Transmission of *Macrophomina phaseolina* in Sunflower by *Cylindrocopturus adspersus*

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A contribution from USDA, Agricultural Research Service, in cooperation with The Texas Agricultural Experiment Station, Texas A&M University, College Station 77843.

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Accepted for publication 18 May 1983.

ABSTRACT

Yang, S. M., Rogers, C. E., and Luciani, N. D. 1983. Transmission of *Macrophomina phaseolina* in sunflower by *Cylindrocopturus adspersus*. Phytopathology 73:1467-1469.

Seventeen of 123 adult *Cylindrocopturus adspersus* (sunflower stem weevil) collected from the internodes and roots of overwintered sunflower (*Helianthus annuus*) plants, and four of 133 adult *C. adspersus* collected from growing sunflower plants in the field yielded *Macrophomina phaseolina*, the causal agent of charcoal rot, on PDA-SP medium. Seven of 168 adults carried sclerotia of *M. phaseolina*. This pathogen was isolated on PDA-SP medium from three oviposition sites of *C. adspersus* on two of 38 field-grown sunflower plants, but was not isolated from subterranean parts of the infected plants. Three of 45 sunflower plants fed on by adult *C. adspersus* showed brown external discoloration of stems and black sclerotia in the pith, typical symptoms of infection by *M. phaseolina*. Control plants did not exhibit symptoms of infection. These results indicate that a small percentage of adult *C. adspersus* carry *M. phaseolina* externally and transmit the pathogen to sunflower during oviposition via that sealed egg cavity in the stalk.

Additional key words: charcoal rot, *Helianthus annuus*, stem weevil.

On the Texas High Plains, *Cylindrocopturus adspersus* (LeConte), a stem-infesting weevil of sunflower, overwinters in the larval stage in the root, crown, and adjacent stalk bases of sunflower (*Helianthus annuus* L.) and emerges as an adult in the late spring and early summer of the succeeding year (2). The adults feed on foliage and lay eggs beneath epidermal tissues in the lower one-third of the sunflower stalk (3). During oviposition, the female chews a small hole through the stalk epidermis, deposits an egg in the cavity, and immediately seals the cavity with an anal secretion. The larva emerges 1–2 wk later, feeds on subepidermal tissue, and tunnels into fibrovascular bundles where subsequent development occurs (3).

Yang and Owen (6), who reported the isolation of *Macrophomina phaseolina* (Tassi) Goid., the causal agent of charcoal rot, from field-collected larvae of *C. adspersus*, assumed that the adults might carry the pathogen from debris in the soil and subsequently transmit the pathogen to sunflower during oviposition in the stems. This study was designed to determine whether adult *C. adspersus* carry *M. phaseolina*. *M. phaseolina* is found in the egg cavity or oviposition site on sunflower stalks, and *M. phaseolina* carried by *C. adspersus* causes charcoal rot in sunflower plants.

MATERIALS AND METHODS

Isolation of *M. phaseolina* from adult *C. adspersus* and oviposition sites. Untreated adult *C. adspersus* collected from the lower stalk and root of overwintered sunflower plants (dead but still standing in the field) in April/May of 1980 and 1981 and from sunflower fields in June of 1981 were placed in petri plates (one adult per plate) of potato-dextrose agar (BBL, Microbiology Systems, Becton-Dickinson & Co., Cockeysville, MD 21030, USA) amended with additional agar (8 g/L), penicillin G potassium (30 mg/L), and streptomycin (100 mg/L) (PDA-SP, both antibiotics were from Calbiochem-Behring, San Diego, CA 92112, USA). Some adult *C. adspersus* that were extracted from overwintered sunflower plants were soaked in 1% NaOCl solution for 20 min before being placed on PDA-SP medium.

Sealed oviposition sites on sunflower plants grown in the field were sampled. The tissues from the oviposition sites were placed on PDA-SP medium (samples from the same plant were placed on the same plate). Four tissue samples were also taken from the inside of each root and crown of the same plant and placed on PDA-SP medium. The tissue samples were surface sterilized in 1% NaOCl solution for 5 min before plating on the PDA-SP medium.

Detection of *M. phaseolina* on adult *C. adspersus* by light microscope. Pupae of *C. adspersus* were taken from the lower stalks and tap root of 80 overwintered sunflower plants in March 1983 and placed in plastic cups that were covered with paper covers. Pupae from the same plants were placed in the same cup. Precautions were taken not to transfer the plant materials into the cup with the pupae. The pupae in the cups were incubated (27.5 ± 0.5 C, 14 hr light and 10 hr darkness) until the emergence of adults. The adults were then transferred to PDA-SP medium in disposable petri dishes (one to three adults from the same cup in the same dish) and examined with the cover on under the microscope (×4 objective, ×10 ocular) for the presence of sclerotia of *M. phaseolina* on the body of adult *C. adspersus*. The plates were incubated on the laboratory benches (25 ± 1 C) for 2 wk. Ten PDA-SP medium plates similarly touched with sterile forceps were used as control.

Feeding of adult *C. adspersus* on young sunflower plants. Adults from overwintered plants and from field-grown sunflower plants were caged on sunflower hybrid cultivar 894 plants in the 2.1 (first alternate leaf formed) and 2.2 (second alternate leaf formed) vegetative stages of growth (5). Five to 10 adult *C. adspersus* and one to three plants in a 22-cm-diameter pot were surrounded by cylindrical transparent plastic cages 15 cm in diameter by 40 cm tall (a total of 25 replicated pots in two experiments). The caged plants were kept on a bench in the headhouse at 21 ± 2 C for 2 days, in the greenhouse at 25 ± 3 C for 5 days, and in a growth chamber at 30 C,

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14 hr light/10 hr dark for an additional 24 days. Adults of *C. adspersus* were allowed to feed on 10 (one plant per pot; five pots) and 35 (two to three plants per pot; total five pots) sunflower plants, respectively, in 1980 and 1981. Ten sunflower plants (two per pot) that were not fed on by adult *C. adspersus* were used as controls in each year. The plants were inspected weekly for 4–5 wk to detect symptoms of infection by *M. phaseolina*. At the end of the tests, the tissue from the tap root and pith of each of the pathogen-infested and control sunflower plants were plated on PDA to detect fungal infection.

Determination of pathogenicity of isolated *M. phaseolina*. Inoculation tests were performed to determine the virulence of isolates of *M. phaseolina* from *C. adspersus* adults. Two isolates of *M. phaseolina* were grown on wheat-grain medium that was prepared by autoclaving 5 g of wheat grains in 15 ml of tap water in a 250-ml flask for 1 to 2 hr on two consecutive days. Twenty sunflower plants (10 for each isolate) were inoculated by inserting a wheat grain infested with sclerotia from a 2- to 3-wk-old culture into the internode pith 30 cm above the soil surface. To simulate oviposition behavior of female *C. adspersus*, holes were made to a depth of 3–5 mm with a 3-mm-diameter sterilized metal needle in the internodes of sunflowers that had flowered. The holes were sealed with plastic adhesive tape. Plants inoculated with an autoclaved wheat-grain served as controls. Inoculated plants were kept at 30 C in a growth chamber and were inspected weekly for 4–5 wk for symptoms of infection by *M. phaseolina*.

Incidence of charcoal rot on sunflower in field plots sprayed with carbofuran and tap water. Sunflower hybrid cultivar 894 was planted in eight four-row plots (each row was 6 m long and spaces between rows were 1 m) on 5 May 1982. Sunflower were then thinned to 30 plants per row on 19 May 1982. Four plots were randomly selected and sprayed with carbofuran (1 kg active ingredient per hectare) and the other four plots were sprayed with water by hand four times at 2-wk intervals from 20 May to 1 July 1982. Sunflower in the center two rows in each plot were harvested by hand on 8 September 1982. The number of plants infected by *M. phaseolina* alone and that parasitized by *C. adspersus* were determined in the field just before harvest. However, the number of sunflower plants parasitized by *C. adspersus* and simultaneously infected by *M. phaseolina* was determined in the laboratory by the method of Yang and Owen (6). All of the sunflower plants parasitized by *C. adspersus* in each plot sprayed with carbofuran and 40 sunflower plants parasitized by *C. adspersus* in each plot sprayed with water were collected and brought to the laboratory for assay of *M. phaseolina*. Four pieces of stalk epidermal tissue and four pieces of pith tissues taken randomly from five basal internodes of each stalk were placed on PDA-SP medium (four pieces per plate). All the plates were incubated for 2 wk on laboratory benches (25 ± 1 C). Formation of sclerotia on the PDA-SP medium indicated the presence of *M. phaseolina* in the stalks.

RESULTS

Isolation of *M. phaseolina* from adult *C. adspersus* and oviposition sites. *M. phaseolina* was isolated from 12 of 70 and five of 53 adult *C. adspersus* that were collected from overwintered sunflower plants in the springs of 1980 and 1981, respectively. Also, *M. phaseolina* was isolated from four of 133 adult weevils that were collected from field-grown sunflower plants in 1981. Three of 62 adult weevils that were collected in 1980 from overwintered sunflower plants, and that had been soaked in 1% NaOCl solution for 20 min, still yielded *M. phaseolina* on PDA-SP medium.

M. phaseolina was isolated from tissues of three oviposition sites located on two of the 38 field sunflower plants, but this fungus was not isolated from the roots and crowns of the two same sunflower plants.

Detection of *M. phaseolina* on adults of *C. adspersus* by light microscope. Seven of the 168 adults of *C. adspersus* from the 80 overwintered sunflower plants carried sclerotia of *M. phaseolina* directly on the body (three adults) and indirectly in the microscopic plant debris adhering to the ovipositor (one adult) or to the tarsus and claws of the hind legs (three adults). The seven adults were taken from six overwintered sunflower plants and yielded *M. phaseolina* on PDA-SP medium. Ten PDA-SP medium plates containing 22 adults taken from ten overwintered sunflower plants also developed *M. phaseolina*. The ten control plates and the other 64 plates containing adults did not develop *M. phaseolina*.

Adult *C. adspersus* fed on young sunflower plants. Among the sunflower plants fed on by adults of *C. adspersus*, two of 10 in 1980, and one of 35 in 1981, respectively, showed brown discoloration of the stalks and black sclerotia in the pith, and the plants were killed by the pathogen. *M. phaseolina* was isolated from the pith but not from tap roots of the three plants. The pathogen was not isolated from the other 42 insect-infested plants nor from the 20 control plants.

Determination of the pathogenicity of isolated *M. phaseolina*. Inoculation tests showed that *M. phaseolina* from adults of *C. adspersus* induced brown and gray discoloration of the stalk and produced black sclerotia in the pith of all 20 inoculated plants. Control plants did not develop symptoms.

Incidence of charcoal rot on sunflower in field plots sprayed with carbofuran and tap water. Carbofuran significantly reduced the number of plants parasitized by *C. adspersus*, and the number of plants simultaneously parasitized by *C. adspersus* and infected by *M. phaseolina* (Table 1). Average yield of sunflower in plots sprayed with carbofuran (5.10 kg per plot) was significantly greater than that of sunflower in plots sprayed with water (3.08 kg per plot).

TABLE 1. Average percent of sunflower plants infected by *Macrophomina phaseolina* and *Cylindrocopturus adspersus* alone or in combination in sunflower plots sprayed with carbofuran and water[a]

Treatment	*M. phaseolina*	*C. adspersus*	*M. phaseolina* + *C. adspersus*
Carbofuran	11	12	4
Water	2	25	45
$t_{0.05} = 2.45$	4.9	−3.0	−5.5

[a] Average of four plots. Each plot had 120 sunflower plants.

DISCUSSION

The results of our study indicate that on the Texas High Plains, some adult *C. adspersus* carry *M. phaseolina* as they emerge from overwintered sunflower roots and stalks, and subsequently transmit the pathogen to sunflower plants while feeding and ovipositioning in the stalks. Apparently, *M. phaseolina*, inoculated in the egg cavity by ovipositioning *C. adspersus*, grows and spreads through the stalk via larval tunnels. Previously, Yang and Owen (6) had isolated *M. phaseolina* from larval *C. adspersus* and some stalks, but not from the subterranean parts of plants harboring larval *C. adspersus*.

Microscopic examination of adult *C. adspersus* emerging from the pupae that were incubated in the cups indicated that adults of *C. adspersus* carried sclerotia of *M. phaseolina* directly on the body and/or indirectly on the plant debris that stuck to the ovipositor or attached to the hind legs. The plant debris was introduced into the cup with the pupae. The adults, after emerging from pupae, contacted the plant debris by walking in the cup. This result indicated that adults of *C. adspersus*, after emerging from pupae under field conditions, could contact and carry sclerotia of *M. phaseolina* by walking in the larval tunnels.

Transmission of *M. phaseolina* in sunflower plants by adults of *C. adspersus* can be reduced by the control of adult *C. adspersus* with carbofuran in the field; control of the adult significantly reduced the number of sunflower plants parasitized by larvae of *C. adspersus* and the incidence of charcoal rot.

M. phaseolina persists in plant debris in the soil and attacks subterranean plant tissues when the sunflower plant is growing under stressful conditions (7). Sclerotia of *M. phaseolina* also occur

on the seed pericarp and under the seed coat (1). As shown in Table 1, transmission of *M. phaseolina* by some adults of *C. adspersus* to sunflower plants on the Texas High Plains does not exclude infection of sunflower plants by *M. phaseolina* through subterranean plant tissues, nor does it exclude transmission of this pathogen by seeds (1).

Short et al (4) reported that about 50% of the sclerotia of *M. phaseolina* extracted from soybean tissues were able to survive submersion for 10 min in 0.5% NaOCl solution, but that mycelia of this pathogen were killed by 15 sec in the same solution. Our study indicates that some sclerotia of *M. phaseolina* carried by adults of *C. adspersus* survived for 20 min in 1% NaOCl solution.

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