Macrophomina phaseolina, Another Cause of the Twin-Stem Abnormality Disease of Soybean

P. R. BRISTOW, Washington State University, Western Washington Research and Extension Center, Puyallup 98371, and T. D. WYLLIE, Department of Plant Pathology, University of Missouri, Columbia 65211

ABSTRACT

Bristow, P. R., and Wyllie, T. D. 1986. *Macrophomina phaseolina*, another cause of the twin-stem abnormality disease of soybean. Plant Disease 70: 1152-1153.

Symptoms of the twin-stem abnormality disease were observed on seedlings of eight of 14 genotypes when apparently healthy seed was sown in vermiculite infested with *Macrophomina phaseolina*. Infected genotypes were Adelphia, Amsoy-71, Calland, Clark-63, Hill, Mandarin, Mukden, and Williams. Disease incidence ranged from 7 to 45%. The genotypes A-100, A.K. (Harrow), Beeson, Cutler, Dunfield, and PI 92-964 did not show the twin-stem symptom and were apparently resistant. This abnormality disease, though reported to be caused by *Sclerotium* sp., may be a general response when seedlings are infected by several seedborne or soilborne pathogens.

Additional key words: charcoal rot, microsclerotia

Charcoal rot (*Macrophomina phaseolina* (Tassi) Goid.) of soybean (*Glycine max* (L.) Merr.) is usually noted after midsummer and is considered a disease of the mature plant (12); however, seedlings and young plants are also attacked (1). Under favorable disease conditions, seedlings damp-off (10). This may result when clean seed is planted into infested soil (10) or when infected seed is used (6). The twin-stem abnormality disease of soybean is reportedly caused by a seedborne species of *Sclerotium* (4). In this paper, we report symptoms of this disease produced in growth chamber experiments on different soybean genotypes when inoculated with *M. phaseolina*. The effects of *M. phaseolina* on other growth parameters have been published (2).

MATERIALS AND METHODS

M. phaseolina, strain S (13), was grown in potato-dextrose broth (PDB) (50 ml/250-ml Erlenmeyer flask) in darkness at 33 C for 3 wk. Mycelia with microsclerotia were washed three times in sterile distilled water and air-dried. The dry fungal mat from each flask was broken apart with a spatula, then suspended in 10 ml of a 2% sucrose solution for 2 days at 33 C. The mass of hyphae and microsclerotia was diluted with sterile distilled water to a volume of 250 ml and blended at slow speed for 15 sec in a Waring Blendor. The contents of one flask were thoroughly mixed with enough sterile vermiculite to fill a sterile clay pot (1,550 cm^3). The number of propagules added to the vermiculite was determined by diluting a known volume of blended inoculum with 0.2% water agar and plating aliquots on chloronebmercuric chloride-rose bengal agar, a semiselective medium for *M. phaseolina* (9). Four extra flasks per experiment were used to determine inoculum density. Inoculum, prepared as above but autoclaved after blending, was mixed with vermiculite to serve as the control. Prepared pots were covered with aluminum foil and incubated for 2–3 days in a growth chamber before seed was planted. Growth chamber conditions both before and after planting were a 15-hr photoperiod (fluorescent plus incandescent light, $2.1–2.2 \times 10^4$ lux) at 29.5 C in light and 24 C in darkness with relative humidity > 50%.

Only undamaged and healthy-appearing certified seeds were used (seed of PI 92-694 was not certified but met the other criteria), and before use, they were surfaced-sterilized (0.5% NaOCl for 1 min followed by three short rinses in sterile distilled water and then air-dried). Seeds were planted four or five per pot 2.5 cm beneath the surface of the vermiculite. Pots were watered daily after planting with 250 ml of either Hoagland's solution (7) or distilled water. Hoagland's solution was used at planting and every other day for the first week, and thereafter, every seventh day. Alternate irrigations were done with distilled water. Excess liquid drained from the pots. Two to five replicate pots were used per treatment for each genotype tested. Experiments were terminated 23–24 days after planting.

RESULTS

No symptoms of the twin-stem abnormality disease were observed on any genotype in the negative controls. The moderately severe type II twin-stem symptom (4) was observed on seedlings of eight of the 14 genotypes tested. Genotypes affected and incidence of the twin-stemming were as follows: Adelphia, 45%; Williams, 25%; Hill, 24%; Mukden, 20%; Calland, 17%; Amsoy-71, 16%; Mandarin, 14%; and Clark-63, 7%. No symptoms of the disease occurred on seedlings of the following genotypes grown in infested vermiculite: A-100, A.K. (Harrow), Beeson, Cutler, Dunfield, and PI 92-694. Results are a summary of six separate experiments, and though not all genotypes were included in each test, at least one symptomatic seedling of the eight genotypes was present in every test that included that genotype.

Seedling emergence was unaffected despite a propagule density of 856 ± 89 cfu/cm^3 of vermiculite.

The germinating seeds were readily colonized by *M. phaseolina*, with microsclerotia forming in the seed coat. The microsclerotia were observed as the seedlings emerged (4–6 days after planting in infested vermiculite). Although microsclerotia vary in size (11), those that formed in the seed coat averaged 44.8 ± 4.1 μm in diameter, similar in size to those reported in plugged xylem vessels (8), and were located in the hollow spaces (33–57 μm) of the "hour glass" cell layer of the seed coat (between the upper palisade cell and the parenchyma cell layers) (12).

The seed coat either remained in the vermiculite as the cotyledons emerged or adhered to one of the cotyledons. The expression of twin stems, however, was not related to the adherence of the seed coat to cotyledons.

Contribution from the Missouri Agricultural Experiment Station, Journal Series No. 10074.

Accepted for publication 22 July 1986 (submitted for electronic processing).

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.

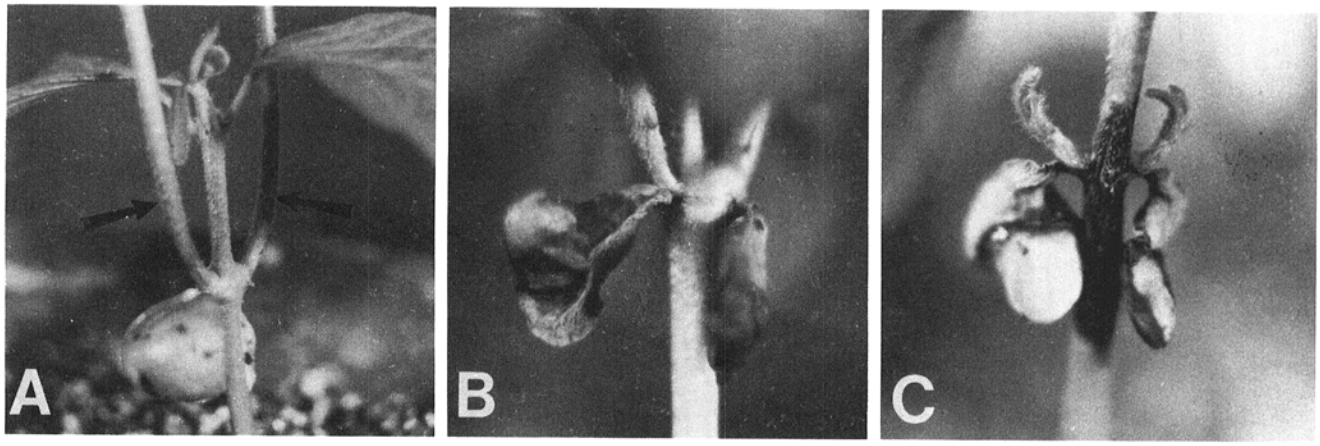

Fig. 1. Responses of soybean seedlings to *Macrophomina phaseolina* 17 days after seeds were sown in infested vermiculite.**(A)** Twin-stem symptom (arrows) and small necrotic spots on cotyledons (Clark-63), **(B)** twin-stems and complete necrosis of cotyledons (Hill), and **(C)** stem lesion formed at cotyledonary node from infected cotyledons (Adelphia).

Symptoms on cotyledons ranged from small black lesions (most common, Fig. 1A) to total necrosis and the formation of microsclerotia (least common, Fig. 1B). Occasionally, a lesion occurred on the stem at the cotyledonary node and developed from infected cotyledons (Fig. 1C). The epicotyl and plumule of only one twin-stemmed seedling died, and it contained microsclerotia. Usually the central epicotyl and plumule remained green but developed slowly and abnormally (Fig. 1A).

DISCUSSION

M. phaseolina was readily isolated from the seed coats of naturally infected soybean seeds with indefinite black spots or blemishes but not from the cotyledons or embryos (6). Dhingra and Muchovej (4) could not isolate *Sclerotium* sp. from seed although it was readily recovered from hypocotyls, primary meristems and primary leaves of symptomatic seedlings. Neither of severe type I nor the less severe type III symptoms (4) were observed in our tests. The type III symptom is characterized by excessive elongation of the first internode and cotyledonary nodes that do not break. Seedlings of all genotypes grown in infested vermiculite, however, were shorter than those in the corresponding negative controls (2).

Gangopadhyay et al (6) reported 44% of seeds infected with *M. phaseolina* and planted in sterile soil produced only a radicle with no hypocotyl extension or cotyledon development. They apparently were observing the severe type I symptom of this abnormality disease where the epicotyl fails to elongate. Meyer et al (10) reported reduced emergence and reddish brown lesions on hypocotyls when seeds were sown in soil infested with different types of inoculum, but they did not mention the twin-stem symptom.

Why twin-stemming occurs is not known, and possibly, the mechanism for *M. phaseolina* and *Sclerotium* sp. is different even though symptoms are the same. *M. phaseolina* may produce toxic metabolites that interfere with apical dominance. A heat-stable phytotoxin partially purified from culture filtrate of *M. phaseolina* and from tissues of inoculated soybean seedlings produce symptoms typical of those observed on inoculated seedlings just before their death (5). *Sclerotium bataticola* produces a non-host-specific toxin that induced necrotic spots on sunflower but not on soybean leaves (3). Virulence of the four test isolates of *S. bataticola* did not correlate with toxin production in culture.

M. phaseolina can cause the twin-stem abnormality disease, and this symptom may be a general response when seedlings are infected by either seedborne or soilborne pathogens.

LITERATURE CITED

1. Ammon, V., Wyllie, T. D., and Brown, M. F., Jr. 1974. An ultrastructural investigation of pathological alterations induced by *Macrophomina phaseolina* (Tassi) Goid. in seedlings of soybean, *Glycine max* (L.) Merrill. Physiol. Plant Pathol. 4:1-4.
2. Bristow, P. R., and Wyllie, T. D. 1984. Reaction of soybean cultivars to *Macrophomina phaseolina* as seedlings in the growth chamber and field. Trans. Mo. Acad. Sci. 18:5-10.
3. Chan, Y. H., and Sackston, W. E. 1973. Nonspecificity of the necrosis inducing toxin of *Sclerotium bataticola*. Can. J. Bot. 51:690-692.
4. Dhingra, O. D., and Muchovej, J. J. 1980. Twin-stem abnormality disease of soybean seedlings caused by *Sclerotium* sp. Plant Dis. 64:176-178.
5. Dhingra, O. D., and Sinclair, J. B. 1974. Isolation and partial purification of a phytotoxin produced by *Macrophomina phaseolina*. Phytopathol. Z. 80:35-40.
6. Gangopadhyay, S., Wyllie, T. D., and Leudders, V. D. 1970. Charcoal rot of soybean transmitted by seeds. Plant Dis. Rep. 54:1088-1091.
7. Hoagland, D. R., and Arnon, D. I. 1950. The water-culture methods for growing plants without soil. Calif. Agric. Exp. Stn. Circ. 347. 39 pp.
8. Ilyar, M. B. and Sinclair, J. B. 1974. Effects of plant age upon development of necrosis and occurrence of xylem sclerotia in soybean infected with *Macrophomina phaseolina*. Phytopathology 64:156-157.
9. 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.
10. Meyer, W. A., Sinclair, J. B., and Khare, M. N. 1974. Factors affecting charcoal rot of soybean seedlings. Phytopathology 64:845-858.
11. Short, G. E., and Wyllie, T. D. 1978. Inoculum potential of *Macrophomina phaseolina*. Phytopathology 68:742-746.
12. Sinclair, J. B., and Shurtleff, M. C. 1975. Compendium of Soybean Diseases. American Phytopathological Society, St. Paul, MN. 69 pp.
13. Wyllie, T. D., and Fry, G. 1973. Liquid nitrogen storage of *Macrophomina phaseolina*. Plant Dis. Rep. 57:478-480.