Diaporthe Stem Canker of Sunflower

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ABSTRACT

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Stem canker, a new disease of oilseed sunflower, was first found in Ohio in 1980. A species of *Diaporthe* and its *Phomopsis* anamorph was isolated from the brown to black, later ash-grey, cankers. Characteristic symptoms were produced in the greenhouse and field on plants wound-inoculated at the time of flowering. Disease losses resulted from premature death and lodging of affected plants.

Stem canker, a new disease of oilseed sunflower (*Helianthus annuus* L.), was first found in Wayne County, OH, in late August 1980 and, subsequently, in widely separated locations of eastern, central, and western Ohio during August 1981 and September 1982. Although the disease was widespread within the state in 1982, it was not detected in all sunflower fields examined. As many as 20% of the plants showed disease symptoms in individual fields. Disease lesions on affected plants superficially resembled those of Phoma black stem (3) caused by *Phoma macdonaldii* Boerema (2) (*Leptosphaeria lindquistii* Frezzi); however, after more critical study, pycnidia of *Phomopsis* sp. were detected. In 1980, an apparently similar stem disease of sunflower caused by a new *Diaporthe* species, *Diaporthe helianthi* Munt.-Cvet. (*Phomopsis helianthi* Munt.-Cvet.), was reported in Yugoslavia (4,5).

Our objectives were to describe the symptoms of this stem canker disease on sunflower, to report results of inoculation tests, and to establish the identity of the pathogen.

MATERIALS AND METHODS

Isolation. Two methods were used to isolate pathogens from diseased sunflower stems. Sunflower stems bearing cankers were split into sections 2–4 cm long and 0.3–0.8 cm wide and incubated at 25 C for up to 30 days in moist chambers (petri dishes containing moistened filter paper, 9 cm diameter, enclosed in plastic bags) until pycnidia formed and/or liberated conidia. Conidia (alpha spores) exuding from pycnidia were transferred to 2% water agar (WA) or to potato-dextrose agar acidified to pH 4.5 with 85% lactic acid (APDA). The second method was to surface-sterilize pieces (about 0.5 cm^2) of diseased tissue (early disease phase, no pycnidia) in 1:1 ethyl alcohol-5.25% sodium hypochlorite for 0.5–1 min then plate on WA or APDA plates. Cultures and moist chambers were incubated at 25 C under either intermittent 8-hr light (900 lux) and 16-hr darkness or continuous fluorescent light (1,400 lux). Stock cultures were maintained on PDA slants.

Pathogenicity tests. Three sunflower cultivars (Dahlgren DO844, RBA 300G, and Stauffer 3101), generally four plants per isolate per test, were used in the pathogenicity studies. Plants were grown in the greenhouse at about 25 C with 12-hr supplemental lighting (about 8,000 lux). More than 100 field-grown sunflower plants (Stauffer 3101) were also inoculated.

For most tests, mass-isolates grown on PDA plates for 10–15 days at 25 C were cut into 7-mm-diameter agar inoculum disks and inserted in wounds (about 0.5 cm^2, extending into the pith) made in sunflower stems with a dissecting needle at the second or third leaf node of plants 7–9 wk old (ie, flower buds just starting to open to full-bloom). Wounds were then covered with petroleum jelly to prevent desiccation. Wounded uninoculated control plants were included in all tests.

RESULTS AND DISCUSSION

Symptoms and signs. In the field, initial symptoms appeared at the point of attachment of a petiole to the stem (Fig. 1A,B) at nodes on the lower half of the stem. Cankers surrounding the petiole base were brown to black and usually had a definite margin. Lesions later enlarged to include several internodes, sometimes showing streaking beyond the lesion margin and often becoming lighter in color (ash-grey) with age. Black pycnidia developed on dead tissues of diseased plants. Pycnidia were scattered or formed irregular rows within and adjacent to cankers (Fig. 2). Subsequently, perithecia were commonly found on diseased, overwintering stems.

Conspicuous early symptoms of the disease were bronzing and necrosis of the interveinal areas of leaves at and above the stem canker (Fig. 1A,B). Leaf necrosis was followed by progressive wilting and drying of leaves and death of stems above cankers. Bronzing and necrosis were limited to leaves and portions of leaf blades directly in line with a stem canker (Fig. 1B), indicating vertical symptoms were limited to vascular bundles specifically affected, with little or no lateral effect. This progressive, unilateral bronzing and necrosis of stem canker-affected plants could usually be distinguished from the sudden flaccid wilting of all leaves caused by *Sclerotinia sclerotiorum* (Lib.) de Bary. As the disease progressed, affected stems rotted and became weakened, resulting in lodging (Fig. 1C). Disease losses resulted from premature death of some affected plants (ie, smaller heads and light-weight seed) and lodging, which increased the number of heads left in the field at harvest.

Fungi isolated. Typical *Phomopsis* pycnidia containing alpha conidia (Fig. 1D) and a lesser proportion of beta conidia (Fig. 1E) developed on cankered stem sections from field-grown sunflower plants collected in 1981. In the late summer and fall of 1982, pycnidia with only beta conidia as well as those with predominantly alpha and those with both alpha and beta conidia were found on cankered field-grown sunflower stems. Because cankered stems were placed in moist chambers within several days of collection, it appears that seasonal environmental conditions may govern the proportions of spore types formed.

Cultures of the *Diaporthe* sp. and its *Phomopsis* anamorph from stem cankers consistently formed pycnidia and, less

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frequently, perithecia on APDA or PDA plates after variable lengths of time at 25 C under either 8 hr of light per day or continuous light. Perithecia were produced both on agar media and on inoculated stem sections incubated in moist chambers. Clavate asci containing eight two-celled ascospores (Fig. 1E) were produced in mature perithecia. In diseased stem tissue, perithecia were separately erumpent with long necks and the entostroma was effused and not limited, indicating that this fungus belongs in the *D. arctii* group as described by Barr (1).

Also, in 1981, a *Phoma* sp. was isolated (five of 25 isolates) from young stem canker tissues (no pycnidia) of field-grown plants. The pycnidia of the *Phoma* sp. on PDA were much smaller (about 165 μm) than the pycnidia of *Phomopsis* sp. (about 500 μm) and contained elliptical single-celled conidia, distinct from the oblong, single-celled, guttulate alpha conidia of *Phomopsis*.

Pathogenicity tests. All three commercial hybrids were susceptible to the 15 isolates of *Diaporthe* sp. tested. Characteristic leaf and stem symptoms developed on wound-inoculated plants in greenhouse tests (Fig. 1F), whereas wounded, uninoculated control plants did not develop symptoms (Fig. 1G). Some variation in virulence among isolates was noted (ie, size of cankers, numbers of leaves affected, and rate of disease progress), and additionally, the severity of symptoms in plants inoculated with the same isolate often varied from plant to plant. Koch's postulates were fulfilled by reisolating the *Diaporthe* sp. and its *Phomopsis* anamorph from inoculated diseased plants, confirming their identity in agar culture or on plant tissue, and reproducing typical symptoms with the reisolated cultures on wound-inoculated sunflower plants.

Field-grown sunflower plants were successfully inoculated at the early-bloom stage (flowers just opening to partially open). This corresponded with the growth stage when stem cankers and leaf necrosis were first noted in naturally infected plants. Characteristic stem cankers and leaf symptoms were produced on inoculated plants in the field (Fig. 1H,I).

None of the five *Phoma* sp. isolated from surface-sterilized stem canker tissue caused more than marginal darkening of stem tissue immediately surrounding inoculation wounds. Isolates from wounded stem areas again produced only limited blackening of tissue surrounding wounds. At best, these *Phoma* sp. isolates were only very weakly pathogenic in wound-inoculation tests and were not considered a causal pathogen of sunflower stem canker disease.

We have established that a *Diaporthe* sp. and its *Phomopsis* anamorph is the primary causal organism of a stem canker of sunflower occurring in Ohio. This pathogen may be identical or closely similar to *D. helianthi*, which causes a stalk disease of sunflower in Yugoslavia (4–6). Some differences between the symptoms described in Yugoslavia (5)

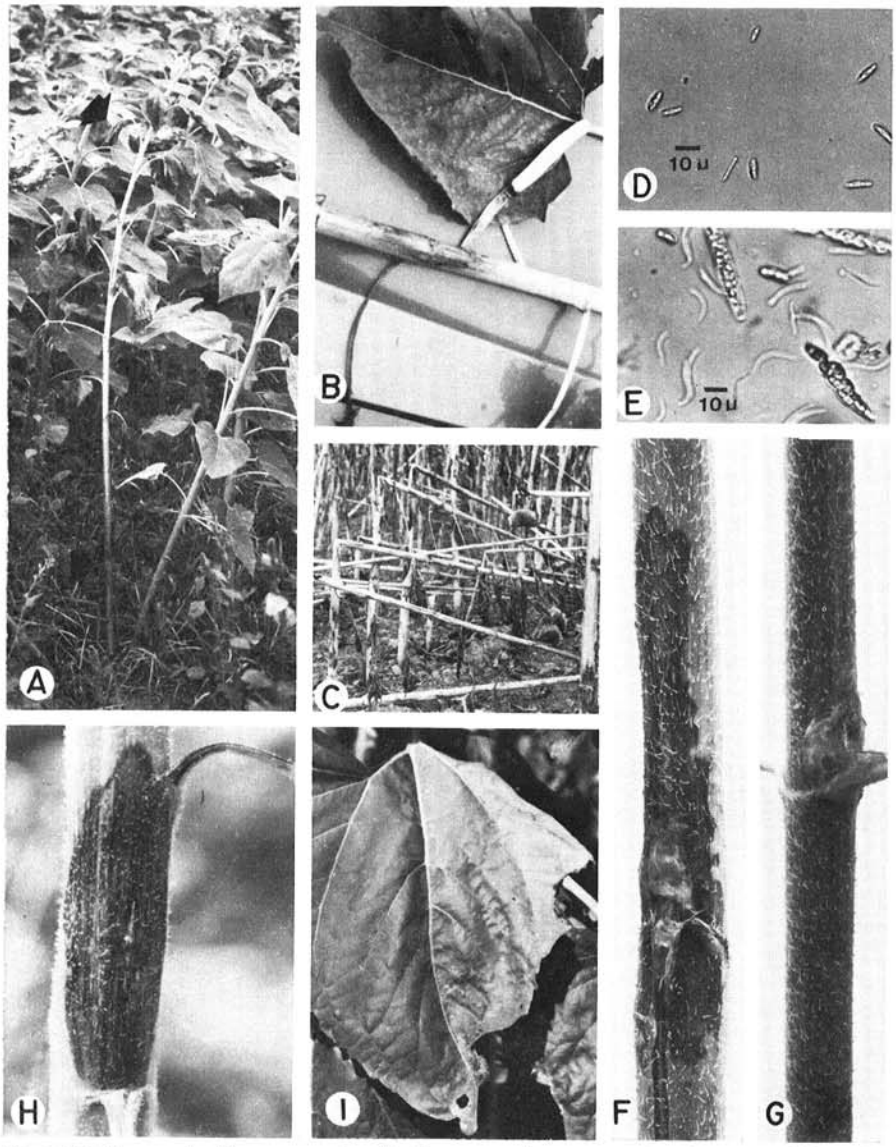

Fig. 1. (A) Stem canker (lower arrow) and leaf symptoms (upper arrow), early stages of disease in field. **(B)** Close-up of early stage stem canker and leaf bronzing-necrosis symptoms. **(C)** Lodging of mature sunflowers in field heavily affected with Diaporthe stem canker. **(D)** Alpha spores of *Phomopsis* stage of *Diaporthe* sp. **(E)** Asci enclosing ascospores of *Diaporthe* sp. and beta spores of *Phomopsis* stage. **(F)** Diaporthe canker symptoms on wound-inoculated sunflower in greenhouse. **(G)** Control stem-wounded sunflower. No symptoms, petroleum jelly covering evident. **(H)** Diaporthe canker symptoms on field wound-inoculated sunflower. **(I)** Leaf symptoms (chlorosis, necrosis, and wilting) on field-grown sunflower wound-inoculated on stem.

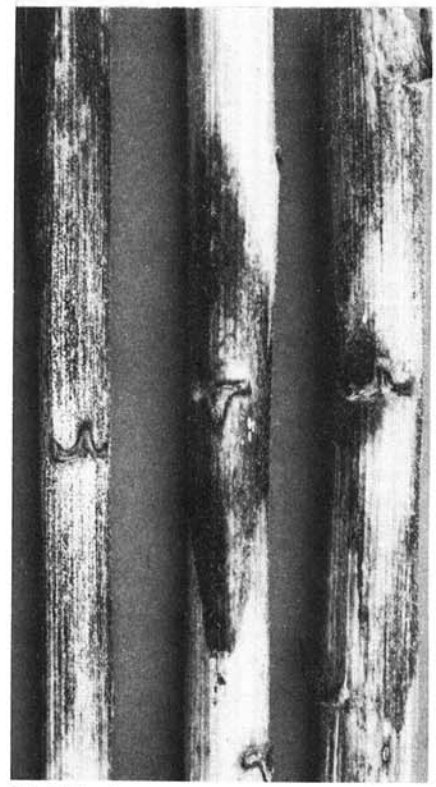

Fig. 2. Canker symptoms on mature sunflower stems showing a range of canker characteristics. Stem on left with pycnidia.

and those found in Ohio included lack of mention of the conspicuous leaf bronzing, necrosis, and wilting in the Yugoslavian report. In addition, discrepancies in the description of the pathogen(s) found in these two areas included the "absolute dominance" of beta spores and infrequent occurrence of alpha spores in pycnidia produced in culture and on plant material in Yugoslavia (6). Reference was also made to the occasional occurrence of C-type conidia in pycnidia formed on plant petioles and stems (6).

In Ohio, pycnidia with predominantly alpha conidia and those with predominantly beta conidia may be found on different areas of the same diseased stem of a field-grown sunflower, but no C-condia have been observed. Preliminary results indicate that the *Diaporthe* sp. and its *Phomopsis* anamorph from sunflower in Ohio may be the same as the *D. helianthi* occurring in Yugoslavia; however, until definitive comparative studies are made, their synonomy cannot be either confirmed or rejected.

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