves were immersed in 75% ethanol for 1 min, and were then rinsed three times with sterile distilled water to remove residual disinfectant. Tissue sections exhibiting spots were aseptically cut into fragments. A total of 100 fragments were placed on PDS medium (39 g L^{-1} Potato dextrose agar [PDA; Oxoid] supplemented with 0.06 g L^{-1} streptomycin sulfate), and the culture plates were incubated at 25 ± 2°C. Each of the resulting distinct colonies was subcultured by transferring a hyphal tip onto a fresh PDA plate for purification.

The isolates obtained (35) were grouped based on colony morphology and pigment production observed after 7 d incubation. Conidium morphology was examined using a Zeiss Axio Imager A2m microscope.

Figure 1. *Magnolia grandiflora* leaves showing leaf spot symptoms.

Two isolates (R3 and R7) were selected as representative strains of the total isolates obtained, and their morphological characterizations were confirmed by molecular analyses. Genomic DNA was extracted from fresh mycelium using the NucleoSpin® Plant II kit (Macherey-Nagel), according to the manufacturer's instructions. Molecular identification of cultures was achieved based on the amplification and sequencing of the gene regions translation elongation factor 1 alpha (EF–1α), RNA polymerase largest subunit (RPB1), and RNA polymerase second largest subunit (RPB2) (O'Donnell *et al*., 2008; Jiang *et al*., 2020). Phylogenetic trees were constructed using the neighbour-joining (NJ) algorithm combined with the Kimura 2-parameter model implemented in MEGA 11 (Tamura *et al*., 2021). Bootstrap analysis was conducted with 1,000 replicates. Sequences of related *Fusarium* species were retrieved from GenBank for comparisons. The dataset consisted of sequences from isolates characterized in this study and ten taxa from the *Fusarium* species complex.

Pathogenicity tests

Pathogenicity tests were carried out on detached healthy *M. grandiflora* leaves to confirm the etiological roles of isolates R3 and R7. For each isolate, 5 mm mycelium plugs collected from 7-d-old PDA cultures were placed on leaf surfaces that had been previously

wounded using a sterile scalpel (wound length 4mm). Inoculation control leaves received sterile PDA plugs. All leaves were then placed in plastic trays with moist paper towels to maintain approx. 90% relative humidity, and were incubated at 25 ± 1°C. Petioles of the leaves were wrapped in wet cotton to prevent desiccation. Each treatment consisted of five replicates per isolate. Development of symptoms was monitored daily, for 3 d after inoculation. Fungal re-isolations were made from symptomatic tissues assess fulfilment of Koch's postulates. The pathogenicity tests were repeated twice to confirm reproducibility.

RESULTS

Isolation and characterization of fungal isolates

Isolates R3 and R7 both produced fast-growing, pale to bright- coloured colonies with dense aerial mycelium. Isolate R3 developed orange to pink aerial mycelium. Macroconidia were hyaline and two to five septate, of lengths 35 to 50 µm, while microconidia were absent. Chlamydospores were abundant, and were globose to oval, and were intercalary and lateral, and measured 7 to 11 µm. Isolate R7 formed white aerial mycelium and produced hyaline, non-septate, single-celled, ovoid microconidia measuring 6 to 8 µm. This isolate also produced abundant globose to oval chlamydospores, measuring 6 to 11 µm. Macroconidia of R7 were falcate, hyaline, one to six-septate, and were 30 to 45 µm in length.

The two isolates were coded as *Fusarium*, and species identity was verified through analysis of the EF1-α, RPB1 and RPB2 sequences using BLAST searches against the NCBI database. Isolate R3 corresponded to *F. venenatum*, and isolate R7 corresponded to *F. clavum*. The phylogenetic analysis based on the concatenated sequences of TEF-1α, RPB1 and RPB2, using the NJ method, was consistent with the result of the BLASTn comparison (Figure 2).

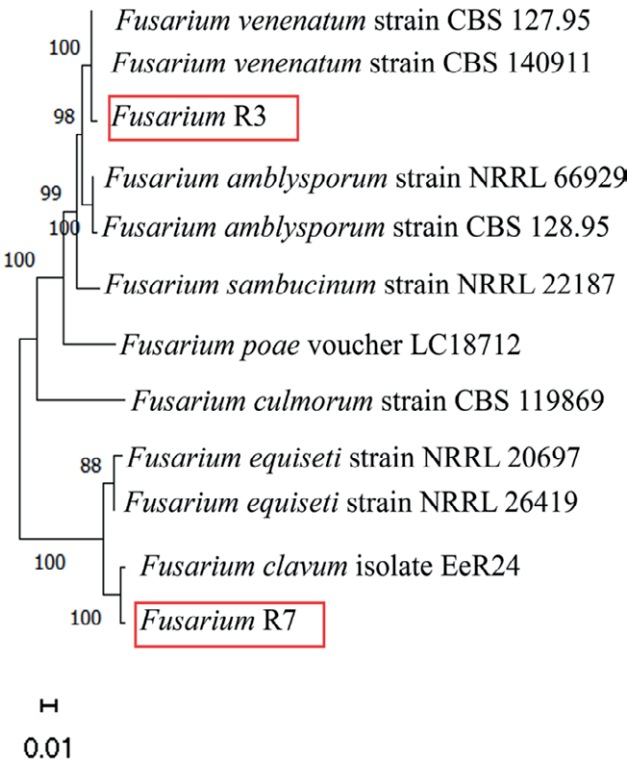

Figure 2. Neighbour-Joining (NJ) phylogenetic tree based on concatenated sequences of TEF-1α, RPB1 and RPB2 genes, including sequences of already known *Fusarium* species and of isolates R3 and R7 obtained in the present study. Bootstrap values greater than 50% (expressed as percentages of 1,000 replications) are shown at the nodes. The isolates characterized in this study are each highlighted with a red-bordered rectangle.

Pathogenicity tests

Three days following inoculations, brown leaf spots appeared on all leaves inoculated with isolates R3 and R7, in both experimental repetitions. No symptoms were observed on control leaves (Figure 3). The symptoms were similar to those previously observed on *M. grandiflora* trees under field conditions. Fungi identical to *F. venenatum* R3 were re-isolated from diseased tissues inoculated with this isolate, and similarly for *F. clavum* R7, confirming Koch's postulates for both fungi.

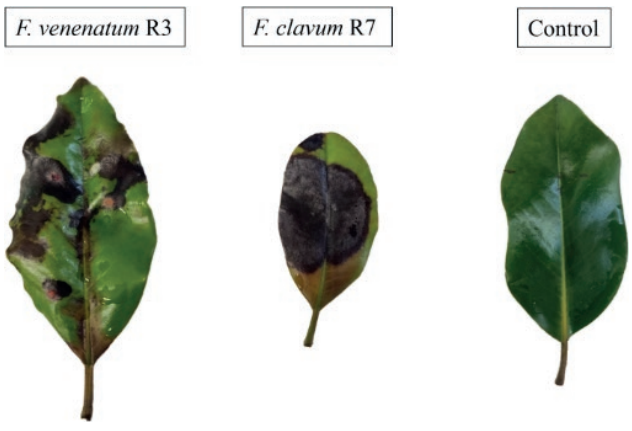

Figure 3. Symptoms on *Magnolia grandiflora* leaves after inoculations with *Fusarium* isolates R3 or R7, compared to an uninoculated control leaf.

DISCUSSION

Magnolia grandiflora is a widely valued ornamental tree in urban and peri-urban landscapes, providing key ecosystem services such as air purification, shade, aesthetic value, and habitats for biodiversity. However, introductions of new pests and pathogens pose significant risks to magnolia health and the benefits provided by these trees. The fungi isolated from leaf spots on *M. grandiflora* during the survey in Viterbo, Italy were identified as *F. venenatum* (isolate R3) and *F. clavum* (isolate R7), based on morphological and molecular analyses.

Fusarium Link (*Hypocreales*, *Nectriaceae*) includes several aggressive plant pathogens that cause root rot, cankers and vascular wilt on a variety of urban trees (Skarmoutsou & Skarmoutsos, 1999; Guo *et al*., 2021). *Fusarium venenatum* has been previously reported in association with dry rot in potato tubers (Stefancyzk *et al*., 2016; De Jesús Díaz Aguilar *et al*., 2023), and root and collar rot, damping-off, and foliar necroses in herbaceous crops (Ayoubi & Soleimani, 2016), citrus trees and strawberry plants. Similarly, *F. clavum* has been associated with leaf spot and blight symptoms, appearing as necrotic lesions that sometimes expand and lead to premature senescence (Sandoval-Denis *et al*., 2018; Matic *et al.,* 2020; Gilardi *et al*., 2021).

The present study has identified, for the first time, natural infections of *M. grandiflora* by *F. venenatum* and *F. clavum*. This is the first documented report of *F. venenatum* and *F. clavum* associated with diseases of *M. grandiflora*. Occurrence of these fungi in symptomatic leaves collected from urban green spaces extends the known host range of both species, and indicates their adaptability to actively colonize foliar tissues, rather than acting merely as secondary saprophytes, highlighting the adaptability of *Fusarium* species to urban tree environments.

These results emphasize the vulnerability of urban trees to emerging fungal pathogens, and the importance of regular disease surveillance in green city areas. To mitigate the impacts of these pathogens, management strategies should emphasize preventive cultural practices that limit pathogen spread and establishment, including minimal and well-timed pruning of dead or diseased branches, rapid removal of symptomatic tissues, and strict application of pruning hygiene methods.

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