Survival of *Macrophomina phaseolina* in Soil and in Residue of Soybean

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ABSTRACT

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Survival of sclerotia of *Macrophomina phaseolina* in soil and in soybean residues was studied under mid-Missouri field conditions from May 1975 to October 1977. Sclerotia produced in soybean tissues survived longer in soil than did sclerotia produced in laboratory culture. Sclerotia from soybean tissues were added to soil to augment the natural infestation level. In fallow soil augmented with sclerotia, populations of germinable sclerotia increased by an average of 11% from January to April and decreased by an average of 3% from April to July. Field populations of sclerotia also increased from January to April, with a decline occurring later each year. Populations of germinable sclerotia in soybean residue on the soil surface increased threefold from October 1975 to July 1976, then declined for 9 mo. Populations of germinable sclerotia in residue pieces buried 10–20 cm below the soil surface were relatively stable. When populations of germinable sclerotia declined in residue pieces, there was a concomitant increase in the number of germinable sclerotia in the contiguous soil. The depth at which sclerotia were buried, either free in soil or within soybean residue, had little effect on their persistence. By October 1977, the populations of germinable sclerotia in fallow soil and in residue pieces were as great, or greater, than the initial infestation levels in 1975. Populations of germinable sclerotia in soil were directly related to the number of consecutive years of planting soybeans and corn in the field and showed a twofold increase from 1975 to 1977. In 1975, 1976, and 1977, severity of charcoal rot of soybean was directly related to the population of germinable sclerotia of *M. phaseolina* in soils, and soybean yields were inversely related to the severity of charcoal rot.

Macrophomina phaseolina (Tassi) Goid. causes charcoal rot in more than 400 plant species (16), including corn, sorghum, and soybeans (5,7). This fungus produces sclerotia in root and stem tissues of its hosts which enable it to survive in soil (2,13). These sclerotia constitute the primary inoculum.

The longevity of sclerotia of *M. phaseolina* is not known (5); however, when subjected to the highly variable weather in Missouri and Nebraska, the populations of germinable sclerotia in soil (3) and in colonized host tissues (5) have been reported to decline with time. Factors that adversely affect the persistence of these propagules include repeated freezing and thawing of soil (3), low carbon:nitrogen ratios in soil (7,8), and high soil moisture (7,8). Population dynamics of sclerotia of *M. phaseolina* in soil or in colonized host tissue have never been carefully examined under field conditions over an extended period of time.

The purposes of this investigation were: to observe at regular intervals over a 2.5-yr period the populations of sclerotia in the soil and in decaying colonized host residue during exposure to mid-Missouri weather conditions in the field, to study the relationship between cropping history of a field and the population of sclerotia of *M. phaseolina* in soil, and to determine if a relationship exists between the number of germinable sclerotia of *M. phaseolina*, severity of charcoal rot of soybean, and soybean yield.

MATERIALS AND METHODS

Soil infestation. This study was conducted on a farm near Columbia, Missouri, where in 1975 the University of Missouri and the U.S. Department of Agriculture began a study of various soybean production systems. The soil was a Mexico silt loam containing approximately 20% clay and having a pH of 5.5. The natural populations of *M. phaseolina* on individual plots (12.2 × 45.4 m) ranged from three to 49 sclerotia per gram of soil in the spring of 1975. In several of our studies, the natural population was augmented with sclerotia of *M. phaseolina* reared in the laboratory or recovered from naturally infested soybean residue as indicated in the procedures. Sclerotia of strain S of *M. phaseolina* (21) were produced in potato dextrose broth (PDB) cultures, as previously described (17). When sclerotia from colonized host tissues were used, soybean roots and stems were ground in a Wiley mill and the sclerotia were separated from the bulk of the soybean residue by dry screening with a 106-μm (150-mesh) sieve.

Soils were artificially infested as follows: Soil samples were collected from selected areas on the farm, passed through a 1.7-mm (10-mesh) sieve, and mixed with sclerotia from PDB culture or from soybean residue. These samples of artifically infested soil were returned to the same areas in the field from which they were collected. There were four selected areas for each experiment, hence four replications. In one experiment, soybean root and stem segments (5–8 cm in length), heavily infested with sclerotia of *M. phaseolina,* were either buried in or placed on the surface of nonaugmented soil. This experiment was replicated five times.

Determination of inoculum density in soil and in host residue. The numbers of sclerotia in fallow soil and in soybean residue segments was determined every 3 mo from July 1975 to October 1977. Soil and soybean residue samples were collected with a tubular soil probe (2.5 cm diameter) so that numbers of sclerotia could be determined at 0–10 cm and 10–20 cm, or 0–5, 5–10, 10–15, and 15–20 cm. One probe sample was collected at each sampling date in each selected area (four replications). Samples were processed as soon as they were air dry.

A modification of the procedure used by Papavizas and Klag (15) was used to estimate numbers of sclerotia in soil. Each soil sample (size varied with experiment) was air-dried and passed through a 1.7-mm sieve. A portion was then weighed and suspended for 10 min in 0.5% sodium hypochlorite (NaOCl) by three comminutions (each 10 sec in duration at 5-min intervals) in a Waring Blendor. This treatment killed the mycelial tissue and left the sclerotia unharmed (18). The soil preparation was poured onto a 90-μm (170-mesh) sieve and a 44-μm (325-mesh) sieve and washed with running tap water for 2–3 min. The washed residues on the 44-μm

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and 30-μm (500-mesh) sieves were transferred into molten Chloroneb-mercuric chloride-rose bengal agar (CMRA) at 45 C, agitated, and immediately poured into 10 petri dishes. The dishes were incubated in the dark at 33 C for 1 wk, and examined for colonies of *M. phaseolina*.

Numbers of sclerotia in residue samples of 25 or more soybean root and stem segments were determined as follows: Four samples from the selected areas were used and treated separately. Residue segments were carefully removed from the soil in which they had been buried, hereafter referred to as the contiguous soil. The segments were washed in running tap water to remove adhering soil particles, air-dried for 2 days and ground in a Wiley mill fitted with a 425 μm (40-mesh) screen. A weighed residue sample was placed in 0.5% NaOCl for 10 min of intermittent comminution (each 10 sec in duration at 5-min intervals) in a Waring Blendor. Then the residue was poured onto a 30-μm sieve, washed in running tap water 1–2 min, and transferred to 250 ml of distilled water for 0.5 hr. The residue then was collected on a 30-μm sieve and suspended in molten CMRA at 45 C, agitated, and immediately poured into petri dishes. The dishes were examined for colonies of *M. phaseolina* after incubation for 1 wk at 33 C in the dark.

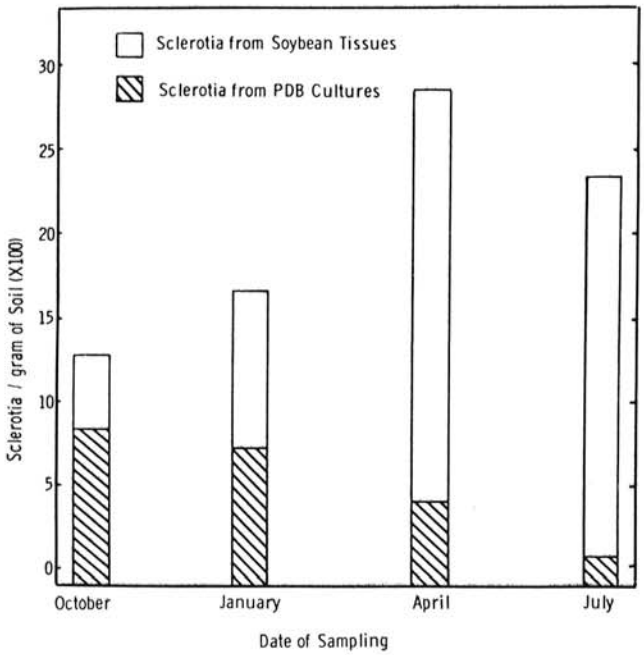

Fig. 1. Populations of germinable sclerotia (>25 μm in diameter) of *Macrophomina phaseolina* in the soil of a mid-Missouri field from October 1975 to July 1976. The natural infestation level of *M. phaseolina* was augmented with sclerotia from potato-dextrose broth (PDB) cultures or from colonized soybean tissue.

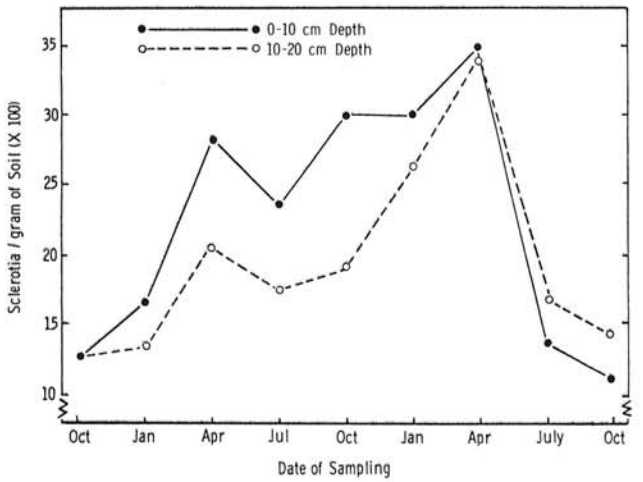

Fig. 2. Populations of germinable sclerotia (>25 μm diameter) of *Macrophomina phaseolina* in the soil of a mid-Missouri field from October 1975 to October 1977. The natural infestation level of *M. phaseolina* was augmented with sclerotia from colonized soybean tissues. Populations were determined at depths of 0–10 and 10–20 cm below the soil surface.

Assessment of disease severity. In 1975, 16 soybean plants (cultivar Williams) from each field with a different cropping history were dug 6 wk after planting; their roots were thoroughly washed in tap water, surface disinfested in 0.5% NaOCl for 1 min, and rinsed in sterile distilled water. For each plant, primary and secondary roots were sectioned into segments 5–10 mm long. The entire primary root, in segments, and 20 randomly selected secondary root segments were plated on CMRA and incubated for 1 wk at 33 C. The percentage of segments yielding *M. phaseolina* was used as a measure of disease incidence. In 1976, disease severity was estimated 10–20 wk after planting (early flower stage) with a more refined method (18) of determining the extent of root colonization. With this procedure, the numbers of sclerotial and mycelial propagules of *M. phaseolina* in root tissue could be determined.

RESULTS

Numbers of viable sclerotia in the soil changed from October 1975 to July 1976 and these changes were related to their origin (Fig. 1). The number of germinable sclerotia in soil augmented with sclerotia from PDB cultures declined from 842 to 80 sclerotia per gram of soil. In contrast, the population of germinable sclerotia in soil augmented with sclerotia from soybean residue increased from 1,270 to 2,360 sclerotia per gram of soil. Because of the unusually poor survival of sclerotia produced in PDB, sclerotia from that source were not used in subsequent studies.

Population changes in soil augmented with sclerotia from soybean tissues. Although the number of germinable sclerotia in soil in October 1977 was not significantly different from the number in October 1975, characteristic seasonal fluctuations were apparent (Fig. 2). In both 1976 and 1977, the number of germinable sclerotia increased from January to April and declined from April to July. The number of germinable sclerotia also increased from July to April and declined from April to October (Fig. 2). The depth at which the sclerotia were located in the soil had little effect on the pattern or the magnitude of these fluctuations.

Sclerotial population changes during fallow in infested field soil. The ability of sclerotia to persist in fallow soil was substantial (Fig. 3). The numbers of germinable sclerotia at all soil depths tested were no less in October 1977 than in July and October 1975. In 1976 and 1977, the numbers of germinable sclerotia in soil increased

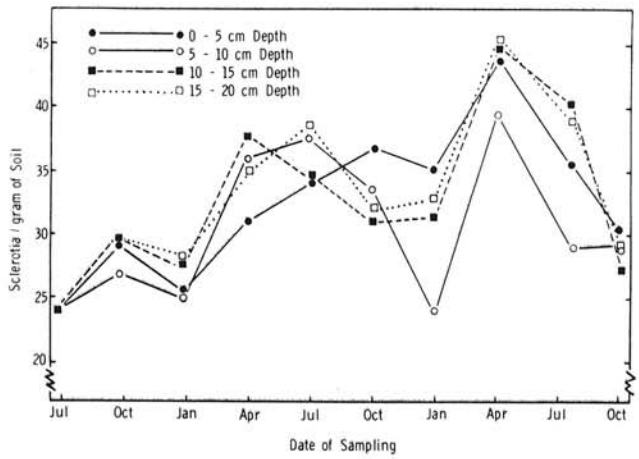

Fig. 3. Populations of germinable sclerotia (>25 μm diameter) of *Macrophomina phaseolina* in a naturally infested fallow soil of a mid-Missouri field from July 1975 to October 1977. Populations were determined at depths of 0–5, 5–10, 10–15, and 15–20 cm below the soil surface.

from January to April, and declined later during both years. The depth at which the sclerotia were located had no apparent effect on the pattern or magnitude of these fluctuations.

Population changes in infested host residue and in the contiguous soil. Populations of germinable sclerotia in soybean residue buried in soil in October 1975 were as high or higher by October 1977. Numbers of germinable sclerotia fluctuated least in residue buried 10–20 cm below the soil surface; fluctuation was greatest in residue on the soil surface (Fig. 4a). Sclerotial population changes in host residue exhibited no clear seasonal pattern at any soil depth. There was a tendency for the number of sclerotia in debris to increase to levels greater than the initial infestation level of October 1975 except at 10–20 cm, although these differences were minimal. The rapidity with which maximum numbers of sclerotia developed in soybean debris was inversely related to the depth at which the residue was buried; maximum populations of germinable sclerotia were recorded first in July 1976 in debris on the soil surface, second in October 1976 in debris buried at 0–10 cm, and third in January 1977 in debris buried at 10–20 cm. The subsequent decline in numbers of sclerotia during the winter of 1976–1977 was accompanied by a simultaneous increase in the number of germinable sclerotia in the soil contiguous to the pieces of soybean debris (Fig. 4b).

Effect of plant canopy on oversummering of sclerotia. Plant canopy had no effect on the populations of sclerotia in infested field soil at the 0–5 cm depth through July and August 1975 (Table 1). The slight increase in numbers of sclerotia from 5 July to 5 August appears to be a general phenomenon associated with soil that has been screened, thoroughly mixed, and returned to the field (P. R. Bristow and T. D. Wyllie, *unpublished*).

Effect of cropping history on soil populations of *M. phaseolina*. The populations of sclerotia in individual fields in 1975 were directly related ($r = 0.98$) to the number of years the field had been planted to corn and soybeans since 1970 (Table 2). By continuously cropping with corn and soybeans, the inoculum densities of sclerotia in the five fields that were monitored increased approximately twofold from 1975 to 1977 (Table 3).

Relation between inoculum density, disease severity, and yield. In 1975 and 1976, the severity (extent of root colonized by *M. phaseolina*) of charcoal rot of soybean was directly correlated with the population density of sclerotia ($r = 1.00$ and 0.94 for 1975 and 1976, respectively) in soil (Table 3). In 1975, 1976, 1977, the soybean yields were inversely related to the density of sclerotia in soil ($r = -0.98$, -0.84, and -0.98, respectively). Though disease severity ratings were not taken in 1977, in both 1975 and 1976, soybean yields were inversely related to the percentage of roots colonized by *M. phaseolina* ($r = -0.98$ and $r = -0.71$). Finally, yields from 1975 to 1977 were related to the total amount of rainfall recorded in July, August, and September; for example, yields were much less when only 1.3 cm of rainfall was measured from 1 July to 30 September, than in 1975 and 1977 when over 20 cm of rainfall fell during the months of July, August, and September (Table 3). Although one would expect yields to increase with adequate moisture, the relationship between numbers of sclerotia in soil and soybean yield remained valid for each year.

DISCUSSION

Charcoal rot of corn, sorghum, and soybeans is most severe in arid soil and when temperatures are high during the growing season (5,9,13). Incidence and severity of charcoal rot of these crops have increased in recent years (5,9,10) and there are several contributing factors. Monocropping and alternate cropping (ie, corn and soybeans) have contributed to the damage caused by *M. phaseolina*. Our data demonstrate that populations of the pathogen in soil are influenced by cropping history and that increase in numbers of sclerotia in soil occur and do adversely affect yield (12) (Table 3).

The ability of *M. phaseolina* to survive in soil under mid-Missouri field conditions was markedly greater with sclerotia obtained from soybean residue than with sclerotia from PDB cultures (Fig. 1). Previous studies on survival of *M. phaseolina* in soil frequently have used sclerotia produced in broth cultures (3,8). Our data demonstrate the importance of using field-produced propagules for studies designed to interpret field results.

High (50 C) and low (−10 C) temperatures both were reported (1,3) to adversely affect survival of *M. phaseolina*. Soil

TABLE 1. Effect of canopy on populations of *Macrophomina phaseolina* in naturally infested soil from 5 July to 5 September 1975

Date	Numbers of sclerotia per gram of soil under current crop canopy		
	None (Fallow)	Soybeans	Corn
5 July	24 (20–28)[a]	24 (20–28)	24 (20–28)
5 August	31 (24–33)	28 (27–30)	27 (25–28)
5 September	30 (26–35)	29 (28–31)	28 (25–30)

[a] Mean and range of sclerotia (> 44 μm in diameter) in four replications per treatment.

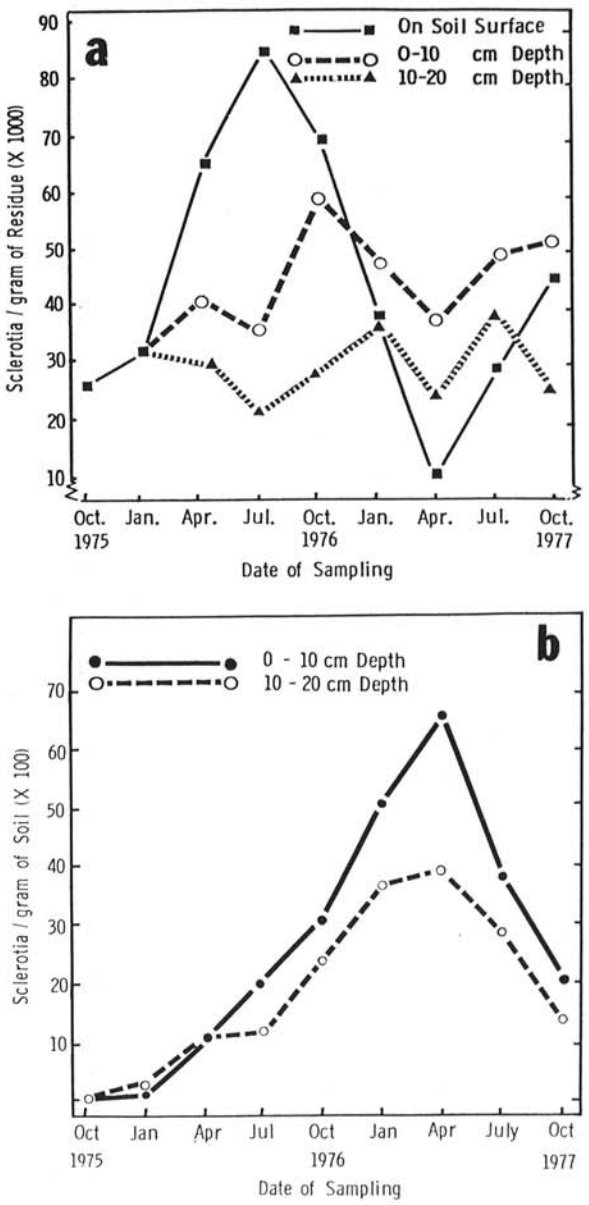

Fig. 4. Populations of germinable sclerotia (>25 μm diameter) of *Macrophomina phaseolina* in a mid-Missouri field from October 1975 to October 1977. Soybean root and stem segments containing sclerotia of *M. phaseolina* were buried in the soil or placed on the soil surface in October 1975. Populations of viable sclerotia **a,** in residue and **b,** in soil were determined at various depths.

TABLE 2. Effect of cropping history on populations of *Macrophomina phaseolina* sclerotia in soil

Field identification	Crop per year				Sclerotia per gram of soil in 1975[a] (no.)
	1971	1972	1973	1974	
2	1954 Fescue			Soybeans	7 (3–15)
3	1954 Fescue			Corn	8 (4–13)
8	1970 Fescue		Soybeans	Soybeans	15 (10–28)
9	1970 Fescue		Soybeans	Corn	17 (10–31)
10	1967 Fescue		Soybeans	Soybeans	17 (11–25)
7	Corn	Soybeans	Soybeans	Corn	24 (16–30)
4	Corn	Soybeans	Soybeans	Soybeans	27 (15–43)
5	Corn	Soybeans	Soybeans	Corn	28 (13–41)
1	Corn	Wheat	Soybeans	Corn	30 (20–45)
6	Corn	Soybeans	Soybeans	Soybeans	32 (17–49)

[a] Mean and range of sclerotia (> 44 μm in diameter) in 12 plots per field.

TABLE 3. Populations of *Macrophomina phaseolina* in soil in relation to colonization of soybean roots and to soybean yields

Year	Field identification	Sclerotia per gram of soil[a] (no.)	Disease incidence (%)		Yields (kg/ha)	Rainfall[e] (cm)
1975	3	8	3[b]		1,816	26.0
	9	17	12		1,547	
	7	24	17		1,345	
	5	28	21		1,211	
	1	30	23		1,278	
1976	2	7	2[c]	1[d]	807	1.3
	8	15	12	8	673	
	10	17	15	9	538	
	4	27	61	14	538	
	6	32	30	29	538	
1977	3	17			2,421	21.8
	9	33			1,749	
	7	43			1,345	
	5	48			1,412	
	1	48			1,278	

[a] Soil populations of sclerotia (>44 μm in diameter) for 1975, 1976, and 1977 were determined in May 1975, September 1975, and April 1977, respectively.
[b] Percent colonized root segments (5–10 mm in length) of 6-wk-old plants.
[c] Numbers of mycelial propagules per gram of root tissue from soybeans in early flower stage of development.
[d] Numbers of sclerotia per gram of root tissue from soybeans in early-flower stage of development.
[e] Rainfall from 1 July through 30 September.

temperatures at midsummer in Missouri apparently are not high enough to decrease the viability of sclerotia (Table 1). Effects of freezing temperatures in the field were not definitive; both increases (Fig. 1–4) and decreases (Fig. 1 and 4) in sclerotial populations were observed immediately following the winter months. However, populations on 1 April 1976 and 1977 may have been much greater than during January and February when freezing and thawing of soil occurred. Moustafa and Wyllie (14) found populations of *M. phaseolina* in soil to be lowest in February, and to increase fourfold by May 1974; however, their methods did not permit differentiation between mycelial and sclerotial propagative units in soil. Perhaps a reduction of sclerotia in soil during the winter months is largely offset by the formation of new sclerotia in early spring.

M. phaseolina survives well in soybean residue (Fig. 4a). The population of sclerotia in soybean residue on October 1977 was as high or higher than in October 1975. Fluctuations of the populations in debris may be affected by the ability of the fungus to survive and grow in the residue and by the release of sclerotia into the soil. The dramatic decrease in numbers of sclerotia in residue on the soil surface from July 1976 to April 1977 (Fig. 4a) would appear to result from the release of sclerotia from residue segments (Fig. 4b). Secondly, Dhingra and Sinclair (7) reported reduced numbers of germinable sclerotia in soybean stem pieces after 3–4 wk at 30 C in soil at 60–100% moisture holding capacity. There were no apparent long-term affects on the survival of *M. phaseolina* in soil at depths down to 20 cm (Fig. 4a). This suggests that survival of sclerotia of *M. phaseolina* in soil (Fig. 2,3, and 4b) or in residue (Fig. 4a) in Missouri may not be greatly affected by the tillage system that is used.

The rapid increase in populations of sclerotia in soils cropped with susceptible hosts (Tables 1 and 3) (12), the persistence of sclerotia in soil (Fig. 3) and in soybean residue (Fig. 4b), and the direct relation between soil populations and yield reduction (Table 3) (4,13,19) suggest that charcoal rot must be controlled by means other than crop rotation. Fumigation (20), addition of organic amendments (8,11), and maintenance of high soil moisture (8) have been suggested as possible methods of control; however, use of a hyperparasite (6) and the incorporation of genes conferring general resistance to the host (18) would likely have wider applicability.

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