Biology of Macrophomina phaseoli in Soil Studied with Selective Media

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ABSTRACT

Two selective media using rice agar as the basal medium, were developed for the isolation and enumeration of *Macrophomina phaseoli* from soil and soybean plant debris. The first, designated as CC, was developed in India and contained chloroneb, methoxyethylmercury chloride (Ceresan Wet), streptomycin sulfate, and potassium penicillin. The second, designated as CMR, was developed at the University of Illinois and contained the same ingredients, except that mercuric chloride was substituted for methoxyethylmercury chloride and rose bengal was added. Recovery of *M. phaseoli* from artificially infested soil was close to 100% with either medium. Assays with CC showed *M. phaseoli* persists in soil under diverse environmental conditions and that populations of the fungus in soil, increased with increased years of continuous soybean cropping. Populations of *M. phaseoli* in soil from Illinois soybean and corn fields, determined with CMR, were higher than those determined using the sclerotial-flotation method. In the absence of a suitable host, *M. phaseoli* populations increased and mycelial inoculum persisted in soil up to 7 days. The test fungus was capable of invading dead stem tissues in soil containing antagonistic microorganisms. The evidence does not support the strict classification of *M. phaseoli* as a root-inhabiting fungus.

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Macrophomina phaseoli (Maubl.) Ashby [*Rhizoctonia bataticola* (Taub.) Butler] causes root and stem rot on soybean, corn, sorghum, and sugar pine (1, 11, 17) and causes losses every year on soybean in the U.S. and India (5, 9). Variation among isolates of *M. phaseoli* from soybean has been described (2). The saprophytic nature of *M. phaseoli* in soil is not well understood (6, 12). Based on Garrett's scheme (3) it has been classified both as a soil-inhabiting and root-inhabiting fungus (12). However, evidence suggests it is primarily a root-inhabiting pathogen with sclerotia as the

principal means of survival. Norton (6, 7) showed that *M. phaseoli* had a limited saprophytic ability because of the antagonism of other soil-borne microorganisms. Smith (12) reported that limited mycelial activity of *M. phaseoli* may occur in soil and even cause the death of pine seedlings, but soil-borne sclerotia caused greater disease incidence than mycelium. Increased sclerotium densities in soil were correlated with increased disease incidence on bean (14). The germination of sclerotia in the soil increased from 5 to 50% with the addition of root exudates (13). Watanabe et al. (14, 15) described a differential flotation technique to assay populations of *M. phaseoli* sclerotia in fumigated and nonfumigated pine nursery soils. This technique requires the culturing of all recovered "sclerotial-like" bodies to determine their identity and viability. Selective media are needed to accurately assay all of the propagules of specific plant pathogens, since saprophytic fungi omnipresent in soil quickly overgrow agar plates and prevent recovery of important pathogens. No selective medium has been developed for the isolation of *M. phaseoli* from infested soil or plant debris.

The purposes of this study were: to develop a selective medium for the isolation of *M. phaseoli* from soybean plant debris and from soils cropped to soybean and other crops; and to investigate the survival of mycelium and sclerotia in soil.

MATERIALS AND METHODS.—*Selective media employed.*—Two selective media for the isolation of *M. phaseoli* were developed following considerable investigating combinations of basal media, fungicides, antibacterial agents, and medium pH. These were used in India and the U.S. for quantitative assays of *M. phaseoli* propagules in artificially infested soil, field soil naturally infested with the pathogen, and soybean (*Glycine max* L.) field stubble.

The first medium, designated as CC, was prepared by amending molten (45-50 C) rice agar (RA) with 150 mg active ingredient chloroneb (1,4-dichloro-2,5-dimethoxybenzene) as Demosan 65 wettable powder (E. I. duPont), 0.25 mg actual mercury as methoxyethylmercury chloride (Ceresan Wet seed dressing, 2.5% mercury, Bayer Co.), 40 mg streptomycin sulfate, 60 mg potassium penicillin, and lactic acid to bring to pH 6.0. The RA was prepared by boiling 10 g of polished rice in 1 liter of distilled water for 5 min; filtering through cheese cloth; and adding 20 g of Difco agar to the filtrate and autoclaving. The second medium, designated as CMR, was prepared in a similar manner with the following added to one liter of RA: 300 mg active ingredient chloroneb; 7 mg mercuric chloride; 90 mg rose bengal; 40 mg streptomycin sulfate; 60 mg potassium penicillin; and lactic acid to bring to pH 6.0. All studies using CC were done at J. Nehru Agricultural University, Jabalpur, India (JNAU). All studies using CMR were done at the University of Illinois, Urbana (UI).

To obtain consistent results, the media must be prepared fresh and mixed well. Culture plates containing fresh CMR should set 24 hr at room temperature before use and then be incubated in the dark (8). Fifteen ml of medium per 9-cm culture plate was used in all experiments. All cultures were incubated at 30 C in the dark, and colony counts made at 7 days unless otherwise indicated. All experiments were conducted at least twice.

Isolates of the pathogen.—The following 12 Indian isolates of *M. phaseoli*, nine from soil and three from other sources, were studied: from JNAU (J-1, J-2, J-3, J-4), Indore (IN-1, IN-2), Gwalior (G), Tikamgarh (T), and Seoni (S); one isolate from a diseased soybean plant at JNAU (J-S); a single isolate from a diseased soybean plant from G. B. Pant University of Agriculture and Technology, Pantnagar, U. P. (U. P.); and one isolate from the Indian Agriculture Research Institute Type Culture Collection No. 583 (IARI). Seven U.S. isolates of *M. phaseoli*, five from soil, and two from diseased soybean plants, also were studied. They were: from the soil of an Illinois soybean field (I-1, I-2, I-3, I-4, I-5); one isolate from a soybean plant from Missouri (M-5); and one from an Illinois soybean plant (I-R). All isolates were maintained on Difco potato-dextrose agar (PDA) slants at 4 C. Isolates were grown on PDA plates at 30 C for 2 days before 4-mm agar discs were removed from the periphery of the colonies for seeding purposes.

Individual sclerotia of *M. phaseoli* were prepared using the following technique. Four agar discs were used to seed 250-ml flasks containing 100 ml of soybean-seed-extract broth (70 g soybean seed plus 20 g dextrose/liter distilled water). After 15 days at room temp (25-28 C), sclerotia were removed using the technique of Smith (10), rinsed with sterile, distilled water, and air-dried.

Mycelium of *M. phaseoli* was prepared by first seeding culture plates containing 20 ml of soybean-seed-extract broth and incubated for 14 hr. The agar plugs were then removed, leaving mycelium and culture media to be placed in a Waring Blendor at low speed for 10 seconds. The culture medium was then separated from the mycelium by low speed centrifugation for 2 min and the resulting mycelial mats were rinsed twice in sterile distilled water. The mats were blotted on filter paper and their fresh weight determined.

Artificially and naturally infested soils.—Either 24, 48, or 72 mg of dried sclerotia of the J-S isolate were mixed with 20-g samples of air-dried, autoclaved (3 hr, 121 C), or nonautoclaved loam soil (pH 7.1) and the mixture sieved through a 2-mm mesh screen. Either 25, 50, or 75 mg of dried sclerotia of the J-1 isolate were mixed with 40-g samples of autoclaved and nonautoclaved silt loam soil (pH 5.6). These samples were crushed to a particle size of approximately 0.5 mm in a mortar before assays were made. Infestations were also made with both sclerotial and mycelial inoculum of the J-1 isolate in a silt loam soil (pH 5.5) before amendments were added.

Six soil samples were taken at random during the growing season (June-September) from each of five adjoining field plots on three different dates at JNAU. The plots had been under continuous soybean

cropping for 1, 2, 3, 4, or 5 years, respectively, and in unattended grassland prior to this time. The soil was a clay loam with a pH of 6.8 - 7.1. Samples were taken from the top 18 cm of soil and combined. Each sample was air-dried at 30 C, sieved through a 2-mm mesh screen, and crushed before assays were made.

Composite samples were collected from seven fields located in different soil zones in Madhya Pradesh (India) near Jabalpur, Gwalior, Indore, Sehore, Tikamgarh, Rewa, and Seoni. These soils were processed using the same procedure as described above.

Six soil samples were collected at random on the same date from four different fields on the UI Agronomy South Farm, Urbana, continuously cropped with one of the following for 6 years or more: wheat (*Triticum* spp.) (silty loam, pH 6.3); corn (*Zea mays* L.) (silty loam, pH 6.2); alfalfa (*Medicago sativa* L.) (silty loam, pH 6.8); and soybean (silty loam, pH 6.7). These were processed as described above.

Tests for pathogenicity.—Pathogenicity studies were made in India using 'Bragg' soybean seedlings. Nine isolates of *M. phaseoli* were grown on soybean-seed-extract broth for 3 days and used to wound-inoculate the hypocotyl just below the cotyledons of ten 3-day-old seedlings. Wounded but noninoculated plants served as controls. Plants were then placed in a growth chamber maintained at 28-32 C with 600 ft-c of light from fluorescent tubes. The number of dead seedlings was recorded daily over a 20-day period.

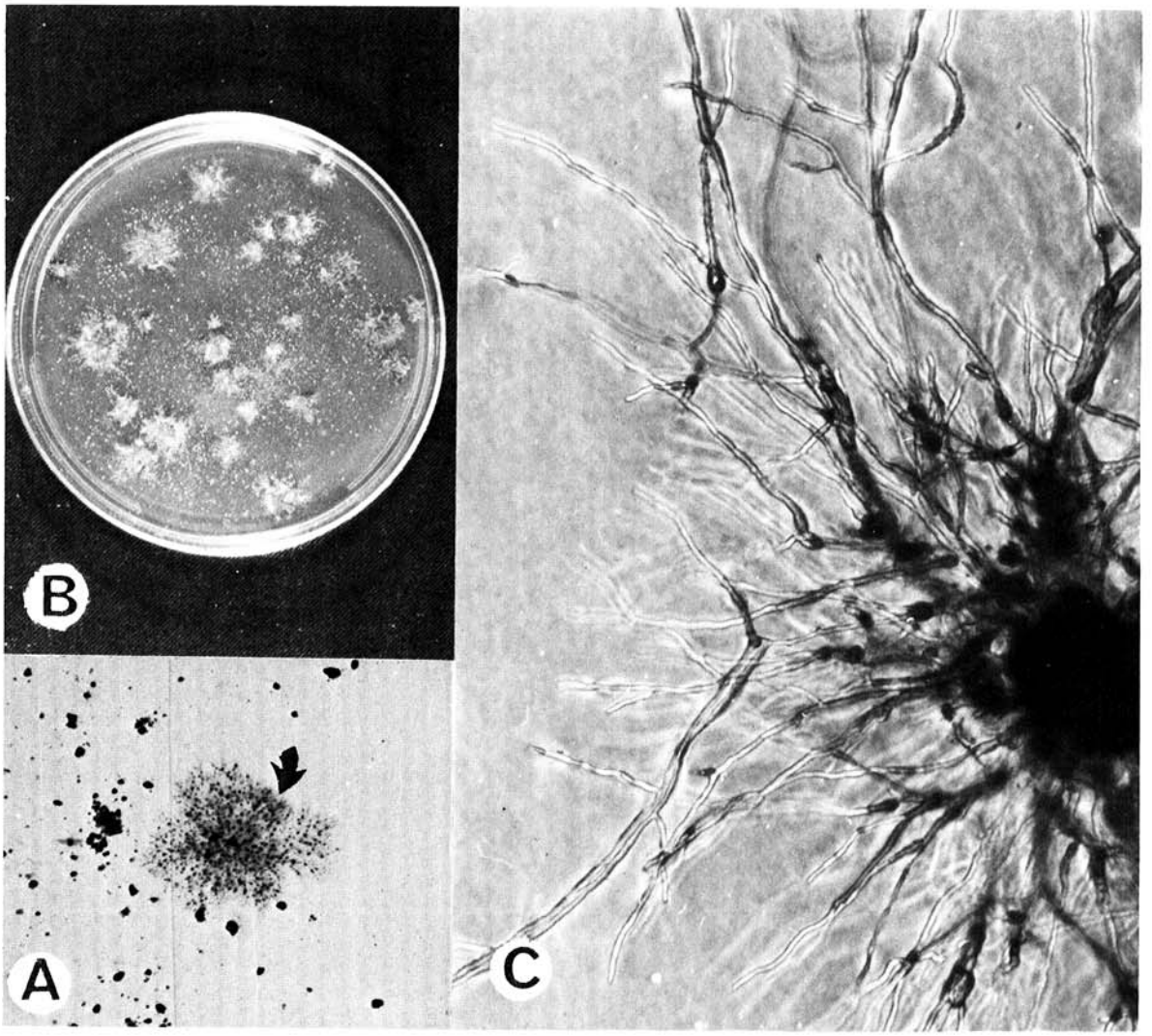

Fig. 1. Development of *Macrophomina phaseoli* on selective media. A) a 7-day-old colony (arrow) showing sclerotia production from a soil sample naturally infested with the fungus and plated on chloroneb-Ceresan Wet medium; B, C) plates of chloroneb-mercuric chloride-rose bengal medium, with B) showing individual colonies 6 days after plating a soil sample artificially infested with the test fungus; and C) mycelial development from a single sclerotium 4 days after plating.

The pathogenicity of five isolates from UI soybean cropped soils was tested in the same way, except five seedlings were grown in vermiculite (Terralite brand) in a growth chamber maintained at 30 C, 3,500 ft-c, 14-hr day, with 60% relative humidity.

RESULTS.—*Efficiency of recovery of M. phaseoli propagules from artificially infested soil.*—Five samples of dried sclerotia of the J-S isolate were weighed and counted, and the number of sclerotia/mg of cultured sclerotia was calculated to be 952 ± 32 at a 95% confidence interval. The soils artificially infested with different quantities of sclerotia of the J-S isolate were assayed on five CC plates. In a similar manner the infested and noninfested, autoclaved soil samples were also plated on five RA plates (to which 40 mg streptomycin sulfate and 60 mg potassium penicillin/liter were added). Approximately 10 mg of soil with or without sclerotia added were spread on the surface of each plate. Oven-dry weights were determined for ten 10-mg samples. RA plates were examined microscopically for the number of colonies per plate at 24 and 36 hr and CC plates at 5 and 8 days.

Ten samples of dried sclerotia of the J-1 isolate were weighed and counted and the number of sclerotia/mg of cultured sclerotia was calculated to be 853 ± 47 at a 95% confidence interval. Approximately 10-mg samples each of soil with or without sclerotia were spread on each of eight CMR plates. The infested and noninfested, autoclaved soil samples were also plated on eight RA plates to which 40 mg streptomycin sulfate, 60 mg potassium penicillin, and 50 mg rose bengal/liter were added. The number of colonies per plate was recorded at 3 and 7 days. The oven-dry weight of ten 10-mg samples was determined.

The CC and CMR media were effective in selecting and making quantitative isolations of *M. phaseoli* from autoclaved and nonautoclaved soils, artificially infested with three amounts of sclerotia. Colonies of the test fungus were recognized by the fourth day of incubation and sclerotia developed in most colonies of all isolates by the eighth day on CC and the sixth day on CMR (Fig. 1). All colonies had rough, irregular margins and colony diameters of 5-15 mm after 8 days and were easily counted. Only a few contaminating fungal colonies developed on some plates of CC and CMR when nonautoclaved, infested soil samples were used. These were highly restricted in growth and distinctive in colony characteristics, and did not interfere with counting *M. phaseoli* colonies.

The mean percent recovery of isolate J-S on CC from autoclaved, infested soil was 96% ± 15 and for RA, 78% ± 8 at a 95% of confidence interval (Table 1). Recovery from nonautoclaved, infested soil was 100% ± 5 at 95% confidence interval. Using CC, it was estimated that the nonautoclaved soil contained an original population of 200 propagules/g of oven-dried soil. This number was used to calculate the total number of sclerotia in the nonsterile soil.

The mean percent recovery of isolate J-1 on CMR from autoclaved, infested soil was 107% ± 8 and for RA, 108% ± 3 at a 95% confidence interval (Table 2). Recovery from nonautoclaved, infested soil with CMR was 100% ± 8 at a 95% confidence interval.

The growth of different M. phaseoli propagules on selective media.—The ability of mycelia, sclerotia, and pycnidia of *M. phaseoli* each to produce colonies was determined by plating the following separately on four plates each of CC and RA: five individual air-dried J-S sclerotia; five 1-mm wefts of J-S mycelium; or five J-S pycnidia removed from a lesion

TABLE 1. Recovery of *Macrophomina phaseoli* (Isolate J-S) on rice agar and chloroneb-Ceresan Wet (CC) selective medium from autoclaved and nonautoclaved artificially infested field soil

Medium	Soil treatment	Sclerotia[a] mixed with 20 g soil (mg)	Estimated: Number of sclerotia added /g soil	Estimated: Population assayed with media/g soil		Percent recovery
Rice agar[b]	Autoclaved	24	1,142	950		83
	Autoclaved	48	2,284	1,775		78
	Autoclaved	72	3,426	2,500		73
					CI[c] =	78±8
CC	Autoclaved	24	1,142	1,150		101
	Autoclaved	48	2,284	1,950		86
	Autoclaved	72	3,426	3,487		102
					CI[c] =	96±15
CC	Nonautoclaved	24	1,342[d]	1,287		96
	Nonautoclaved	48	2,484[d]	2,512		101
	Nonautoclaved	72	3,626[d]	3,650		101
					CI[c] =	100±5

[a] Calculated number of sclerotia/mg was 953±32 at a 95% confidence level.
[b] Rice agar contained the following/liter: 40 mg streptomycin; and 60 mg penicillin.
[c] CI = confidence level of 95%.
[d] Includes 200 propagules/g estimated as natural infestation.

TABLE 2. Recovery of *Macrophomina phaseoli* (Isolate J-1) on rice agar and chloroneb-mercuric chloride-rose bengal (CMR) selective medium from autoclaved and nonautoclaved artificially infested field soil.

Medium	Soil treatment	Sclerotia[a] mixed with 40 g soil (mg)	Estimated: Number of sclerotia added /g soil	Estimated: Population assayed with media/g soil		Percent recovery
Rice agar[b]	Autoclaved	25	533	587		110
	Autoclaved	50	1,066	1,142		107
	Autoclaved	75	1,599	1,753		109
					CI[c] =	108±3
CMR	Autoclaved	25	533	600		113
	Autoclaved	50	1,066	1,112		104
	Autoclaved	75	1,599	1,675		105
					CI[c] =	107±8
CMR	Nonautoclaved	25	533	525		98
	Nonautoclaved	50	1,066	1,025		96
	Nonautoclaved	75	1,599	1,675		105
					CI[c] =	100±8

[a] Calculated number of sclerotia/mg was 853±47 at a 95% confidence level.
[b] Rice agar containing the following/liter: 40 mg streptomycin; 60 mg penicillin; and 50 mg rose bengal.
[c] CI = confidence level of 95%.

on a soybean seedling infected with the test fungus. Also plated from a field infested with *M. phaseoli* were five 5-mm sections of surface-sterilized (0.2% sodium hypochlorite, 5 min) stubble and roots of soybeans, which possibly contained all three propagule types. Five dried sclerotia from isolates: J-1 (JNAU), I-R (from roots of soybean grown in Illinois), and M-5 (soybean stem isolate from Missouri) were transferred to five CMR and potato-dextrose agar (PDA) plates. Five surface-sterilized, 5-mm sections of corn and soybean stubble were plated on four CMR and PDA plates, respectively. The number of *M. phaseoli* colonies was recorded after 3 and 7 days.

All of the Indian isolates of *M. phaseoli* except for J-S were compared for their ability to grow on RA and CC. Each isolate was transferred to six plates each of RA and CC. Diameter of colonies were measured after 3 and 5 days.

Colonies of *M. phaseoli* developed from all of the mycelia, sclerotia, and pycnidia placed on CC and RA plates, as well as from approximately 50% of the soybean-stubble sections plated on CC and from corn and soybean stubble sections plated on CMR. Only a small number of colonies developed from similar sections plated on RA and PDA, and these were difficult to count because of fast-growing contaminating colonies of other fungi. All the sclerotia of isolate J-1 and 94% of the sclerotia from isolate I-R and M-1 produced colonies on PDA and CMR plates.

All 11 Indian isolates produced colonies with a mean diam of 15-20 mm after 7 days on CC and RA after 3 days the mean diam in mm for each isolate were: S, 52; IN-1, 55; IARI, 63; UP, 66; IN-2, 68; and a range of 70-73 for all others.

Quantitative estimations of populations in naturally infested soils.—The soil samples from plots under different periods of continuous soybean cropping and from fields located in different soil zones in Madhya Pradesh were spread in 10-mg quantities over the surface of ten culture plates containing CC medium. The number of colonies was counted after 8 days and oven-dry wt were recorded. The soil samples from the Illinois fields under different crops were plated in 10-mg quantities on 10 CMR plates and oven-dry wt also were determined for 10 different samples.

The use of CMR for the recovery of *M. phaseoli* from soil was compared with the differential flotation technique described by Watanabe et al. (15). Six 0.10-mg soil samples from each of the soybean and corn plots were used to float out sclerotium-like bodies on cellulose discs and then these bodies were transferred to PDA. The number of *M. phaseoli* colonies was counted after 7 days.

All of the soil samples collected from different locations in Madhya Pradesh (MP) and assayed on CC contained *M. phaseoli*. The estimated population of propagules/g of oven-dry soil by location was: Rewa, 52; Sehore, 92; Indore, 66; Seoni, 52; Tikamgarh, 155; and Gwalior, 114. The estimated population of propagules/g of oven-dry soil increased with increased number of years of continuous soybean cropping in Jabalpur: 1 year, 80; 2 years, 120; 3 years, 149; 4 years, 149; and 5 years, 165.

M. phaseoli was recovered on CMR from all UI Agronomy Farm soils regardless of cropping history. The estimated population of propagules/g of oven dried soil by cropping history was: soybean, 108; corn, 31; wheat, 24; and alfalfa, 15. The estimated population found when the flotation technique (15) was used was 24/g for soybean and 7/g for corn.

The nine soil isolates of *M. phaseoli* from the state

of Madhya Pradesh were all found to be pathogenic to 'Bragg' soybean seedlings. The percent of seedlings killed within 20 days after inoculation for the following isolates was: T, 70; IN-1, 70; G, 70; J-1, 60; S, 55; IN-2, 55; J-3, 50; J-2, 35; and J-4, 35. The five isolates from UI field soils showed the following percent of killed seedlings within 7 days: I-5, 90; I-4, 70; I-2, 60; I-3, 50; and I-1, 50.

Recovery of M. phaseoli from amended and nonamended soil.—Eight 50-mm petri plates containing 10 g of air-dried soil were either infested or noninfested with 10 mg of dried sclerotia of the J-1 isolate of *M. phaseoli*. Ten-mg samples from each of these plates were then plated on each of six CMR plates. Four infested and four noninfested soil plates were then either amended or nonamended with a 0.5% solution of soybean-stem extract (35 g soybean stem/1 liter distilled water) on a wt/wt basis. A total of 3 ml of sterile distilled water was added to each plate and then incubated for 7 days at 30 C. The soil samples were then removed from all plates and air-dried in a 30 C oven. Six CMR plates were then used to plate out 10-mg samples from each of the air-dried soil samples.

Ten mg fresh weight of mycelium of J-1 and I-S isolates was suspended in water and added to each of four culture plates containing 10 g of either amended or nonamended, air-dried soil. Amended and nonamended soils without mycelium served as controls. All soil plates received a total of 3 ml of water/plate. A sterile transfer needle was used to remove ten 3- to 5-mg samples of soil from all plates immediately after the mycelial suspensions were added. Five samples were placed at equidistant points on each of two CMR plates. In addition, five approximately 20-mg samples of soil were placed on 5-cm filter paper discs for drying at 40 C for 16 hr. Samples of 25 sclerotia of isolate J-1 and 15 cellulose discs with 0.5 ml of a water suspension of washed mycelium (60 mg fresh weight/30 ml water) of isolates I-5 and J-1 were either plated on CMR and PDA or dried. Five 3- to 5-mg dried soil samples were placed at equidistant points on each of two CMR plates along with the cellulose discs and individual sclerotia. This plating of wet and dry samples was repeated after 3.5- and 7-day incubation of the soil plates at 30 C. The number of colonies developing from the five different points on the CMR and PDA plates was recorded after 7 days of incubation.

Amended and nonamended soil samples infested with an average of 426 sclerotia/g oven-dry soil increased to 514 and 573 propagules/g, respectively, after 7 days. Noninfested, amended, and nonamended soil samples had approximately 10 propagules/g

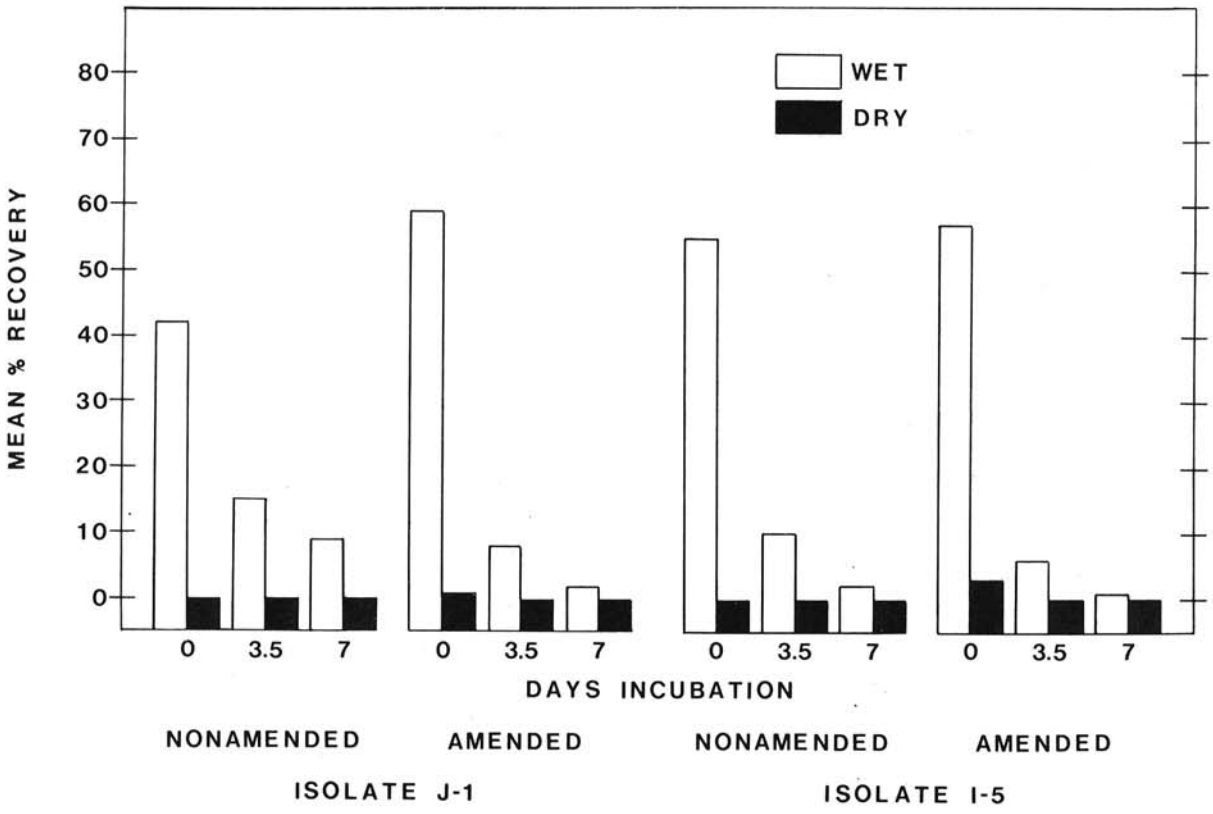

Fig. 2. Average percent of colonies of *Macrophomina phaseoli* recovered from amended and nonamended soil mixed with mycelium from either of two isolates of the test fungus, incubated for 0, 3.5, or 7 days in a wet condition and then assayed in both a wet and dry condition using chloroneb-mercuric chloride-rose bengal selective medium.

before the 7-day incubation and showed an increase of only a few propagules/g after this period.

Recovery of *M. phaseoli* was greater in the wet amended than nonamended soil samples when assayed on CMR after infestation with mycelial suspensions of J-1 and I-5 isolates (Fig. 2). There was a decrease in the percentage of recovery from all wet samples after 3.5 and 7 days of incubation. Recovery rate was higher at 3.5 and 7 days with the J-1 isolate. All the cellulose discs containing wet mycelial suspensions and the 25 sclerotia of J-1 isolate produced colonies on CMR.

Recovery of the test fungus from dried soil samples was below that of the wet, with only a single colony recorded from the samples amended with J-1 isolate and three from samples amended with I-5 isolate. One out of 15 discs with mycelial suspension and all of the 25 sclerotia produced colonies after being dried for 16 hr. The number of colonies recovered from noninfested, amended, and nonamended wet and dry soil samples varied from 1-2%.

Evidence of saprophytic colonization of sterile stem sections.–Isolation of M. phaseoli from sterile soybean stem sections.–The saprophytic nature of isolate J-1 was studied by placing ten 5-mm stem sections of 6-week-old plants, which were boiled for 30 min in distilled water, rinsed, and then autoclaved, in 10 g of soil noninfested (control) or infested with 10 mg of dried sclerotia. There were four replications of each treatment. The soil was moistened with 3 ml of sterile distilled water. The stem sections were removed after 7 days of incubation at 30 C, washed under tap water, surface sterilized with 0.2% sodium hypochlorite for 3 min followed by a sterile water rinse, and each section was plated on CMR.

The test fungus grew on CMR from 36% of the surface sterilized stem sections buried in infested soil and was not isolated from any of those buried in noninfested soil.

DISCUSSION.–The CC and CMR media were successfully used for the selective isolation and enumeration of *M. phaseoli* from a wide range of soil types and soybean debris in India and the U.S. Recovery was close to 100% from artificially infested, autoclaved, and nonautoclaved soils and thus *M. phaseoli* can be quantitatively assayed directly from soils even in the presence of soil-borne microorganisms reported to be antagonistic to it (7).

The media offer a fast and effective means to assay populations of *M. phaseoli* by direct soil plating. Fungal populations vary greatly among soil types. Some of the soils assayed on CMR resulted in only *M. phaseoli* colonies, whereas others gave rise to contaminant colonies. These were restricted and did not interfere with the counting of *M. phaseoli* colonies. Individual colonies of *M. phaseoli* on these media were recognized by restricted growth, rough colony margins, and production of small, black sclerotia. CMR was preferred to CC because sclerotia formed earlier and the light-colored young mycelium stood out against the red color due to rose bengal in the medium.

Higher populations of *M. phaseoli* were detected with CMR than with the recently reported sclerotial flotation technique (15) which requires the culturing of individual sclerotia to verify viability and identity. Possibly the flotation technique does not separate all of the sclerotia from organic debris in the Illinois soils. However, CMR does not differentiate between colonies arising from sclerotia and other propagules of *M. phaseoli*.

A wide range of *M. phaseoli* isolates and the different types of propagules developed into colonies on these media. There is still the possibility that other isolates not tested may vary in their ability to grow on these media.

The fungitoxic materials used in these media did not reduce the recovery ability of *M. phaseoli* as shown by the same level of recovery from known samples of infested autoclaved soils on RA and the two selective media. Mercuric chloride, used in CMR, was a good substitute for Ceresan Wet which is no longer available in the United States. Rose bengal was fungistatic to a number of contaminants sometimes encountered in Illinois soils but not in India.

It is generally believed that *M. phaseoli* persists in the soil as sclerotia formed in infected host tissue and later released into the soil during the decaying process (12). Wyllie & Calvert (16) reported that sclerotial formation in infected soybean plants is conditioned by flowering and pod set and that other variables such as temperature and moisture were subordinate in their effect. The increased populations of *M. phaseoli* with increased years of soybean cropping were probably due to the formation of sclerotia in infected soybean tissues, which remain in the soil after harvest. Many sclerotia were observed in decaying tissues and *M. phaseoli* was isolated from these using CC and CMR. This is the first report of increased inoculum levels resulting from increased years of cropping in an area that was in unattended grassland prior to the introduction of cultivated crops. *M. phaseoli* was found to be widely distributed in Madhya Pradesh from areas with variable soil types and annual rainfall, indicating that this fungus can persist under highly diverse environmental conditions.

The highest population of *M. phaseoli* found in the Illinois soils studied was from a field cropped for over six years to soybeans. The next highest was in a field cropped continuously for six or more years to corn, followed by other fields cropped continuously in wheat or alfalfa. Corn and soybean are common hosts to *M. phaseoli* and can be seriously affected under favorable conditions, whereas wheat and alfalfa, although hosts for this fungus, have not had known serious losses (17). A similar increase in population of *M. phaseoli* in soils planted to the susceptible white fir was reported (13), but not when moderately resistant ponderosa pine was planted.

Populations of *M. phaseoli* increased more in amended than in nonamended, artificially infested field soils. Most of this increase was apparently caused by the germination of some sclerotia or growth from mycelial fragments. The soybean stem extract used as an amendment may have initially

stimulated sclerotial germination as described for pine root exudates (13). The increase in the populations in nonamended soil does not agree with the less than 5% germination of sclerotia previously determined using the buried membrane filter (13). These differences may have resulted because the 21 C incubation temperatures used previously (13) were too far below the 30 C optimum used in the present study.

Recovery of *M. phaseoli* was greater in wet amended soil samples after infestations with mycelial suspensions. This indicates that the amendments of soybean stem extract may have initially stimulated the growth of *M. phaseoli* in soil samples. The substantial recovery from wet, nonamended but not from dry soil samples after the incubation periods indicated that the fungus persisted in the form of mycelium which was not resistant to drying. The general reduction in recovery from all the samples, especially those that were amended, can be explained by the populations of actinomycetes and bacteria which may be antagonistic to *M. phaseoli* (4).

M. phaseoli was isolated from some of the soybean stem sections buried in artificially infested soils. These sections were boiled and autoclaved so that large quantities of soluble substrates resembling root exudates would not be added to the soils. Because the sclerotia were thoroughly mixed with the soil no appreciable linear growth was required for *M. phaseoli* to invade these pieces. The isolation of *M. phaseoli* from the surface-sterilized sections illustrates that this fungus penetrated some of these dead tissues in the presence of other competitive and antagonistic soil microorganisms.

This is the first report of increased populations of the test fungus and the persistence of mycelial inoculum in the soil in the absence of a suitable host and the invasion of dead tissue by *M. phaseoli* in soil. This evidence along with that of Smith's (12) on the infection caused by mycelial inoculum does not support the strict classification of this organism as a root-inhabiting fungus. The ability to develop away from the living host is an important consideration where attempts are made to control this pathogen.

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