Factors Affecting Charcoal Rot of Soybean Seedlings

W. A. Meyer, J. B. Sinclair, and M. N. Khare

Graduate Research Assistant and Professor, Department of Plant Pathology, University of Illinois, Urbana 61801; and Associate Professor, Department of Plant Pathology, J. Nehru Krishi Vishwa Vidyalaya, Jabalpur, M. P., India, respectively. Senior author's present address: Warren's Turf Nursery, Palos Park, Illinois 60464.

A portion of the senior author's Ph.D. thesis.

Supported in part by the Illinois Agricultural Experiment Station, the U.S. Agency for International Development Grant No. csd-1922, and J. Nehru Krishi Vishwa Vidyalaya, Jabalpur, M. P., India.

Accepted for publication 14 January 1974.

ABSTRACT

During the rainy season in India, soil in field plots was infested with either mycelium or sclerotia of *Macrophomina phaseolina,* or mixed with soybean stubble containing *M. phaseolina*. The emergence of soybean seedlings was significantly reduced and the percentage of emerged infected seedlings was increased. Yields were not reduced. Symptoms on seedlings in field plots appeared first as reddish-brown lesions on the hypocotyls close to the cotyledons, then turned ashy-gray to black. Sclerotial and mycelial inoculum were almost equally effective in causing seedling disease under controlled and field conditions. Seedling disease was greatest at 30 and 35 C although some infections occurred on soybean seedlings at 20 and 25 C. Infected seedlings may serve as a latent source of inoculum for the mature-plant phase of the disease over a wide temp range. The percentage of diseased seedlings increased slightly with an increase in number of viable *M. phaseolina* propagules in soil.

Phytopathology 64:845-849.

Additional key words: damping-off, *Sclerotium rolfsii.*

Macrophomina phaseolina (Tassi) Goid [*Rhizoctonia bataticola* (Taub.) Butler] causes a root and stem rot on various crops (1, 5, 10, 15). It causes a seedling and mature plant disease on soybean in India (6), and annual losses on mature soybeans in the U.S. (9). It was reported to cause 50% losses on soybean seedlings in Yugoslavia, but symptoms were not described (1). *M. phaseolina* infections on conifer seedlings (4) and other crops (4, 7, 12) are favored by high temp (35 C). Moisture stress in addition to high temp favor charcoal rot in sorghum and cotton (2, 3). Temperature was considered not to have an effect on infection of stem-inoculated soybean plants (14).

Sclerotial inoculum of *M. phaseolina* in soil resulted in significantly greater mortality of pine seedlings than mycelial inoculum (11). Sclerotial densities in soil were correlated with increased disease incidence on bean (13). With the use of selective media, *M. phaseolina* populations in soil were shown to increase, and mycelial inoculum to persist up to 7 days in the absence of a suitable host (8). Soil populations also were shown to increase with increased years of continuous soybean cropping (8).

This study shows: (i) the effect of three types of inoculum (mycelium, sclerotia, and infected soybean stubble) of *M. phaseolina* on emergence of soybeans in the field; (ii) the symptoms produced by *M. phaseolina* on soybean seedlings; (iii) the influence of temp and soil treatment on ability of mycelial and sclerotial inoculum to infect soybean seedlings; and,

(iv) the effect of different sclerotial densities on the incidence of soybean seedling disease. Field studies were conducted by J. Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur, M.P., India. Laboratory studies were made at JNKVV and the University of Illinois.

MATERIALS AND METHODS.—An isolate, J-S, of *M. phaseolina* from a diseased soybean seedling was used (8). Sclerotial and mycelial inocula were prepared using methods similar to those of Meyer et al (8). Four 5-mm Difco potato-dextrose agar (PDA) disks from a 2-day-old culture were placed in 250-ml flasks containing 100 ml of soybean seed-extract broth (SEE) (8) and incubated at room temp (24-28 C). Mycelial inoculum was prepared by comminuting a 3-day-old mycelial mat from a single flask in a Waring Blendor and suspending it in 1 liter of distilled water. After 15 days of growth, the fungal mats, which consisted primarily of sclerotia, were removed, comminuted in a Waring Blendor, and suspended in either 1 or 2 liters of distilled water. A third type of inoculum consisted of soybean stubble collected from fields planted to soybeans for three consecutive years. The stubble was washed with water and chopped into approximately 5-mm pieces. Half of the stubble pieces were autoclaved (3 h, 121 C). Three inoculum types (mycelial, sclerotial, and stubble) were used to infest field plots. Samples from autoclaved and nonautoclaved stubble pieces were plated on chloroneb-Ceresan-rice agar (CC) (8). Approximately half of the nonautoclaved stubble pieces produced colonies of *M. phaseolina,* and 30% produced colonies of *Sclerotium rolfsii.* No colonies of pathogenic fungi were produced by the remaining stubbles.

Field trials.—The JNKVV field plots were planted in a well-drained soil (pH 7.1), which had been in nonattended grassland for at least 10 yr. The population of *M. phaseolina* from soil samples was assayed on CC medium and estimated to be 72 propagules (sclerotia, mycelium, and/or pycnidia)/g oven-dried soil (8). Six infestation treatments were arranged in a randomized-complete-block design with four replicates, each 6 m long, with nine rows spaced 45 cm apart. Treatments were applied to the middle five rows in each plot in a 10-cm band and 6-cm deep prior to planting on July 1 (3 wk after the beginning of monsoon rains). The treatments applied/m of row were: 1 liter water (control); 10 g autoclaved stubble (control); sclerotia from a flask in 1 liter water; half the sclerotia from a flask in 1 liter water, mycelial suspension from a flask in 1 liter water; and 10 g of nonautoclaved stubble. Each plot was planted with 200 hand-selected (cracked and discolored seeds were removed) soybean seeds (cultivar 'Bragg'). *Rhizobium* sp. inoculum was applied to all seed prior to planting. Each plot was surrounded with a ditch (3-cm wide and 20-cm deep) to prevent washing of soil-borne inoculum from one plot to another during the heavy monsoon rains.

TABLE 1. The effect of different kinds of propagules of *Macrophomina phaseolina* added to field plot soils on emergence lesion formation, stand, yield, and presence of sclerotia in mature stems of 'Bragg' soybean

Treatments[s]	Emergence[t]	Percent with lesions	Final[u] stand	Yield[u] kg per hectare	Percent[v] stems with sclerotia
Water (1 liter)	163 a[w]	1 d	142 a	3,428 a	52 c
Autoclaved soybean stubble (10 g)	134 bc	1 d	110 b	3,332 a	75 bc
Sclerotial suspension[x]	123 cd	14 b	103 b	3,172 a	92 ab
Sclerotial suspension[y]	144 b	11 b	126 a	3,487 a	92 ab
Mycelial suspension[z]	112 d	18 a	102 b	3,190 a	95 a
Nonautoclaved soybean stubble (10 g)	131 bc	9 c	109 b	3,446 a	92 a

[s] A 10-cm band treatment in the soil previous to planting.
[t] Mean emergence out of 200 seeds planted/6 m row in five rows.
[u] Mean final stand and yield taken on middle three rows/plot.
[v] Based on 10 plants per replication. Plots contained 72 propagules/g oven-dried soil.
[w] All means followed by the same letter do not differ significantly ($P = 0.05$) from each other. Based on Duncan's multiple range test.
[x] Sclerotia from one culture flask suspended in 1 liter of water.
[y] One half the sclerotia from one culture flask suspended in 1 liter of water.
[z] Mycelium from one culture flask suspended in 1 liter of water.

Controlled temperature studies. – Inoculum of a second isolate, J-1, was prepared as described above. Fresh-weight samples (1.0 g) of either sclerotia or mycelium were suspended in 4 ml of sterile, distilled water and mixed with 200 Bragg soybean seed. Seeds mixed with distilled water served as controls. Ten seeds from each treatment were planted in each of three 19-cm diam clay pots containing autoclaved and nonautoclaved soil-sand (3:2) mixture (silt loam, pH 5.6). All pots were placed in a controlled environment chamber programmed for either 20, 25, 30, or 35 C at 3,767-4,305 lumens/m^2, a 14-h day and 60-70% relative humidity (RH) for 12 days. The experiment was done twice with three replications for each treatment. The percentage of diseased seedlings was determined by isolations from surface-sterilized (0.2% sodium hypochlorite for 5 min) 5-mm sections of the tap root and hypocotyl of all seedlings after plating on PDA containing antibiotics (40 mg streptomycin sulfate + 60 mg potassium penicillin) and incubated 4 days at 30 C.

Sclerotium densities and seedling disease.–Sclerotial inoculum was prepared as described above, using isolate I-5 (8), except that sclerotia were air-dried before weights were recorded. Autoclaved soil, mixed with autoclaved silica sand (1:1, v/v) was stored in a greenhouse flat, kept moist and turned weekly for 2 mo to allow recolonization by microorganisms. To each of three 900-g samples of soil was added either 0.25, 0.5, or 1.0 g of dried sclerotia. Six 1.0-g samples were randomly selected from these infested and noninfested (control) soils and each sample plated on 10 plates containing CMR (chloroneb-mercuric chloride-rose bengal) (8) medium in approximately 10-mg portions. Oven-dry weights were determined for ten 10-mg soil samples. The remainder of the soil from each of the three infested samples and the noninfested control was divided and placed separately in three 18-cm plastic pots. Ten Bragg soybean seeds/pot were planted and all pots placed for 10 days in a growth chamber programmed for 28-30 C, 14-h day at 1,076 lumens/m^2 and 60% RH. The percentage diseased seedlings was determined by isolations after 12 days as described above.

RESULTS AND DISCUSSION.–The percentage of diseased seedlings was determined from the middle five rows of each field plot 10 days after planting. Both sclerotial and mycelial inoculum were almost equally effective in reducing emergence and increasing the percentage of diseased seedlings under field (Table 1) and controlled conditions (Table 2). The ability of both forms of inoculum to cause a significant amount of seedling disease is contrary to the report of Smith (11), who stated that sclerotial inoculum caused much greater mortality of pine seedlings than did mycelial inoculum. This disagreement may be due to differences in isolates and host reaction. *M. phaseolina* mycelium can persist in soil for 7 days (8), which would be sufficient time to cause seedling infection. The undiluted sclerotial inoculum significantly reduced emergence below that for the diluted one.

TABLE 2. The effect of growth chamber temp on percent diseased seedlings from soybean seed either noninoculated or inoculated with mycelial or sclerotia of *Macrophomina phaseolina*, and then planted in either autoclaved or nonautoclaved field soil

Inoculum[x]	Soil treatment	Mean percentage diseased seedlings[y]			
		20 C	25 C	30 C	35 C
Water	Autoclaved	45 a[z]	18 a	37 a	38 a
Mycelium	Autoclaved	65 bc	65 b	97 c	91 c
Sclerotia	Autoclaved	55 ab	53 b	92 c	81 c
Water	Nonautoclaved	80 c	47 b	78 b	55 b
Mycelium	Nonautoclaved	78 c	48 b	93 c	89 c
Sclerotia	Nonautoclaved	83 c	45 b	95 c	80 c

[x]1 g fresh weight of either sclerotia or mycelium in 4 ml sterile, distilled water/200 seed.

[y]Percentage of diseased seedlings consisted of both the seedlings which did not emerge and those that did, but which had lesions that yielded *M. phaseolina* upon isolation. Data combined from two experiments, three replications of 10 seeds for each treatment/experiment.

[z]Percentages followed by the same letter were not significantly different (P = 0.05) according to the Duncan's multiple range test.

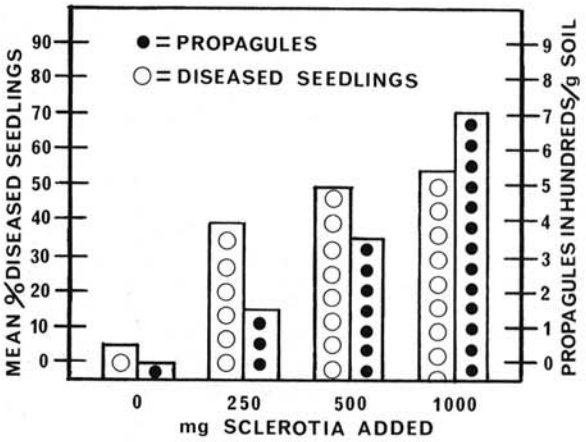

Fig. 1. Percentage of diseased soybean seedlings and number of viable propagules detected using chloroneb-mercuric chloride-rose bengal selective medium from soil infested with three amounts (mg) of *Macrophomina phaseolina* sclerotia initially [LSD for percentage of diseased seedlings (P = 0.05) = 11; and the r value for recovery = 0.99].

There was a significantly greater percentage of emerged seedlings with hypocotyl lesions in infested plots than in controls (Table 1). The highest percentage occurred in mycelium-infested plots. Lesions from which *M. phaseolina* was readily isolated were usually located on hypocotyls just

below the cotyledons and appeared reddish-brown at first (similar to those produced by *Rhizoctonia solani*), then became silver-gray, and finally dark-brown to black. Isolations were made from plants in the border rows, which were also infested with *M. phaseolina*. Similar lesions were found on 10-15% of soybean seedlings growing in other fields on the same farm, which had been in continuous soybeans for 3 or 4 yr. There was no difference in emergence between plots infested with autoclaved and nonautoclaved stubble. The addition of autoclaved stubble appeared to increase the activity of *M. phaseolina* and *Sclerotium rolfsii* which reduced emergence. In plots infested with nonautoclaved stubble, 5% of the emerged seedlings were infected with *S. rolfsii*. *S. rolfsii* was isolated from approximately 30% of the stubble pieces used to infest the plots. This suggested that soybean stubble could serve as an inoculum source for both *M. phaseolina* and *S. rolfsii*.

Counts taken 3.5 mo after planting, and just prior to harvest, on the middle three rows (5 m long) showed that stands in all treatments were significantly lower than those in controls, except for the lowest sclerotial inoculum level (Table 1). The same rows were hand harvested and produced no significant differences in yield (Table 1).

Ten mature plants were selected at random from each treatment after harvesting, split lengthwise and examined for the presence of *M. phaseolina* sclerotia. The mean percentage of plants with sclerotia in stems was higher from *M. phaseolina*-infested plots than controls (Table 1).

The high percentage of seedling infections and plants with sclerotia in the stems from plots infested with *M. phaseolina* suggested for the seedling infection may be a latent source of inoculum for the mature-plant phase of this disease. Temperature was reported to have no effect on infection of stem-inoculated soybean plants (14).

There was increase in the percentage of diseased seedlings from infested seed over noninfested seed at all growth chamber temp in autoclaved and nonautoclaved soils except for infested seed in autoclaved soil at 20 C (Table 2). The high percentage of diseased seedlings in all treatments including the control at 20 C may be explained by the poor germination of seeds at that temp, contamination of the soil by *M. phaseolina*, or seed-borne organisms active at that temp. There was little difference in percentage of diseased seedlings between the two types of inoculum. Hypocotyl lesions from infested seeds were identical to those observed on seedlings in field plots only at 25, 30, and 35 C. The average soil temp at JNKVV at planting time was 30 C. This temp would be favorable for the development of *M. phaseolina* infections on soybean seedlings. The lack of high temp in most U.S. soils at planting time may account for the apparent lack of lesion development on soybean seedlings.

Isolations were made on CC medium at 3 and 8 wk after planting from roots and hypocotyls of 10 plants selected at random from the outer two noninfested rows in each plot. The percentage of plants infected with *M. phaseolina* was 5-15% from control plots and 30-57% from infested plots. There was apparently a native population of *M. phaseolina* propagules in the field soils. Increasing the inoculum significantly increased seedling disease.

In growth chamber studies, the number of viable propagules of *M. phaseolina* recovered from soil samples was directly proprotional to the amount of sclerotia added and as the inoculum levels were increased, the mean percentage of diseased seedlings increased (Fig. 1). However, the percentage of diseased seedlings increased only slightly at the higher inoculum levels (Fig. 1). A significant correlation was reported between sclerotial densities of *M. phaseolina* in soils and mortality of beans (13). With increased years of continuous soybean cultivation, the populations of *M. phaseolina* was shown to increase (8). Under such conditions, seedling disease and thus mature plant disease caused by *M. phaseolina* could increase.

M. phaseolina has been reported to cause charcoal rot on several mature crops including soybean (7, 9, 14, 15). Formation of sclerotia in stem tissue in the field has been used as a criterion to determine whether a plant is infected (14). Wyllie (14) reported that sclerotia formation in soybean was conditioned solely by flowering and pot set, but that plants could become infected long before obvious sclerotia are present. The number of plants infected in our field plot at 3 and 8 wk after planting indicated that natural infections can be present without obvious symptoms. Infections at the seedling stage can occur over a range of 20-35 C and may serve as an important source of inoculum for plants at any age under favorable environmental conditions. Charcoal rot is usually more severe under hot, dry conditions, which promote early crop maturity (14). This disease on corn, cotton, and sorghum does not become severe until they begin to mature (2, 3, 7). During the field studies at JNKVV, the average RH was 90% and there was 175 cm of rainfall during the growing season. Thus, crop maturity was delayed and in combination with the high moisture, the potential damage caused by *M. phaseolina* was reduced.

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