Populations of Macrophomina phaseoli in Soil as Affected by Fumigation and Cropping

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ABSTRACT

A technique for the direct isolation of *Macrophomina phaseoli* from soil by the differential flotation of microsclerotia is described. Using this technique, the effects of soil fumigation and subsequent cropping on the populations of *M. phaseoli* in soil were determined. Populations of microsclerotia, greatly reduced by soil fumigation, increased very little after 2 years in fallow soils or soils cropped to ponderosa pine. In soils sown to white fir, a more susceptible species, there was a greater increase in the *M. phaseoli* populations. These studies indicate that with proper soil fumigation, the seed beds need not be fumigated every year in order to control *M. phaseoli* losses. Phytopathology 60:1717-1719.

Macrophomina phaseoli (Maubl.) Ashby, a widely distributed plant pathogen, is the causal agent of the charcoal root disease of many hosts, including coniferous seedlings and cotton (3, 8, 11). This pathogen overwinters and survives adverse conditions by means of microsclerotia formed in the hosts' roots and lower stems, which are eventually liberated into the soil. The microsclerotia lie dormant in the soil until a new host crop is planted (4, 7). Recent studies have shown that soil fumigation prior to planting is an effective means of controlling this disease (9, 10). Those studies, however, tested fumigants in reference to host infection, response, and mortality only, and left untested the direct effects of the fumigant on the pathogen and its population of microsclerotia in the soil. Furthermore, little or no information is available on the population increases of *M. phaseoli* in cropped areas following soil fumigation. Such formation would aid in determining the future fumigation requirements of infested land.

In the fall of 1963, fumigation trials were conducted in the nursery at the Institute of Forest Genetics in Placerville, California, where the charcoal root disease had been prevalent. White fir, *Abies concolor* (Gord. & Glend.) Lindl., and ponderosa pine, *Pinus ponderosa* Laws., were the two species planted in these trials in the spring of 1964. Fallow areas were also included. The trials were maintained for 2.5 years. The seedling mortality and infection data are contained in an earlier paper (10).

In the present study, a flotation technique was developed for the direct quantitative isolation of microsclerotia of *M. phaseoli* from soil. Using this technique, the microsclerotial populations of *M. phaseoli* were determined in the soils of the various treatments of the abovementioned fumigation trial. Other workers have devised various flotation-type techniques to separate propagules of plant pathogens from soil. Rogers (5) isolated the sclerotia of *Phymatotrichum omnivorum* from soil by differential flotation in sugar solutions. Chinn et al. (1, 2) were able to separate the spores of *Helminthosporium sativum* from soil and determine their populations in infested wheat fields by flotation in mineral oil.

The purpose of this study was to determine (i) the long-term effects of soil fumigation on the populations of *M. phaseoli* in soil, (ii) the buildup of populations of *M. phaseoli* after fumigation in planted and nonplanted land, and (iii) the relation of host susceptibility to population increases of the pathogen.

MATERIALS AND METHODS AND RESULTS.—*Isolation technique.*—The isolation technique developed depends upon the differential flotation of microsclerotia of *M. phaseoli* from the majority of other soil particles of air-dry soil. Soil samples were air-dried at 30 C, a temp favorable for *M. phaseoli* (6). A 0.10-g quantity of air-dry soil was put into a 3-ml glass vial, and 2.5 ml of a 25% (w/v) ammonium sulfate solution was added. This was allowed to stand for 10 min; the microsclerotia floated to the surface, and most other soil particles settled to the bottom. The microsclerotia were removed from the vial by the addition of more ammonium sulfate solution, which caused the solution to overflow with the microsclerotia into a 9-cm filter paper funnel. The sclerotia were immediately rinsed by passing 100 ml of sterile distilled water through the filter paper. A 30-min test soaking in a 25% solution of ammonium sulfate did not reduce the viability of cultured microsclerotia. The filter paper was removed from the funnel and placed open and flat in a sterile petri dish. The microsclerotia were tentatively identified and counted under the dissecting scope at ×30. The counting of the microsclerotia was facilitated by marking the filter paper with lines 5 mm apart prior to its use.

The final identification and viability of the microsclerotial-like particles was accomplished by culturing them on potato-dextrose agar (PDA). As the microsclerotia were counted they were removed aseptically from the filter paper and transferred to PDA plates or slants. The identity and viability were then determined from the developing colonies. If bacterial or fungal contamination became a problem, the microsclerotia were treated while still on the filter paper in the funnel with a 0.0525% solution of sodium hypochlorite for 30 sec, followed by two rinses with distilled water. This treatment greatly reduced the number of

contaminants without lowering the viability of the microsclerotia.

The effectiveness of the flotation technique as a quantitative sampling method was tested in the following manner. Cultured microsclerotia formed in potato-dextrose broth and separated in a Waring Blendor (7) were rinsed and air-dried. Ten samples of microsclerotia were weighed and counted. The number of microsclerotia/g of cultured microsclerotia was calculated to be 922 ± 138 at a 95% confidence level. Ten samples of air-dried cultured microsclerotia weighing between 1.5 and 100 mg were mixed with 20 g of air-dried Salinas soil. This Salinas soil had been previously found free of microsclerotia of *M. phaseoli*. The number of microsclerotia added per g of soil was calculated for each sample. Three or eight 0.1-g samples were taken from each of the 10 soil-microsclerotia mixtures and assayed for microsclerotia by the flotation technique. The identity of the sclerotia was verified by culturing, and the number recovered was compared to the computed number added on a per-g-of-soil basis.

The average percentage of microsclerotia recovered by the flotation technique from the 10 artificially infested soil samples was 111 ± 35% at a 95% confidence level. The percentage recovery from the samples ranged from a low of 77% to a high of 134% (Table 1).

Soil assay of fumigation trials.—In the fall of 1963, 12 fumigation test plots, each 24 ft long and 5 ft wide, were laid out at the Institute of Forest Genetics nursery. Three soil treatments, each replicated 4 times, were randomly assigned among the 12 test plots. The three treatments were: (i) soil fumigation with Trizone (61% methyl bromide, 31% trichloronitromethane, and 8% propargyl bromide) at a rate of 200 lb./acre; (ii) soil fumigation with Pathofume (57% methyl bromide and 43% trichloronitromethane) at 325 lb./acre; and (iii) a nontreated check. Details of the fumigation are in an earlier paper (10). Three subplots: (i) a white fir sowing, (ii) a ponderosa pine sowing, and (iii) a fallow area were placed within each of the 12 plots. The plots were sown in the spring of 1964, and the seedlings were removed in the summer of 1966.

TABLE 1. The recovery of the microsclerotia of *Macrophomina phaseoli* from soil artificially mixed with cultured microsclerotia by the flotation method

Microsclerotia/g soil			
Wt (mg) for mixing	Calculated no. added	No. after reisolation	% Recovery
0.075	70	54[a]	77
0.080	75	83[a]	111
0.090	84	93[a]	111
0.093	87	108[a]	124
0.175	163	166[a]	102
0.200	186	221[a]	119
0.405	377	371[a]	98
0.465	432	580[a]	134
2.500	2,323	2,627[b]	113
5.000	4,645	5,817[b]	125
			Avg 111 ± 35[c]

[a] Average of 3 samples.
[b] Average of 8 samples.
[c] 95% confidence level.

TABLE 2. Average number of viable microsclerotia of *Macrophomina phaseoli*/g soil in fumigated and nonfumigated nursery soils planted to white fir, ponderosa pine, or left fallow

Fumigation treatment	Microsclerotia/g soil[a]		
	Fir	Pine	Fallow
Nonfumigated check	35	17	17
Trizone,[b] 200 lb./acre			
Pathofume,[c] 325 lb./acre			

[a] Each figure is the average of 24 samples, 6 combined samples from each of 4 plots.
[b] Trizone: 61% methyl bromide, 31% trichloronitromethane, and 8% propargyl bromide.
[c] Pathofume: 57% methyl bromide and 43% trichloronitromethane.

On 4 April and 26 October 1966, before and after seedling removal, soil samples were taken from six positions in each subplot in all 12 plots to a depth of 6 inches, using a ¾-inch-diam soil auger. The six samples of each subplot were combined after collection. The samples were taken to the laboratory where they were air-dried, sieved through a 1-mm screen, and assayed for microsclerotia of *M. phaseoli* by the flotation technique.

The average number of microsclerotia/g soil for each fumigation and sowing treatment is shown in Table 2. The highest population of viable microsclerotia, 35 microsclerotia/g soil, was found in the nonfumigated white fir-sown subplots. The lowest populations, from 0-3 microsclerotia/g soil, were found in the fumigated fallow and pine-sown soils. In general, the nonfumigated soils contained more microsclerotia than the fumigated, and the fir-sown soils contained more than either the fallow or pine-sown areas.

DISCUSSION.—The flotation technique proved to be an efficient means of isolating microsclerotia of *M. phaseoli* from soil. The over-recovery of *M. phaseoli*,

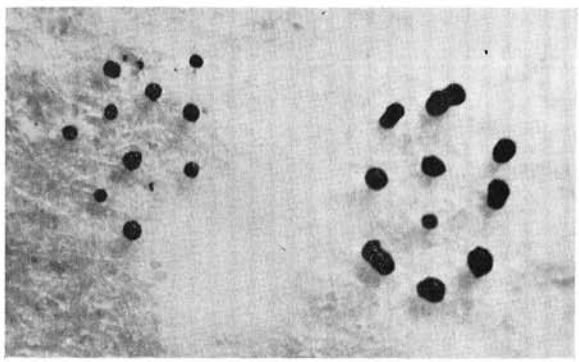

Fig. 1. Comparison of microsclerotia from plant tissue (left) and liquid culture (right). Both sets of microsclerotia originated from the same isolate from Placerville nursery. The tissue-formed microsclerotia were grown in bean stems, and the cultured microsclerotia were formed in potato-dextrose broth. The average sizes of 10 microsclerotia from plant tissue and liquid culture were 99 × 108 μ and 104-146 μ, respectively.

cultured microsclerotia from the Salinas soil, 111 ± 35%, may have been the result of the breaking apart of large elongate microsclerotia. The rather large differences noted in the sizes and wt of cultured microsclerotia may have resulted in variable amounts of sclerotia being added to the soil, and hence may be partially responsible for the variations in recovery in the test of the flotation technique (Fig. 1).

The results of the population assays indicate that soil fumigation with combinations of methyl bromide and chloropicrin effectively reduced the population of microsclerotia of *M. phaseoli* in soil. Where the soil fumigation was adequate, as in the 325 lb./acre treatment, the population buildup was rather slow and was still quite low even after 2 years of cropping. The population was the greatest in the soils planted to the most susceptible host, in this case white fir. This influence of crop susceptibility on populations in soil can be seen when one compares the average number of infections/root sample shown by Smith & Krugman (10) with the population of microsclerotia in the same soil after 2 years' cropping (Table 2). In the soils planted to ponderosa pine, a species which possesses moderate resistance, there appeared to be no difference in the population in nonfumigated soils when compared to the fallow soils.

The data obtained in this study also indicate that when the dosage is adequate, the populations of *M. phaseoli* in fumigated soils are still low after 2 years even with a highly susceptible crop, and as a result the soil need not be fumigated every year to obtain adequate disease control.

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