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NEW DISEASE REPORT

First report of leaf spot disease caused by Xenodidymella glycyrrhizicola on Glycyrrhiza glabra

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Glycyrrhiza glabra (liquorice) is a perennial shrub belonging to the Fabaceae family. It is a native of Mediterranean areas and primarily cultivated for its extracts which are used in the pharmaceutical and food industries, and in the manufacture of food supplements (Hayashi & Sudo, 2009). Several foliar diseases, such as powdery mildew, rust and leaf spots, have been reported to endanger the long-term viability and production of liquorice (Farr & Rossman, 2023). In 2022, a leaf spot disease was observed on c. 40% of the two-year-old plants in a liquorice field in Ankara, Turkey. Leaf spots were brown-to-black in colour, sometimes with a light brown centre. Lesions coalesced to large areas of blighted tissue with age (Figure 1a). Branches and petioles also became covered in oval lesions (Figure 1b).

Pieces (5 × 5 mm) from lesion margins were surface sterilised in 70% ethanol (30 seconds) and 1% NaOCl (1 minute) and rinsed three times with sterile water. Phoma-like colonies were isolated from surface-sterilised parts of diseased tissues on potato dextrose agar (PDA). One representative isolate (Xg_Gg01), purified using the hyphal tip method, was selected for molecular, morphological and pathogenic characterisation and transferred to plates containing fresh PDA, oatmeal agar (OA), and malt extract agar (MEA) for seven days at

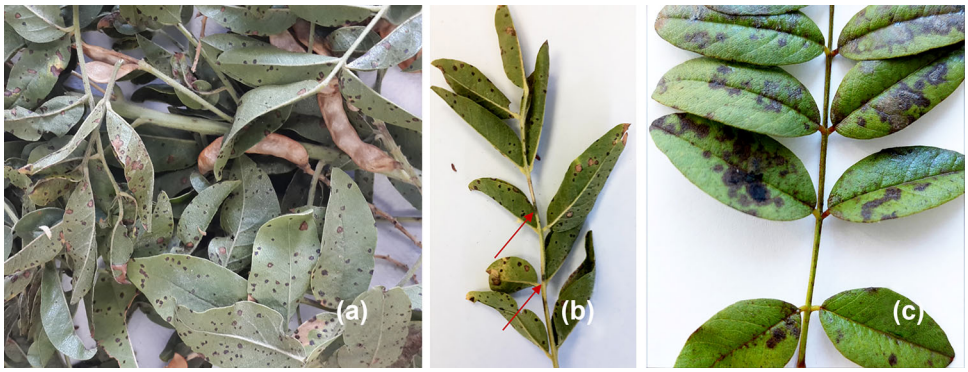


FIGURE 1 Disease symptoms on *Glycyrrhiza glabra* collected: Spot and lesions on leaf (a) and petiole (b) in the field, spots and lesions on inoculated plants at seven days after inoculation (c).

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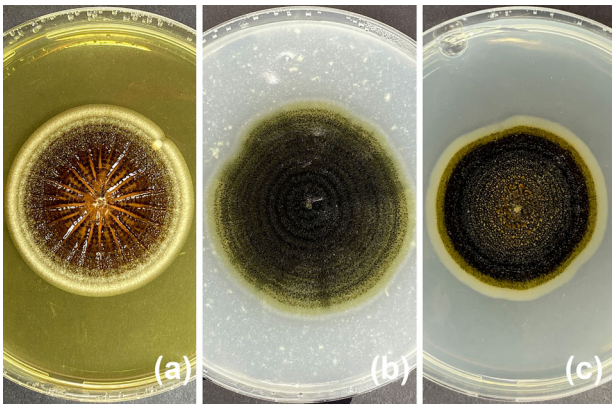


FIGURE 2 Colony morphology of *Xenodidymella glycyrrhizicola* isolate Xg_Gg01 after seven days' growth on malt extract agar (a), oatmeal agar (b), and potato dextrose agar (c).

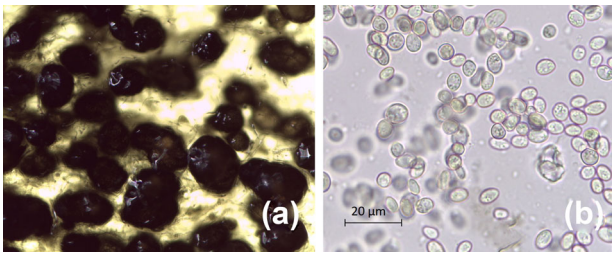


FIGURE 3 *Xenodidymella glycyrrhizicola* isolate Xg_Gg01, pycnidia forming on oatmeal agar (a), conidia (b).

25°C with 12 hr light and 12 hr dark cycles. Colonies on MEA reached about 45 mm in diameter, margin regular and white, brown towards the centre, which was brown and orange due to conidial discharge from pycnidia (Figure 2a). Colonies reaching about 50 mm in diameter on the OA were marginally regular and olivaceous, with a colour change to black due to pycnidia formed towards the centre (Figure 2b). Colonies on the PDA reached about 43 mm in diameter, and the margin was regular and pale olive; the middle and centre were black due to dense pycnidia, and light brown conidial discharge was observed in the centre (Figure 2c). Pycnidia were mostly superficial or immersed in agar, ostiolate, 230–470 × 190–365 μm, and globose or subglobose and brown, becoming irregular-shaped and black with age (Figure 3a). Conidia were aseptate, variable in size and shape, oblong, broadly ellipsoidal, hyaline, becoming pale brown with age and measured 6.1 ± 9.3 × 3.4 ± 6.4 μm (Figure 3b).

The internal transcribed spacer (ITS) of ribosomal DNA, partial β-tubulin gene (*TUB2*) and RNA polymerase second largest subunit (*RPB2*) loci of isolate Xg_Gg01 were amplified and bidirectionally sequenced with the universal primer sets; ITS1/ITS4 (White *et al.*, 1990), BT2a/ BT2b (Glass & Donaldson, 1995), and 5f2 (Reeb *et al.*, 2004)/7cr (Liu et al., 1999), respectively. A BLAST search of the resultant sequences (GenBank Accession Nos. OQ568230 for ITS, OQ571627 for *TUB2*, and OQ571628 for *RPB2*) showed 99.61–100% nucleotide identity with the holotype strain CBS 684.97 of *X. glycyrrhizicola* from New Zealand. The combined maximum-likelihood tree of

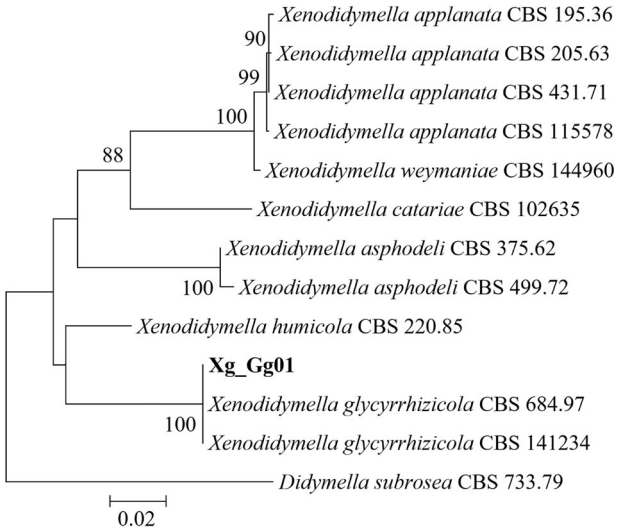


FIGURE 4 Phylogenetic tree inferred from a maximum likelihood analysis based on a concatenated alignment of ITS, *TUB2*, and *RPB2* sequences of *Xenodidymella* spp. isolates and outgroup sequences. Bootstrap support values above 50% are given at the nodes. The isolate obtained in this study is printed in bold font. The tree is rooted to *Didymella subrosea* CBS 733.79.

ITS, *TUB2*, and *RPB2* regions with 1000 bootstrap replicates showed that the isolate from the current study was clustered with strains of *X. glycyrrhizicola* derived from GenBank with strong support (Figure 4).

Koch's postulates were fulfilled by spraying 10 ml of a spore suspension (10^6 spores/ml prepared in a 0.1% Tween-20 solution) of isolate Xg_Gg01 on each of five two-month *G. glycyrrhizicola* plants in trial pots. The plants were covered with plastic bags for 24 hr post inoculation. After seven days, spots were observed on the inoculated plants' leaves and petioles (Figure 1c). No symptoms were observed on control plants, sprayed with sterile distilled water only. A morphologically identical *X. glycyrrhizicola* was isolated from affected tissues.

To the best of our knowledge, this is the first report of *X. glycyrrhizicola* associated with leaf spots on *G. glabra* (Farr & Rossman, 2023). The fungus was previously reported on *Glycyrrhiza lepidota* in Iran (Ahmadpour *et al.*, 2022) and New Zealand (Hou et al., 2020). However, *G. glabra* constitutes a new host record for this pathogen in Turkey and worldwide.

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