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NEW DISEASE REPORT

First report of *Diaporthe miriciae* and *Diaporthe masirevicii* causing soybean stem canker in Uruguay

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KEYWORDS
Soybean stem canker, Diaporthe, Soybean pathogens

In 2022 and 2023, samples of soybean stems showing symptoms of soybean stem canker were collected in the Departments of Rio Negro, San José and Colonia, Uruguay. A total of fifty-three isolates were obtained from the margins of necrotic lesions on the stems (Fig. 1). These isolates were cultured on potato dextrose agar (PDA), revealing morphological characteristics similar to those of *Diaporthe* species. Fungal DNA was extracted from pure isolates, and the internal transcribed spacer (ITS) region of the ribosomal DNA along with the translation elongation factor 1-alpha gene (tEF1a) were amplified and sequenced according to Mena et al. (2020). In addition to *D. caulivora* and *D. longicolla*, four isolates of *D. miriciae* (syn. *D. ueckerae)* and two isolates of *D. masirevicii* were identified. All sequences were deposited in GenBank: ITS (Accession Nos. OR944537-OR944624) and tEF1a (OR966576-OR966626). *Diaporthe miriciae* and *D. masirevicii* exhibited aerial mycelia with a radiate growth pattern on PDA and displayed yellow pigmentation with cream to pale brown at the centre on the reverse side of the plates (Fig. 2). At 30 days, reproductive structures became evident over scattered circular black stromata (Fig. 2). Both isolates produced black and globose pycnidia. Alpha conidia measured 6.5-8.0 × 2.0-2.8 μm, being unicellular, hyaline, smooth, ellipsoid to ovoid, biguttulate, and aseptate. Beta conidia, exclusive to *D. masirevicii*, measured 25–35 × 1.0-2.0 μm, and appeared hyaline, flexuous to hamate. Perithecia, black and smooth, were clustered in groups, and

FIGURE 1 Symptoms of soybean stem canker under field conditions

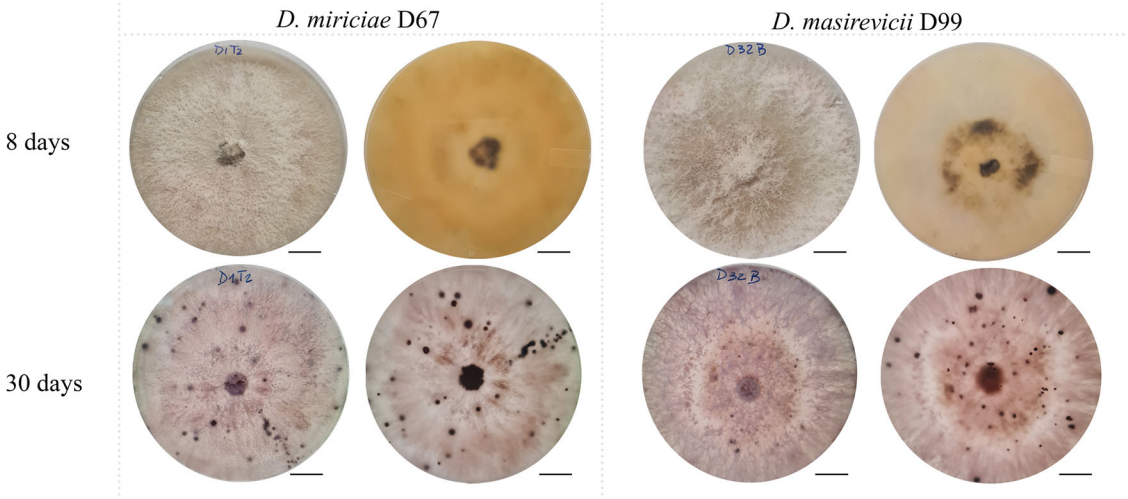

FIGURE 2 Morphological characteristics of *D. miriciae* D67 and *D. masirevicii* D99 isolates grown on potato dextrose agar medium (PDA) at 8 and 30 days. Scale bar=13 mm.

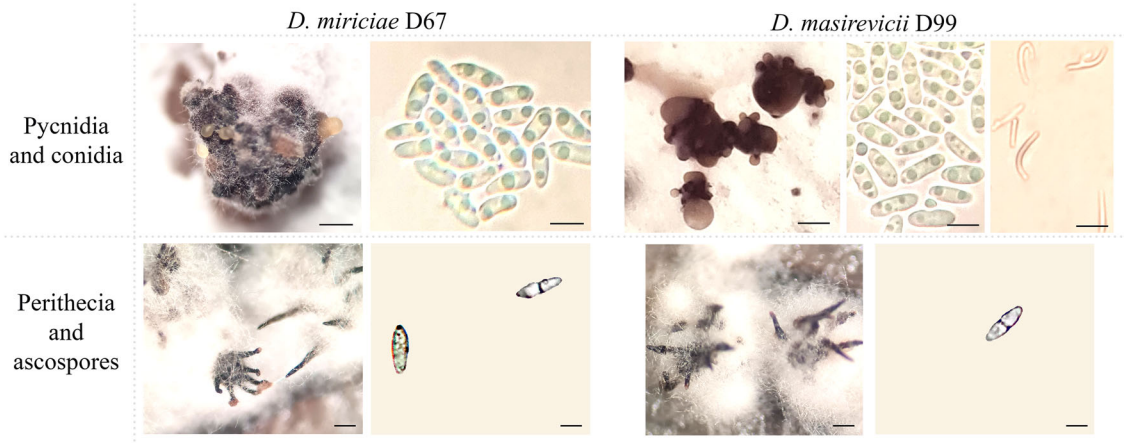

FIGURE 3 Reproductive structures of *D. miriciae* D67 and *D. masirevicii* D99 isolates. Scale bar: Pycnidia=500 μm; Alpha conidia=5 μm; Beta conidia=25 μm; Perithecia=180 μm; Ascospores=3.5 μm.

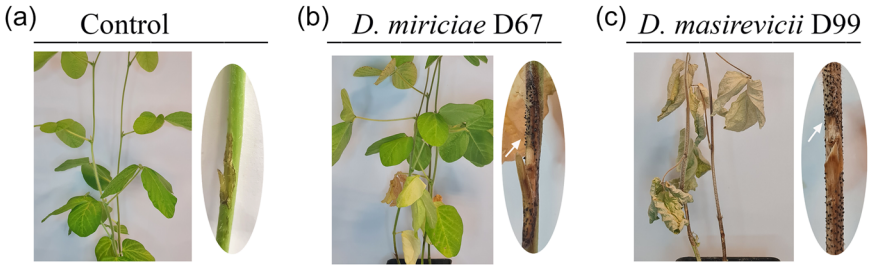

FIGURE 4 Disease symptoms in soybean stems at 11 days post-inoculation (dpi). Control plants without pathogen (a), inoculated with *D. miriciae* D67 (b) and *D. masirevicii* D99 (c) isolates. Pycnidia are highlighted with a red arrow in B and C.

ascospores measured 8.6 × 2.1 μm, being two-celled, medianly septated, hyaline, smooth, often biguttulate, and ellipsoid to fusoid (Fig. 3).

Pathogenicity tests were performed by inoculating a mycelial agar plug (5 mm diameter) of *D. miriciae* D67 and *D. masirevicii* D99 onto the wounded stem of ten susceptible soybean plants cv. Williams (Fig. 4). Dark brown necrotic lesions, resembling soybean stem canker symptoms observed in the field, were detected on inoculated soybean stems at 11 days post-inoculation (dpi), with pycnidia appearing around the wound (Fig. 4). No symptoms developed on control stems which were mock inoculated with a sterile agar plug. Successful re-isolations were

achieved from all the inoculated stems. Confirmation of *D. miriciae* and *D. masirevicii* was established by tEF1a sequencing.

Diaporthe miriciae and *D. masirevicii* have been isolated previously as endophytes or pathogens causing leaf spots, fruit rots and dieback in various plant species (Thompson et al., 2018; Dong et al., 2021; Hongsanan et al., 2023). Recently, *D. miriciae* was reported as the causal agent of soybean stem canker in Colombia (López-Cardona et al., 2021). To the best of our knowledge, this is the first report of *D. masirevicii* causing soybean stem canker globally and the first of *D. miriciae* as a pathogen causing this disease in Uruguay. Given the economic significance of this disease, further research on the pathogenicity of and detection methods for *Diaporthe* species are needed to develop effective management strategies.

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