

## NEW DISEASE REPORT

# First report of *Diaporthe ambigua* causing Phomopsis stem canker on sunflower in Argentina

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**KEYWORDS**

Fungi, Pathogen detection

Phomopsis stem canker, a disease emerging on sunflower (*Helianthus annuus*) in response to environmental change, has already been described in Argentina with *Diaporthe helianthi* as the main aetiological agent (Ridao *et al.*, 1994). During a survey (2020–2021) in the Argentinian sunflower-growing region of Pampeano, 208 sunflower plants with canker symptoms were collected from 75 cultivated fields (Fig. 1). Necrotic triangular dark leaf lesions invaded the stem, forming a conspicuous brown to black canker at the corresponding nodes.

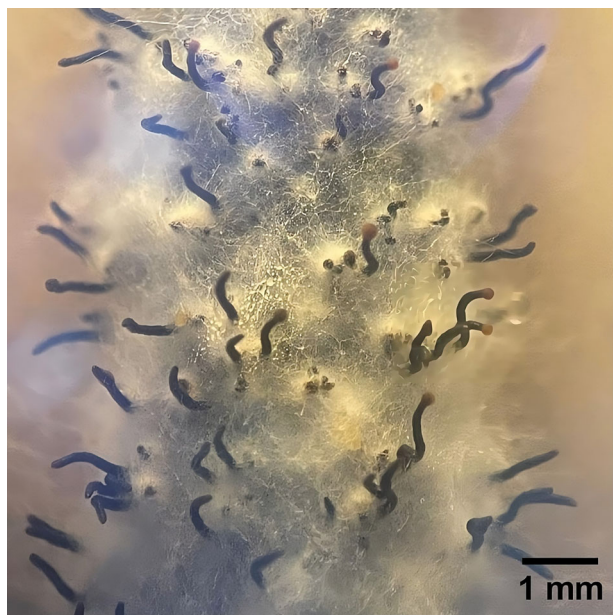
The infected stems were surface sterilised with 70% (v/v) ethanol for 30 seconds and in 1% (w/v) sodium hypochlorite for one minute. Tissue fragments were excised aseptically from the internal leading lesion's edge, transferred to potato dextrose agar (PDA) with a sterilised soybean stem fragment, and incubated at 25°C for 14 days for anamorph development and 40 days for teleomorph maturation (12-hour photoperiod, UV light, 345–400 nm). *Diaporthe ambigua* was identified based on the following features. Colonies on PDA, flat, floccose, white, presenting coriaceous and dark stromatic patches arranged in an annular pattern; reverse pale, dark under the stromata. Mycelium consisting of a dense dirty white to cream-coloured layer supporting floccose hyphal tufts. Perithecia globose, solitary with a long neck (Fig. 2). Asci unitunicate, cylindrical-clavate with a refractive apical ring, 8-spored, 42–46 × 6–7 μm in size. Paraphyses unbranched, constricted at the septa, tapering towards the apex with a rounded tip, extending above the asci. Ascospores bicellular, hyaline, smooth, 11–



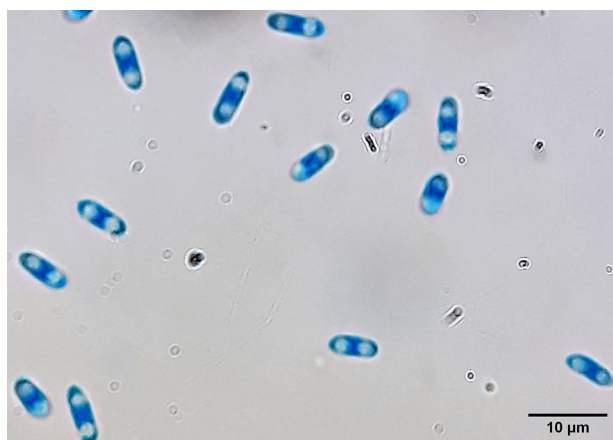
**FIGURE 1** Sunflower plants with canker symptoms in the field in Pampeano, Argentina

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**FIGURE 2** Perithecia of *Diaporthe ambigua*



**FIGURE 3** Alpha conidia of *Diaporthe ambigua*

$13 \times 2\text{--}3\ \mu\text{m}$  with four guttules. Pycnidia, globose, solitary or in groups. Conidiophores simple, composed of a globose basal cell and an integrated conidiogenous cell. Alpha conidia ellipsoidal, with slight central constriction and two conspicuous guttules, obtuse apex and papillose base, smooth walled ( $6\text{--}7 \times 2\text{--}3\ \mu\text{m}$ ) (Fig. 3). Beta conidia not seen.

The internal transcribed spacer (ITS) and translation elongation factor 1- $\alpha$  (EF1- $\alpha$ ) were amplified and sequenced using primers ITS1/ITS4 (White *et al.*, 1990) and EF1-728F/EF1-986 (Carbone *et al.*, 1999) respectively. The sequences were deposited in the GenBank database, ITS (Accession No. ON099487) and EF1- $\alpha$  (ON146034). After BLASTn analysis, the Argentinian isolate showed more than 99% identity with *D. ambigua* (NR\_119434.1 and KC343736.1).

To investigate pathogenicity, two experiments (15 plants each) were conducted in the greenhouse. A susceptible sunflower hybrid (ACA 869 DM) was inoculated at the V8 stage using the stem wounding method described by Mathew *et al.* (2018). After 14 days, all inoculated plants showed cankers with necrosis and wilting, while control plants remained symptom free. *Diaporthe ambigua* was reisolated consistently from artificially infected plants using the methods above. *Diaporthe ambigua* was isolated from sunflower in Italy (Gomes *et al.*, 2013) but to our knowledge, this is the first report of *D. ambigua* in Argentina.

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