Tillage Effects on *Macrophomina phaseolina* Population Density and Soybean Yield

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ABSTRACT

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Reduced-tillage production systems have become popular with soybean farmers in the United States. During 1992 to 1994, soil and soybean root samples were collected from disk-tillage, no-tillage, and moldboard plow treatments in an experiment conducted since 1979 at the University of Tennessee in Jackson. The objective was to determine if reduced-tillage production systems affected charcoal rot incidence and severity. There were no differences at planting in soil population density of *Macrophomina phaseolina* in the 0- to 15-cm layer of soil among treatments. However, the soil population density of *M. phaseolina* in disk-tillage and no-tillage plots was significantly greater in the 0- to 7.5-cm layer of soil than in the 7.5- to 15-cm layer. Similarly, soil population densities were significantly greater in the 0- to 7.5-cm layer of soil in no-tillage than in either disk-tillage or moldboard plowed plots. Tillage did not affect the number of *M. phaseolina* infection sites in 6-week-old plant roots or the percentage of root segments of physiologically mature plants colonized by *M. phaseolina*. There was no significant correlation between soybean yield and *M. phaseolina* soil population density. These data suggest that long-term tillage did not affect charcoal rot incidence and severity.

Charcoal rot, caused by *Macrophomina phaseolina* (Tassi) Goidanich, is a serious problem of soybean, *Glycine max* (L.) Merr. (23). For example, the estimated total yield loss due to this disease in Argentina, Brazil, Canada, Paraguay, and the United States during 1994 was 1.23 million metric tons, valued at \$272.26 million (\$220.50/metric ton) (21). In the United States alone, the yield loss attributable to charcoal rot in 1994 was 0.28 million metric tons, valued at \$60.65 million.

There are few strategies for control of charcoal rot in soybean. There are no resistant genotypes, although the rates of pathogen colonization may differ among soybean cultivars (11). Crop rotation is of limited benefit as a control tactic for charcoal rot because this fungus has a wide host range (8). However, lower soil population densities of *M. phaseolina* occur as soybeans are grown less frequently in rotation with either corn or cotton in southeast Missouri (3). Moreover, cotton in

rotation with soybean consistently reduced the population density of *M. phaseolina* more than did corn-soybean rotations. These results suggest that only long-term rotations of soybean with cotton or corn would be effective in decreasing the population density of *M. phaseolina* to non-damaging levels. Alternative methods are needed to reduce the density of this fungus in soil.

The soil population density of *M. phaseolina* can be affected by tillage. Rothrock and Hargrove (13) determined that the population density of this fungus in the upper 15-cm layer of soil was significantly greater under grain sorghum rows in no-tillage than in moldboard plow plots 4 years after the tillage treatments were established.

The effect of tillage systems on soybean diseases is important because there is a trend toward reduced-tillage production systems in the United States (6). Tillage before planting a full-season soybean crop can affect the incidence of brown spot (19), stem canker (14,19), and sudden death syndrome (22). In addition, tillage just prior to planting soybean after harvest of winter wheat affected the incidence of brown spot and bacterial blight (20). The population dynamics of soilborne pests such as soybean cyst nematode (1,17) and *Rhizoctonia* spp. (12) can also be affected by tillage.

The objective of this project was to determine if tillage treatments affected soybean yields and soil and soybean root population densities of *M. phaseolina*.

Data were collected during 1992 to 1994 from long-term tillage plots established in 1979 at the University of Tennessee in Jackson. Charcoal rot symptoms had been observed previously on some plants scattered through the test site, but no yield loss was attributed to this disease.

MATERIALS AND METHODS

A long-term tillage experiment involving continuous soybean was initiated in 1979 at the University of Tennessee at Jackson on a Lexington silt loam soil (fine-silty, mixed, thermic Typic Paleudalf). Treatments were no-tillage, moldboard plow (0.25 m deep) followed by disking, and disking alone (0.1 m deep). Each year, plots were tilled just prior to planting soybeans in late May. Vegetation in all plots was chemically killed with paraquat (Zeneca Ag. Products, Wilmington, DE) at 0.71 kg/ha about 3 weeks prior to soybean planting. Residual weed control in all soybean plots was accomplished by preemergence application of alachlor (Monsanto Company, St. Louis, MO) at 3.36 kg/ha and metribuzin (Miles Inc., Kansas City, MO) at 0.42 kg/ha. All plots were treated with alachlor, metribuzin, and paraquat, and all treatments except no-tillage were cultivated for supplemental weed control.

The tillage treatments were replicated four times in a randomized complete block design. Each plot was 6 m wide and 18 m long. From 1979 to 1982, the soybean cv. Forrest was planted in six rows (1-m spacing) per plot. From 1983 to 1984, four rows each of the soybean cvs. Bedford and Forrest (0.75-m spacing) were planted in each plot. During 1984 to 1991, eight rows of cv. Hutcheson (0.75-m spacing) were planted per plot, and cv. TN 4-86 was planted from 1992 to 1994. The middle two rows of each plot were harvested at maturity by combine, and yield was adjusted to 13% moisture.

Charcoal rot symptoms were not observed on the aboveground portions of plants in this experiment during 1992 to 1994. So charcoal rot disease data were not collected.

During 1992 to 1994, soybean plots were sampled for soil population density of *M. phaseolina* at planting. In 1992, four sets of soil cores were collected from each plot. The four sets consisted of 10 soil cores collected from within the two center rows and 10 from between the two center rows of each plot, and each core (2.5 cm

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diameter × 15 cm deep) was separated at collection into two portions by depth, 0 to 7.5 cm and 7.5 to 15 cm. The cores were bulked by depth and by location relative to the row and analyzed for microsclerotia of *M. phaseolina*. Soil cores were collected at planting in 1993 as in 1992, except cores were not bulked by depth or location relative to the row but were analyzed separately. No significant differences were observed in the population density of this fungus within individual plots of any treatment in 1993, so the 1993 subsample means were used in further analysis. There were no significant differences in *M. phaseolina* population density between the in-row and between-row samples during 1992 and 1993. Consequently, the 1994 soil samples collected from each plot were a composite of 10 cores collected from within and between rows (5 cores from each location), and these were separated at collection into two portions by depth, 0 to 7.5 cm and 7.5 to 15 cm.

Microsclerotial soil estimates were made by a modification of the procedure described by Short et al. (15). Soil samples were air-dried and then passed through a 45-μm-mesh sieve. A 5-g portion of the sieved soil was suspended in a 500-ml flask containing 250 ml of 0.5% sodium hypochlorite. The flask containing the suspension was placed on a rotating shaker (120 cycles per min) for 10 min. The suspension was then poured onto a 325-μm-mesh sieve, and the debris was rinsed with tap water for 30 s. The residue on the sieve

was transferred to a 250-ml flask, and 100 ml of the selective medium Cholorneb-Mercury-Rose Bengal agar (CMRB) was added (7). The suspension was gently swirled and then divided among five petri plates. The petri plates were incubated in the dark at 33°C for 5 to 7 days. Microsclerotia densities were calculated from the number of colony-forming units on the plates and adjusted to a per gram of air-dried soil basis. The colonies were identified as a ring of fluffy white mycelium surrounding a central area with black sclerotia.

Early season root infection by *M. phaseolina* was investigated during 1993 and 1994. Ten arbitrarily selected plants, V6 growth stage (2), were removed from the outside two rows of each plot. The intact roots were surface disinfested in 0.5% sodium hypochlorite for 60 s and then placed on a thin layer of CMRB agar in large trays (22.5 × 45 cm). CMRB agar, 50°C, was then poured over the top of the root system, and the tray was covered to prevent drying of the medium and contamination. Roots were incubated at 32°C for 5 days, and the number of colonies of *M. phaseolina* was then recorded. Each colony was considered an infection site on roots. The number of colonies developing on roots 0 to 7.5 cm from the hypocotyl-root transition zone was kept separate from the number on roots 7.5 to 15 cm from this zone. The numbers of colonies on roots 0 to 7.5 cm or 7.5 to 15 cm from this transition zone are hereafter referred to as infec-

tion sites on roots from 0 to 7.5 cm or 7.5 to 15 cm deep in the soil, respectively.

Late season colonization of soybean roots by *M. phaseolina* was investigated during 1992 to 1994. Six plants, growth stage R7 (2), were removed from the outside two rows of each plot. Roots were washed to remove soil, and 12 1.0-cm root segments per intact root system were arbitrarily selected. Root segments were surface disinfested in 0.5% sodium hypochlorite for 1 min, rinsed in distilled water for 10 s, and plated on CMRB agar. Root segments were not separated by soil depth because the mass of roots on these plants made differentiation by soil depth very difficult. After 2 to 3 days at 32°C, the number of root segments colonized by *M. phaseolina* was determined. The percentage of the 12 segments from which *M. phaseolina* was isolated was used in the analysis.

The organic matter content of each soil sample collected in 1993 was determined using the Walkley-Black method (9).

ANOVA was used to analyze all data, and mean separation was by an *F* test protected LSD (5). SAS (SAS Institute, Cary, NC) was used for all analyses. Data were pooled where appropriate for analysis of means over years.

RESULTS

Analysis of variance for soil population density of *M. phaseolina* data collected in 1992 to 1994 indicated that year and soil depth effects were significant (Table 1). The overall population density was significantly lower ($P = 0.05$) in 1994 (45 CFU/g soil) than in 1992 (61 CFU/g soil) and 1993 (67 CFU/g soil). Significant interactions were observed between tillage and soil depth (Table 1). When averaged over years, the soil population density of *M. phaseolina* was significantly greater within the 0- to 7.5-cm than the 7.5- to 15-cm soil depth for no-tillage and disk-tillage plots (Table 2). The population densities in plowed soil were similar between the two soil depths. The population density was significantly greater in the 0- to 7.5-cm layer of soil in no-tillage than in disk-tillage plots and lowest in moldboard plow plots. There were no significant differences in population density in the 7.5- to 15-cm layer between no-tillage and disk-tillage.

Analysis of variance for the number of sites infected on V6 growth stage plant roots collected in 1993 and 1994 indicated that year and soil depth effects were significant (Table 1). The number of early season infection sites per 10 plants over soil depths was significantly greater ($P = 0.001$) in 1993 (74 infection sites) than in 1994 (44 sites) and significantly greater over years ($P = 0.05$) in the 0- to 7.5-cm (62 sites) than the 7.5- to 15-cm (56 sites) soil layer. Tillage, however, did not affect the number of sites infected.

Analysis of variance for the percentage of R7 growth stage plant root segments

Table 1. Analysis of variance for soil population density of *Macrophomina phaseolina* and for the number of *M. phaseolina* infection sites on roots of V6 growth stage plants for year, tillage, and soil depth^a

Source of variation	Soil population density		Infection sites on plant roots (no.)	
	df	Mean square ^b	df	Mean square ^b
Year (Y)	2	0.157 *	1	10,980.75 **
Rep	3	0.026	3	303.98
Error A	6	0.031	3	330.15
Tillage (T)	2	0.017	2	589.15
Y × T	4	0.038	2	57.56
Error B	18	0.017	12	752.27
Depth (D)	1	2.439 ***	1	520.08 *
Y × D	2	0.002	1	0.33
T × D	2	0.447 ***	2	36.02
Y × T × D	4	0.009	2	17.27
Error C	27	0.017	18	117.17

^a Tillage treatments were no-tillage, disk-tillage, and moldboard plow. Soil depths were 0 to 7.5 and 7.5 to 15 cm.

^b Significance levels: *, $P = 0.05$; **, $P = 0.001$; and ***, $P = 0.0001$.

Table 2. Tillage and soil depth effects on soil population density of *Macrophomina phaseolina*, CFU/gram of dry soil, averaged over years^a

Depth	Tillage		
	No-tillage	Disk-tillage	Moldboard plow
0.0 to 7.5 cm	99.58	78.33	61.38
7.5 to 15 cm	22.99	31.98	50.92
LSD _(0.05) depth means for same tillage	= 13.50		
LSD _(0.05) across tillage same or different depth	= 13.12		

^a Years were 1992, 1993, and 1994.

colonized by *M. phaseolina* during 1992 to 1994 indicated that only year effects were significant (Table 3). The percentage of R7 growth stage plant root segments colonized by *M. phaseolina* was significantly less ($P = 0.007$) in 1992 (74%) than in either 1993 (90%) or 1994 (88%).

Significant interactions ($P = 0.0001$) were observed between tillage and soil depth for soil organic matter (Table 4). Organic matter in disk-tillage and no-tillage soil was significantly greater in the 0- to 7.5-cm than the 7.5- to 15-cm soil depth (Table 5). There were no significant differences in organic matter observed between soil depths in plowed soil, and there were no significant differences in organic matter level in the 7.5- to 15-cm depth among tillage treatments.

There was no significant correlation between organic matter and population density of *M. phaseolina* in the 0- to 15-cm soil layer. However, there was a significant and positive correlation ($P = 0.0001$) between organic matter and population density of *M. phaseolina* in the 0- to 7.5-cm-deep layer of soil.

Soybean yields were similar between tillage treatments and years (data not shown). The yields averaged 1,998 kg/ha over all years and tillage practices.

DISCUSSION

Aggregation in *M. phaseolina* population density has been observed in some fields (10,11); however, the degree of aggregation decreased after one and two diskings (11). Our data did not indicate

aggregation in *M. phaseolina* population density within individual plots. This may be due to the length of time, 14 years, this test was conducted before soil samples were first collected. The uniformity in *M. phaseolina* population density between in-row and between-row samples of disk-tillage and moldboard plow plots may be due to tillage distribution of the fungus. The reason for the similarity of *M. phaseolina* population density between in-row and between-row samples in no-tillage plots is not clear. Rows in these plots were planted in the previous years stubble, and the plots had not been tilled for 14 years. Moreover, the area between soybean rows was never planted with soybean, and plots were free of weeds that may have been hosts for *M. phaseolina*. However, soybean roots growing into the area between rows and the decomposing crop residue that fell between rows during harvest may have been the source of this fungus (16).

This is the first report of variation in *M. phaseolina* soil population density by tillage and soil depth. Rothrock and Hargrove (13) determined that the soil population density of *M. phaseolina* was greater in the 0- to 15-cm layer of soil in no-tilled than in tilled soil planted with grain sorghum. They did not measure the population density at other soil depths. The population density of *Rhizoctonia solani* AG 4 was not affected by tillage (13). Differences in population density of fungi between tillage treatments may be more evident if soil samples were collected from various depths.

The greater population density of *M. phaseolina* in the 0- to 7.5-cm layer of the no-tillage treatment than in other tillage treatments in our experiment may be due to reduced soil compaction. Analysis indicated that the soil bulk density was lower in no-tillage than in plowed plots at this Jackson site in 1992 and 1993 (18). The soil population density of *M. phaseolina* declined as soil compaction increased in central Missouri (4).

The greater population density of *M. phaseolina* in the 0- to 7.5-cm layer of soil in no-tillage may be due to the greater concentration of *M. phaseolina*-infested residue on the soil surface in no-tillage than in other treatments. Organic matter content and *M. phaseolina* population density in the 0- to 7.5-cm soil layer were significantly and positively correlated, and the percentage of organic matter was sig-

nificantly greater in the upper layer of soil from no-tillage than from other treatments. The greater percentage of organic matter in the no-tillage treatment than in other treatments was presumably from residue of previous crops, including stems, petioles, leaves, and shallow roots, accumulating in this soil layer. There were no differences in root length by tillage and depth at this site in 1992 and 1993 (18), but root mass and volume were not measured by soil depth.

The significantly greater number of *M. phaseolina* infection sites in V6 growth stage soybean roots during 1993 than during 1994 was probably due to the significantly greater soil population density of this fungus at planting in 1993 than in 1994. However, in this experiment the at planting population density of *M. phaseolina* did not affect the percentage of R7 growth stage soybean roots colonized by this fungus. The soil population density of *M. phaseolina* was significantly greater in 1992 than in 1994, but the percentage of R7 growth stage soybean roots colonized by this fungus was greater in 1994 than in 1992. Factors other than at planting population density may influence late-season infection of soybean roots by *M. phaseolina*. Soil moisture and temperature may have contributed to differences in infection and colonization rates of roots by *M. phaseolina* among years, but these data were not collected during 1992 to 1994.

No correlation was observed between soybean yield and at planting soil population density of *M. phaseolina* in the upper 0 to 15 cm of soil. This was probably due to the similarity in soil population of *M. phaseolina* in the 0- to 15-cm layer of soil observed among treatments. Although the population density in the 0- to 7.5-cm layer of soil was significantly different among treatments, there were no differences in the population density in the 0- to 15-cm layer among treatments. The effect of tillage imposed for short periods, 2 to 4 years, on *M. phaseolina* population density in monoculture soybean is unknown. Tillage treatments had been in place in this study 13 years before data were first collected. Over these 13 years, the population may have increased greatly, declined due to the buildup of antagonistic soil microbes, and then reached equilibrium.

In this study, colonization of soybean roots occurred regardless of tillage system. This suggests that efforts to develop cultivars resistant to charcoal rot must be en-

Table 3. Analysis of variance for the percent of R7 stage plant root segments colonized with *Macrophomina phaseolina* for year and tillage^a

Source of variation	df	Mean square ^b
Year (Y)	2	0.087 *
Rep	3	0.005
Error A	6	0.01
Tillage (T)	2	0.016
Y × T	4	0.008
Error B	18	0.005

^a Tillages were no-tillage, disk-tillage, and moldboard plow. Years were 1992 to 1994.

^b Significance level: *, $P = 0.0072$.

Table 4. Analysis of variance for soil organic matter for tillage and soil depth^a

Source of variation	df	Mean square ^b
Tillage (T)	2	2.648 *
Rep	3	0.678
Error A	6	0.183
Depth (D)	1	21.318 **
T × D	2	4.519 **
Error B	9	0.059

^a Tillages were no-tillage, disk-tillage, and moldboard plow. Depths were 0 to 7.5 and 7.5 to 15 cm.

^b Significance levels: *, $P = 0.05$ and **, $P = 0.0001$.

Table 5. Tillage and soil depth effects on percent soil organic matter after tillage treatments were in place 14 years

Depth	Tillage		
	No-tillage	Disk-tillage	Moldboard plow
0.0 to 7.5 cm	1.68	1.51	0.89
7.5 to 15 cm	0.78	0.67	0.85

LSD depth means for same tillage = 0.159.

LSD across tillage same or different depth = 0.295.

hanced to protect the U.S. soybean crop from this disease since control strategies and systems for this disease, including the use of tillage, are very limited.

The relationship between soil organic matter levels in no-tillage and in mold-board plow treatments after 14 years of the experiment was similar to the relationship after 4 years (19). The organic matter in the levels of soil studied in the no-tillage plots appears to change little after an initial increase during the first few years the practice is imposed.

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