Symptomatology and Detection of *Macrophomina phaseolina* in Sunflower Plants Parasitized by *Cylindrocopturus adspersus* Larvae

S. M. Yang and D. F. Owen

Research plant pathologist, Conservation and Production Research Laboratory, Agricultural Research Service, U.S. Department Agriculture, P.O. Drawer 10, Bushland TX 79012; and research scientist, Texas A&M University, Agricultural Research and Extension Center, Route 3, Lubbock 79401, respectively.

Approved for publication by the director, The Texas Agricultural Experiment Station, Texas A&M University, College Station 77843.

Mention of a trademark or proprietary product constitutes neither a guarantee or warranty of the product by the U.S. Department of Agriculture nor approval to the exclusion of other products that also may be suitable.

Accepted for publication 10 November 1981.

ABSTRACT

Yang, S. M., and Owen, D. F. 1982. Symptomatology and detection of *Macrophomina phaseolina* in sunflower plants parasitized by *Cylindrocopturus adspersus* larvae. Phytopathology 72:819-821.

The typical symptoms and signs of charcoal rot of cultivated sunflower (*Helianthus annuus* L.) in the Texas High Plains areas are a gray discoloration of the stem and formation of many minute, black sclerotia on the surface of the stem and in the pith tissues. Sunflower stems infected with *M. phaseolina* and parasitized by *C. adspersus* (stem weevil) larvae have a black-to-brown discoloration with or without typical gray areas on the surface of the stem. Pith tissue may be black and partially or completely disintegrated. Portions of the lower stem are hollow. Mixtures of frass and fragments of pith tissue are observed in the lower stem and upper taproot. *M. phaseolina* was isolated from all plants with typical symptoms on potato-dextrose agar amended with antibiotics. The fungus was also detected in most plants parasitized by stem weevil larvae, even when the stems lacked gray discoloration. The pathogen was isolated from internal tissues of the taproots, stem epidermis, pith, stem weevil larvae, and frass, but not necessarily from all these in a given plant. Isolates of *M. phaseolina* from plants parasitized and unparasitized by stem weevil larvae, and from stem weevil larvae, caused brown-and-gray discoloration of charcoal rot in inoculated sunflower plants kept in a growth chamber.

As oilseed sunflower (*Helianthus annuus* L.) production has increased on the Texas High Plains, losses caused by charcoal rot (*Macrophomina phaseolina* (Tassi) Goid.) and by sunflower stem weevil (*Cylindrocopturus adspersus* (LeConte) Coleoptera) larvae also have increased. Sunflower plants parasitized by stem weevil larvae do not show symptoms of charcoal rot as described by Nyvall (2) and Zimmer and Hoes (6). It has not been determined whether sunflower plants parasitized by stem weevil larvae can be infected by *M. phaseolina* or whether stem weevil larvae can vector *M. phaseolina.*

Different symptoms have been described for charcoal rot on cultivated sunflower (2,6). According to Zimmer and Hoes (6), the predominant symptoms are gray-to-black discoloration and shredding of plant tissue at the base of the stem and the top of the taproot, with the lower stem hollow and rotten. Nyvall (2) described charcoal rot symptoms and signs as a discoloration of the lower stem, shredding of the vascular bundles (fibers), and presence of many black sclerotia. He did not specify the color of the base of the stem.

This paper describes the common symptoms of charcoal rot of cultivated sunflower on the Texas High Plains. It also reports the isolation of *M. phaseolina* from the plants parasitized by larvae of the stem weevil and from the surface of stem weevil larvae. Knowledge of charcoal rot symptomatology and methods for detecting *M. phaseolina* in sunflower plants parasitized by stem weevil larvae will be valuable for diagnosing charcoal rot and selecting breeding lines for resistance to *M. phaseolina* in the field.

MATERIALS AND METHODS

Symptomatology. Sunflower plants with discolored stems were collected at random from two experimental plots and a commercial field. To relate *M. phaseolina* with stem discoloration and with parasitization (infestation) by larvae of the stem weevil, 300 sunflower stems were examined externally and internally for signs of disease and the presence of stem weevil larvae.

Detection of *M. phaseolina.* The pathogen was isolated on potato-dextrose agar amended with streptomycin and penicillin (PDA-SP, 10 to 15 ml per plate) (both antibiotics were obtained from Calibiochem-Behring, San Diego, CA 92112). The PDA-SP medium was prepared by boiling sliced tissues of potato (200 g) in 500 ml of distilled water for 1 hr, straining the potato juice into melted agar (23 g), adding dextrose (20 g) and distilled water to make up 1 L of medium. The medium was autoclaved at 121 C for 20 min. Penicillin G potassium salt (30 mg) and streptomycin (100 mg) in 5 ml of distilled water were added aseptically to the medium just before pouring.

Larvae-parasitized plants were sampled for the pathogen by removing five tissue pieces each from the internal part of the taproot, stem epidermis, and pith tissue between the first (close to the soil) and seventh internodes. Larvae from the root or basal part of stems, and mixtures of frass and fragments of pith at the base of the stem also were sampled for the presence of *M. phaseolina.* When available, five larvae from a given plant were used; some plants had fewer larvae. Samples were not surface sterilized and larvae were first placed on a piece of clean paper to reduce the transfer of frass before being transferred to the PDA-SP medium by using clean forceps. All instruments were dipped in alcohol and flamed between samplings. For plants with symptoms and signs of charcoal rot, but without larvae, samples were taken only from the stem epidermis or from the pith.

To be assured that *M. phaseolina* appearing on the PDA-SP medium was grown directly from sampled tissue and larvae, and not contaminated from the air or transfer forceps, the PDA-SP medium in several plates was also touched by the clean transfer forceps at five different places per plate. All plates were kept on laboratory benches (21 ± 2C) for 14 days. Formation of black, effuse colonies with sclerotia indicated the presence of *M. phaseolina* on the plate.

Determination of the virulence of isolated *M. phaseolina.* Inoculation tests were performed to determine the virulence of isolates of *M. phaseolina* to sunflower. Isolates of *M. phaseolina*

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

from stem weevil larvae, and from infected plants harboring and not harboring larvae, were transferred to new PDA-SP medium and subsequently to wheat-grain medium. The wheat-grain medium was prepared as described by Yang and Thomas (5) except that the wheat grains were not soaked overnight in water. The medium was prepared by autoclaving 5 g of wheat grains in 15 ml of tap water in a 250-ml flask for 1–2 hr on two consecutive days. For inoculation of sunflower, a wheat grain with sclerotia from a 2- to 3-wk-old culture was inserted into a hole in the internode and sealed with plastic adhesive tape. Holes were made to a depth of 0.3 to 0.5 cm with a metal needle (0.3 cm in diameter) in the internode either near the ground, or in the middle part of a stem of sunflower plants that had flowered. Plants inoculated with autoclaved wheat grain served as control. Inoculated plants were kept at 30 C in a growth chamber and were inspected weekly to detect symptoms and signs of *M. phaseolina* infection.

RESULTS

Symptomatology. Field-collected sunflower plants were classified into three groups based on discoloration of the stem surface and the presence of stem weevil larvae or their tunnelings in the stems.

Group I. Sunflower plants in this group showed common symptoms and signs of charcoal rot, but were not parasitized by stem weevil larvae. Symptoms and signs of infection includes gray discoloration of the stem and the presence of numerous minute, black sclerotia in the pith and on the inside wall below the discolored areas in the hollow stem. Some plants had black and brown discoloration of the lower stem and gray discoloration extending up from the third internode. Shredding of the epidermal stem tissues (Fig. 1) and hollowing of the lower part of the stem were evident in plants after flowering and when the plants were mature.

The symptoms and signs associated with charcoal rot for the other two groups of sunflower plants parasitized by stem weevil larvae were as follows:

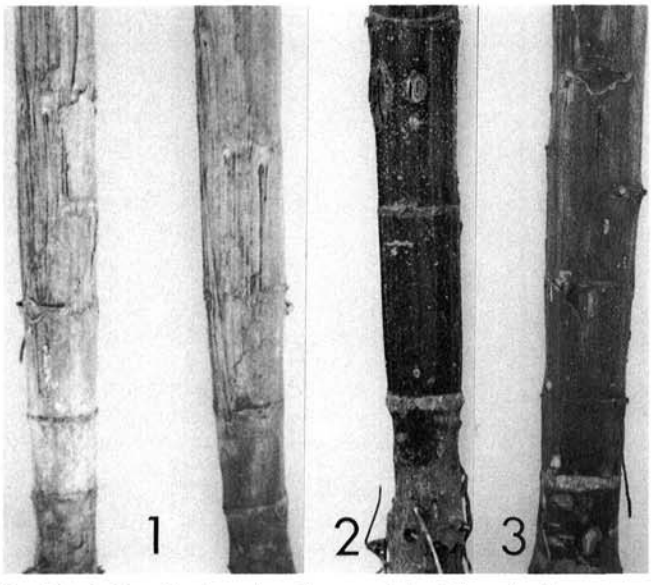
Group II. Stems exhibited black-to-brown discoloration of the stem from the base (Fig. 2) to the middle or upper part. Pith tissue sometimes was partially or completely disintegrated and discolored, usually black. In most infected plants, portions of the lower stem were hollow. Mixtures of frass and fragments of pith tissue were present in the lower stems and upper taproot. Sclerotia of *M. phaseolina* were not readily visible on the stem epidermis or in the pith tissue because of the black discoloration.

Group III. The symptomatology of infection in plants of this group was similar to that described for plants in Group II except, there was a gray to dark-gray discoloration of some internodes (Fig. 3). Some larvae-parasitized plants had only gray discoloration; these could be easily identified in the field as diseased with charcoal rot because sclerotia were visible on the stem epidermis and in the pith.

Detection of *M. phaseolina*. *M. phaseolina* was isolated from all tissue samples taken from plants that showed typical charcoal rot symptoms and signs and not parasitized by stem weevil larvae. This pathogen was also isolated from 64 and 81% of the plants parasitized by stem weevil larvae in Groups II and III, respectively (Table 1). *M. phaseolina* was detected on PDA-SP medium from all plant parts sampled from larvae-parasitized plants, but not every sample from a given plant yielded this pathogen. Samples were not taken from plants in Group III showing only gray discoloration since sclerotia were visible on the discolored area.

Tissue samples from plants showing typical charcoal rot symptoms and not parasitized by stem weevil larvae yielded sclerotia of *M. phaseolina* on PDA-SP medium within 4 days. Plant tissues (Fig. 4) and larvae from larvae-parasitized plants also yielded sclerotia of *M. phaseolina* within 1 wk. *M. phaseolina* from larvae-parasitized plants and from larvae was not different from that isolated from unparasitized plants having common symptoms and signs of charcoal rot. Among the total of 848 larvae tested, 246 (29%) were positive for *M. phaseolina*. *M. phaseolina* was not detected on PDA-SP plates that were touched only by clean forceps, indicating that the *M. phaseolina* in the plates came from the samples.

Determination of virulence of isolated *M. phaseolina*. Virulence tests showed that *M. phaseolina* from stem weevil larvae and from larvae-parasitized and unparasitized plants induced brown-to-gray discoloration with the production of sclerotia in inoculated plants. Control plants did not develop charcoal rot symptoms.



Figs. 1–3. 1, Gray discoloration of stem and shredding of epidermal stem tissue, common symptoms of charcoal rot on cultivated sunflower not parasitized by stem weevil (*Cylindrocopturus adspersus*) larvae on Texas High Plains. **2,** Black discoloration on the surface of stem of a sunflower plant infected with *Macrophomina phaseolina* and also parasitized by stem weevil larvae. **3,** Black discoloration with gray areas on the surface of stem of a sunflower plant infected with *M. phaseolina* and also parasitized by the stem weevil larvae.

TABLE 1. Isolation on potato-dextrose agar amended with penicillin and streptomycin of *Macrophomina phaseolina* from stem weevil larvae and from different parts of stem weevil-parasitized sunflower plants

Isolation from	% Stem weevil-parasitized plants yielding *M. phaseolina* on PDA-SP medium[a]	
	Group II	Group III
Stem weevil larvae only	2	1
Internal part of taproot only	8	2
Mixture of frass and fragments of pith only	1	2
Stem weevil larvae and one or more plant parts[b]	19	7
Stem weevil larvae and one or more plant parts[b] and frass and fragments of pith tissues	13	43
Two or more plant parts[b] only	0	6
Two or more plant parts[b] and frass and fragments of pith tissues	21	20
Total	64	81

[a] Each group contained a total of 100 plants taken from two experimental fields and one commercial field. Group II, sunflower plants with black and brown discoloration of stem, and Group III, had symptoms similar to those in Group II except that gray to dark gray discoloration appeared in some internodes between the first (near soil surface) and seventh nodes. Group I (not in Table), sunflower plants not parasitized by stem weevil larvae had gray discoloration of the stems and many minute, black sclerotia on the surface of stems and in the pith. *M. phaseolina* was isolated from 100% of plants in Group I.

[b] Plant parts tested were the internal part of taproot, stem epidermis, and pith.

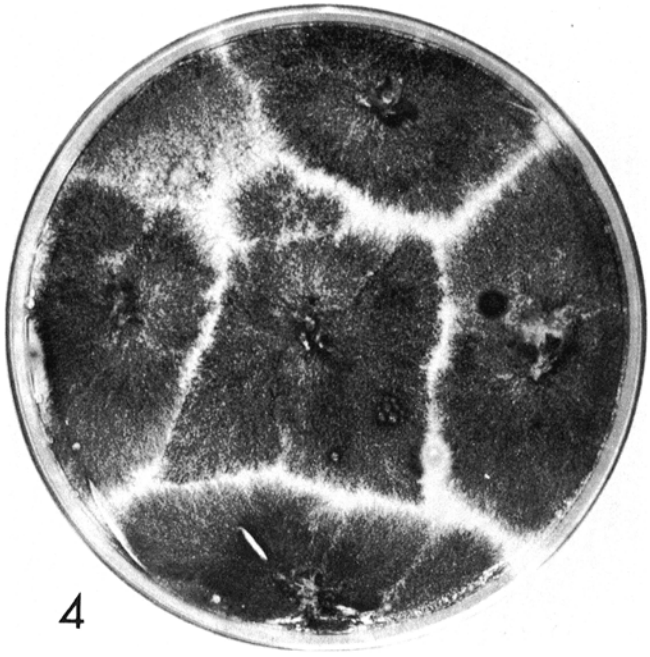


Fig. 4. Colonies of *Macrophomina phaseolina* on potato-dextrose agar amended with antibiotics from pith tissues of a sunflower plant parasitized by a stem weevil larvae.

DISCUSSION

Charcoal rot symptoms in sunflower may vary with plant maturity at the time of observation. Charcoal rot of cultivated sunflower not parasitized by stem weevil larvae on the Texas High Plains is characterized by a combination of symptoms described by Nyvall (2) and Zimmer and Hoes (6). However, we observed that the gray discoloration is not limited to the base of infected stems as described by above authors.

Sunflower plants parasitized by stem weevil larvae and infected by *M. phaseolina* showed external black-to-brown discoloration on the stems. Injury to the stems by tunneling larvae may facilitate the growth of saprophytic fungi, which may contribute to the development of the black-to-brown discoloration. The role of saprophytic fungi in the development of typical symptoms of charcoal rot on the stems of plants harboring stem weevil larvae needs further study.

The incidence of *M. phaseolina* on larvae in stem weevil-parasitized sunflower plants and in larvae-parasitized plants that do not show typical symptoms of charcoal rot in the field can be determined by culturing samples on PDA-SP medium. Tissue samples should be taken from more than two sites within a plant for an accurate estimate, because our studies showed that not all samples from a given infected plant yielded *M. phaseolina* on PDA-SP medium (Table 1). The use of selective media developed for direct isolation of *M. phaseolina* from soil (1,3) may increase the recovery of *M. phaseolina* from tissue samples collected from stem weevil larvae-parasitized sunflower stems and thus may reduce the number of tissue samples required.

Stem weevil larvae often were detected in early-planted sunflower, but were rarely found in late-planted sunflower on the Texas High Plains (4). The high incidence of *M. phaseolina* in sunflower plants parasitized by stem weevil larvae indicates that planting early to reduce loss from charcoal rot as recommended by Zimmer and Hoes (6) may not be effective in the Texas High Plains areas where plants may become parasitized by stem weevil larvae. The charcoal rot pathogen isolated from larvae or from tissues of parasitized sunflower plants was as virulent as that isolated from sunflower plants showing typical charcoal rot symptoms and not parasitized by the stem weevil larvae. In our laboratory, plans have been made to further investigate the significance of stem weevil larvae in the development of charcoal rot.

LITERATURE CITED

1. Meyer, W. A., Sinclair, J. B., and Khare, M. N. 1973. Biology of *Macrophomina phaseolina* in soil studied with selective media. Phytopathology 63:613-620.
2. Nyvall, R. F. 1979. Diseases of sunflowers (*Helianthus annuus* L.). Pages 341–350 in: Field Crop Disease Handbook. H. A. Nyvall, ed. AVI Publishing Co., Westport, CT.
3. Papavizas, G. C., and Klag, N. C. 1975. Isolation and quantitative determination of *Macrophomina phaseolina* from soil. Phytopathology 65:182-187.
4. Rogers, C. E., and Jones, O. R. 1979. Effect of planting date and soil moisture on infestation of sunflower by larvae of *Cylindrocopturus adspersus*. J. Econ. Entomol. 72:529-531.
5. Yang, S. M., and Thomas, C. A. 1981. Comparison of techniques for inoculating sunflower heads with three species of *Rhizopus*. Phytopathology 71:458-460.
6. Zimmer, D. E., and Hoes, J. A. 1978. Diseases. Pages 225–262 in: Sunflower Science and Technology. J. F. Carter, ed. Am. Soc. Agron., Crop Sci. Soc. of America, and Soil Sci. Soc. of America, Madison, WI.