A New Sunflower Disease in Texas Caused by *Diaporthe helianthi*

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ABSTRACT

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A stem canker and leaf spot of cultivated sunflower (*Helianthus annuus*) caused by the fungus *Diaporthe helianthi* (asexual stage *Phomopsis helianthi*) was observed for the first time in Texas. Symptoms on stems are light brown lesions with dark brown margins; lesions are variable in size and shape. Pycnidia are produced in affected areas. Lesions may also occur on leaf scars, petioles, and leaves. Pathogenicity of the fungus was demonstrated through inoculations to hybrid sunflower cultivars in the greenhouse. This is the first report of the disease in the United States.

In September 1982, 5 and 11%, respectively, of the sunflower plants in two commercial fields at Dimmitt, TX, showed symptoms of stem rot that were different from charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid. (7,8) and phoma stem rot caused by *Phoma macdonaldii* Boerema (2,4,8). Diseased plants had lesions on stems and petioles and showed wilting and dying of the leaves. An investigation was made to determine the cause of this disease. In this paper, we describe the symptoms of the disease, the pathogen, and report the results of pathogenicity tests.

MATERIALS AND METHODS

Tissue pieces taken from sunflower stem and petiole lesions were soaked in 1% NaOCl solution for 0.5–2 min and washed in sterile distilled water for 5 min. The surface-sterilized tissue pieces were transferred to potato-dextrose agar (PDA) in petri dishes (four pieces per plate) and the plates were incubated on laboratory benches at 22 ± 2 C.

Pieces of mycelium from the periphery of colonies that developed on PDA were transferred to fresh PDA, autoclaved sunflower leaves and stems, or cornmeal agar for identification. The cultures were placed under near-ultraviolet light for 14 hr and in darkness for 10 hr daily to stimulate sporulation.

Pathogenicity of the isolated fungus was tested by inoculating five 4- to 8-wk-old sunflower hybrid cultivars (S304A, SG342, SG372, SG378, and TT894A) growing in 30-cm-diameter pots (three pots per cultivar, two to four plants per pot) in the greenhouse. Test plants were inoculated with mycelial mats from 3-day-old cultures or ascospores from 30-day-old cultures grown on PDA. Inoculum was introduced into the stem through a hole made with a sterile needle (0.2 mm in diameter) or was placed on the surface of the stem epidermis without wounding, after which the inoculation sites were wrapped with sterile moistened cotton wool and covered with aluminum foil. In other inoculations, 2- to 3-wk-old sunflower seedlings (TT894A) growing in 30-cm-diameter pots (six per pot) were sprayed with ascospore suspensions (10^4/ml) with an airbrush, incubated in a mist chamber for 2 days, and moved to greenhouse benches. Sunflower plants (one pot per cultivar) inoculated with plain agar or sprayed with sterile distilled water served as a control. Inoculated plants were incubated in the greenhouse (22 ± 3 C) for 14–21 days and development of symptoms was recorded. The inoculation tests were repeated three times.

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RESULTS AND DISCUSSION

Field symptoms. Light brown to gray-brown lesions with dark brown margins (outlines) developed on stems. Lesions also occurred on leaves, petioles, and leaf scars. Some internode lesions coalesced with those at leaf scars so that the lesions extended beyond more than one internode. Some stem lesions coalesced and girdled the stems. Some severely infected leaves turned brown, curled, and fell prematurely. Pycnidia were produced in the light brown or gray-brown areas of the lesions on the senescent sunflower.

Causal organism. A fungus that produced both pycnidia and perithecia on PDA was consistently isolated from the stem and petiole lesions. Pycnidia that developed on PDA within 7 days were dark brown, aggregate or solitary, subglobose, 317 × 255 μm in diameter (average of 50), and ostiolate. Hyaline and ellipsoidal alpha pycnospores were not observed on PDA and cornmeal agar. Beta pycnospores were 28.4 × 1.7 μm

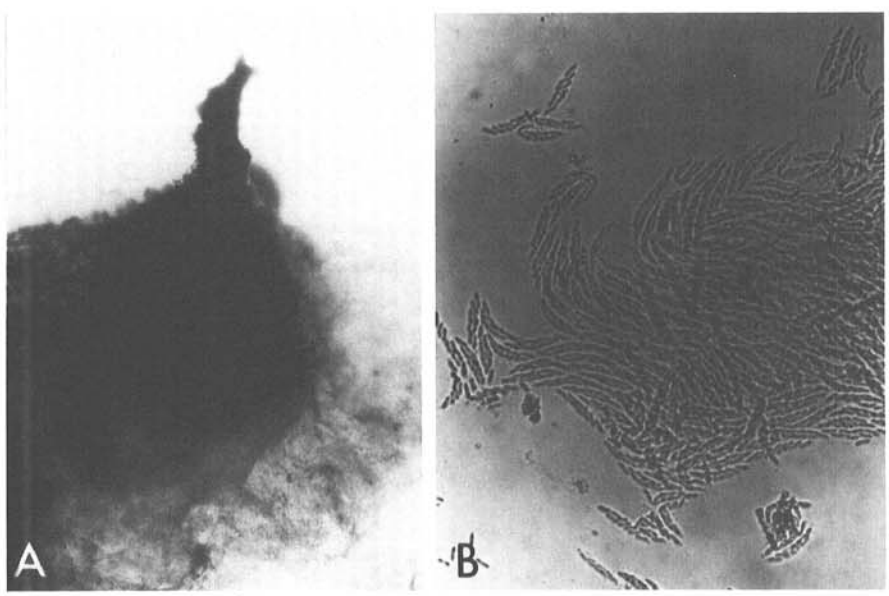

Fig. 1. Perithecium, asci, and ascospores of *Diaporthe helianthi*. **(A)** Perithecium with beak. **(B)** Asci with eight ascospores.

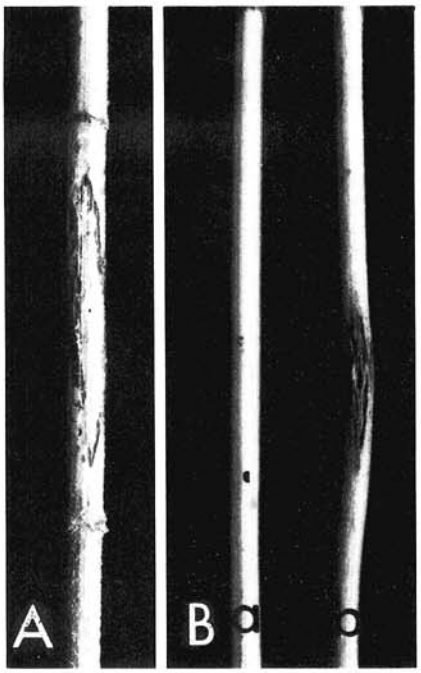

Fig. 2. Symptoms on sunflower stems inoculated with *Diaporthe helianthi*. **(A)** Light brown lesions with dark brown outline on stem. **(B)** (a) Control and (b) inoculated stem; split at inoculation site.

(average of 100), hyaline, filiform, and sigmoid; they were produced abundantly on PDA. Perithecia produced on PDA within 26 days were subglobose, 506×372 μm in diameter with a dark beak 293 × 113 μm (average of 50) (Fig. 1A). Asci were 54.3 × 9.7 μm (average of 50), clavate, cylindrical, with refractive body in the apical wall, with eight ascospores (Fig. 1B). Ascospores were 14.2×5.1 μm (average of 50), subelliptical, and one septate. The fungus was identified as *Diaporthe helianthi* Munt.-Cvet. et al (asexual stage *Phomopsis helianthi* Munt.-Cvet. et al) based on its morphological characteristics (5,6). The culture was verified to species by M. Muntanola-Cvetkovic, Institute for Biological Research "Sinisa" Stankovic, Belgrade, Yugoslavia, and by S. C. Jong, American Type Culture Collection (ATCC), Rockville, MD 20852, and one has been deposited at ATCC (ATCC 52472).

The fungus produced many pycnidia but no perithecia on autoclaved sunflower stems and leaves and produced very few pycnidia on cornmeal agar. Beta pycnospores were produced abundantly on these substrates. Very few alpha pycnospores (8.4×3.3 μm, average of 15) were produced on the autoclaved sunflower stems and leaves.

Pathogenicity tests. Many small, reddish brown lesions 3 mm or less in diameter developed on sunflower stems that were inoculated without artificial injuries. These lesions coalesced and became light brown with dark brown margins. Sometimes the lesions enlarged and girdled the stem. When the sunflower stems were inoculated through wounds, large brown to dark brown lesions 1–3 cm long were produced (Fig. 2A). The centers of these lesions later became light brown. Some stems split or broke at the site of inoculation (Fig. 2B). Disease from the inoculation sites on the stem progressed toward leaf scars, petioles, and leaves and produced pycnidia in the light brown areas 1–2 mo after inoculation. All control plants remained healthy.

When 2- to 3-wk-old seedlings were sprayed with ascospores, many brown lesions with chlorotic margins developed on the leaves. Infected leaves became brown, some curled from the tip, and died prematurely. Small brown lesions also developed on petioles and stems. Alpha pycnospores were not used in the inoculation tests because alpha spores were not produced on PDA. *D. helianthi* was reisolated on PDA from the lesions of inoculated sunflower plants. No organism was isolated from the control plants.

Diaporthe stem canker occurred in sunflower in Ohio (3), but the identity of the pathogen was not identified to species (3). This is the first verified report of *D. helianthi* on cultivated sunflower in Texas and also in the United States. This disease was recently reported on cultivated sunflower in Yugoslavia (1,5,6).

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