Quantitative Recovery of *Macrophomina phaseolina* Sclerotia from Soil

J. D. MIHAIL, Graduate Research Assistant, and S. M. ALCORN, Professor, Department of Plant Pathology, University of Arizona, Tucson 85721

ABSTRACT

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A selective medium containing chloroneb and streptomycin sulfate in potato-dextrose agar was developed for the quantitative isolation of *Macrophomina phaseolina* sclerotia from soil. Populations as low as one sclerotium per gram of soil could be detected with this medium. The medium was also found to be suitable for isolating *M. phaseolina* from infected plants.

Macrophomina phaseolina (Tassi) Goid. is a sclerotium-producing fungus that causes charcoal rot of corn, soybean, and other crops in arid and semiarid areas (1). Several techniques have been developed for recovering sclerotia from soil. One method (7) involves flotation recovery of sclerotia, which are then identified in culture; the procedure is cumbersome if many soil samples must be assayed. Other methods (2–4,6) involve selective or semiselective media but were insufficiently sensitive or selective for our needs when we worked with a loam field soil.

We report here a selective technique for the quantitative isolation of *M. phaseolina* sclerotia from soil. We compared the effectiveness of our procedure with those of McCain and Smith (2), Meyer et al (3), and Papavizas and Klag (4).

MATERIALS AND METHODS

Isolation medium. The selective medium contained Difco potato-dextrose agar (39 g/L), Difco Bacto agar (10 g/L), chloroneb (Demosan 65WP) (100 μg a.i./ml), and streptomycin sulfate (250 μg a.i./ml). The chemicals, dissolved in sterile, distilled water, were added after autoclaving when the medium was added to a soil slurry (described below). The final unadjusted pH of the medium was 6.0.

Preparation of soils. The addition of streptomycin sulfate and chloroneb inhibits competitive growth by bacteria and Oomycetes, respectively. A loam field soil with a pH of 7.25 (51.9% sand, 33.0% silt, and 15.1% clay) was used for all reported tests. Air-dried soils were crushed through a 2-mm sieve and then divided into samples of 1–25 g, depending on the expected population of sclerotia. If large numbers were expected (more than 50 sclerotia per gram), samples of 1–5 g were used; otherwise, 25-g samples were used.

Each sample was mixed in 250 ml of 0.525% sodium hypochlorite in a blender three times for 30 sec at 3-min intervals. The sodium hypochlorite was used to exclude soil bacteria and those fungi present as hyphae. This mixture was washed with distilled water through a 45-μm sieve, and the residue was backwashed into a 250-ml flask for a final volume of 5–10 ml or 40–50 ml for soil samples of 1–5 g and 25 g, respectively. To this slurry, 100 ml of molten, cooled (50–55 C) agar medium was added. Chloroneb and streptomycin sulfate were then added from fresh (50 μg a.i./ml, 5%), aqueous solutions such that their respective concentrations in the mixture were 100 μg a.i./ml and 250 μg a.i./ml. The mixture was incubated at 31–32 C in the dark in five or six petri dishes. *M. phaseolina* colonies were identified within 3–4 days as a ring of fluffy white mycelium surrounding a central area with black sclerotia.

Efficiency of the medium. Sclerotia of *M. phaseolina* were produced in liquid culture (5). After 11 days, sclerotial mats were collected, dried, and gently ground with a mortar and pestle. The sclerotia were added to a loam field soil that had been passed through a 2-mm sieve, dried overnight at 75 C, and then cooled. After thorough mixing, dilutions of 1:10 and 1:100 (v/v) were made with autoclave-sterilized sand. Five subsamples of each dilution were assayed in each of two tests replicated five times.

The same artificially infested soil and naturally infested field soil were used to compare the sensitivity of our medium with those of Papavizas and Klag (4) and Meyer et al (3). Five subsamples of each soil were assayed with each medium in each of two trials replicated five times.

The efficiency of our medium was also tested by determining the vertical distribution of *M. phaseolina* sclerotia in naturally infested field soil. Samples were collected at three depths (0–15 cm, 16–30 cm, 31–60 cm) by scraping the exposed wall of a pit. Soil samples from each depth were thoroughly mixed and air-dried; five subsamples from each level were assayed. This experiment was repeated with soil similarly collected from a second pit excavated in the same field.

RESULTS AND DISCUSSION

The number of sclerotia per gram of

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Table 1. Comparison of selective media for isolating sclerotia of *Macrophomina phaseolina* from artificially infested and naturally infested soils

Soil	Medium[a]	Sclerotia per gram[b]	Contamination[c]
Artificially infested	MP	141.2 ± 15.8	None
	PK	124.4 ± 16.6	Light
	MSK	124.4 ± 36.9	None
Naturally infested	MP	0.3 ± 0.1	None
	PK	0.4 ± 0.8	Heavy
	MSK	0	None

[a] MP = Difco potato-dextrose agar (39 g/L), Difco Bacto agar (10 g/L), chloroneb (100 μg a.i./ml), and streptomycin sulfate (250 μg a.i./ml). PK is the medium of Papavizas and Klag (4). MSK is the medium of Meyer et al (3).

[b] Mean of five replicates per treatment.

[c] Light = three to five colonies less than 5 mm in diameter. Heavy = five or more colonies or fewer colonies at least 5 mm in diameter.

fresh, naturally infested field soil and per gram of the same soil air-dried for 1 or 8 days was 1.2 ± 0.29, 1.4 ± 0.25, and 1.2 ± 0.4, respectively. Similar results were obtained in a second test. Because air-drying had no effect on the recovery of sclerotia, field soils collected subsequently were air-dried.

In preliminary assays of field soil using the technique of McCain and Smith (2), profuse growth of other fungi made detection of *M. phaseolina* difficult, and this procedure was not tested further. We then used both artificially and naturally infested field soils to compare our medium (MP) with those of Papavizas and King (PK) (4) and Meyer et al (MSK) (3) (Table 1). Both PK and MSK media were almost as effective as the MP medium in detecting *M. phaseolina* sclerotia in artificially infested field soil. However, the MSK medium was not sensitive enough to detect the low populations encountered in naturally infested field soil. Growth of other organisms was minimal on all media when artificially infested soil was sampled. However, with naturally infested field soil, growth of other organisms on the PK medium was so great that recognition of *M. phaseolina* colonies was very difficult; such growth was not a problem with the MP or MSK media. At least 6 days were required before *M. phaseolina* colonies were detectable on PK and MSK media, while only 3–4 days were required for detection on MP medium.

Additional tests were conducted to determine the sensitivity of the MP medium. In the first, an artificially infested field soil was found to contain 138.6 ± 11.2 sclerotia per gram. When this soil was diluted 1:10 and 1:100 with sterile sand, 13.3 ± 1.9 and 1.1 ± 0.2 sclerotia per gram were detected. Similar results were obtained when this test was repeated. In the second test, the vertical distribution of sclerotia in naturally infested field soil at 0–15 cm, 16–30 cm, and 31–60 cm depths was found to be 0.75 ± 0.72, 0.62 ± 0.08, and 0.07 ± 0.07 per gram, respectively. Similar results were obtained in the second test.

The selective medium is also suitable for isolating *M. phaseolina* from infected plants. Surface-sterilized (30 sec in 0.525% sodium hypochlorite) stem and root sections of *Euphorbia lathyris* L. were placed in petri dishes containing solidified medium. After incubation for 3–4 days at 32–33 C, colonies characteristic of *M. phaseolina* were readily observed. The medium remained suitable for selective isolation after being stored for 4 mo at 12 C.

Because of the small number of antimicrobial chemicals used in MP (PK and MSK both have five), the sensitivity of the medium, the rapid growth on it by *M. phaseolina*, and its shelf life, the MP medium appears useful for isolating *M. phaseolina*.

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