

# Influence of Crop Rotation on Population Density of *Macrophomina phaseolina* in Soil Infested with *Heterodera glycines*

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## ABSTRACT

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Soil populations of *Macrophomina phaseolina*, cause of charcoal rot of soybean and various rots and blights of a large number of cultivated plants, might be managed by crop rotation if the density of infective microsclerotia in soil could be reduced below damaging levels. The soybean cultivar Forrest was grown in monoculture and in a 2-, 3-, or 4-yr rotation with corn, cotton, and grain sorghum from 1980 to 1984 in soil infested with the soybean cyst nematode, *Heterodera glycines*. This was to determine the population dynamics of *M. phaseolina* in response to cropping history and the quantitative effect of fungus and nematode on soybean yields. Highest population densities of *M. phaseolina* were found after soybeans were grown, but often not until early spring or at planting the following year, after crop residue had decomposed. Lower densities of *M. phaseolina* occurred as soybeans appeared less frequently in rotations. Cotton, in rotation with soybean, consistently reduced the population density of *M. phaseolina* more than corn-soybean rotations. The effect of sorghum on microsclerotial density was intermediate between corn and cotton. Soybean yield could be related to densities of *M. phaseolina* and *H. glycines* in 1984, but not in 1983. There was no evidence of an interaction between *H. glycines* and *M. phaseolina* in 1983 or 1984 with respect to yield. Crop rotation may be an effective method of reducing charcoal rot in soybeans, even though the other crops in the rotation are hosts of *M. phaseolina*.

*Macrophomina phaseolina* (Tassi) Goid. is known to cause a seedling blight, charcoal rot, and root and stem rot of a wide range of cultivated crops (14), usually under conditions of high soil temperatures (1,8) and low soil moisture (6,12,13). Charcoal rot of soybean (*Glycine max* (L.) Merr.) has been increasing in severity and prevalence in Missouri since it was first noticed in 1963. Today it can be found throughout the state, in all soil types, and in all locations where soybeans are grown.

Attempts to find resistance to *M. phaseolina* in soybean have been unsuccessful, although earlier studies on seedling resistance (2) seemed promising. Unfortunately, this resistance did not carry over into the mature plant. Cultural practices, therefore, remain the only immediate hope for controlling the pathogen. Apparently, crop rotations only recently have been considered an option with a pathogen that has such a

wide host range (10). Isolates of *M. phaseolina* that differed in their sensitivity to chlorate colonized corn (*Zea mays* L.) in preference to soybeans (10). If host preference among isolates of *M. phaseolina* proves to be the rule, then reduction of the appropriate microsclerotial phenotype may also increase yields. Scant information is available on the relative amounts of microsclerotia produced on different hosts or on the possible interactions between *M. phaseolina* and nematodes. Low microsclerotial density in soil typically results in decreased soybean charcoal rot. The current study was undertaken to determine the influence of crop rotation schemes on population levels in soil of *M. phaseolina*, as affected by the presence of the soybean cyst nematode,

*Heterodera glycines* Ichinohe.

## MATERIALS AND METHODS

The soybean cultivar Forrest (maturity group V) was grown in monoculture and in a 2-, 3-, or 4-yr rotation with corn, cotton (*Gossypium hirsutum* L.), and grain sorghum (*Sorghum bicolor* (L.) Moench) at the Delta Research Center, Portageville, MO (Table 1). Soil at the experimental site was infested with soybean cyst nematodes; nematode population behavior and associated soybean yield reduction were examined in a previous report (5). Forrest is resistant to the wild pathotype of cyst nematodes (7), but nematode reproduction occurred during the experiment (5). The experiment began in 1980 following a soybean crop on the experimental site in 1979. Data from October 1982 to May 1985 are presented here. Temperature and precipitation were measured at a weather station 4 km from the experimental site. The soil was a Tiptonville silt loam with an alluvial sandy loam overwash. In 1984, the pH was 5.7, organic matter was 1.5%, K averaged 603 kg/ha, and P<sub>2</sub>O<sub>5</sub> averaged 133 kg/ha, according to Bray's P1 test.

Field plots were four rows wide on 96-cm centers, 12.1 m long, and were bordered by perennial grass strips (*Festuca arundinacea* Schreber) to isolate treatment effects. Soybeans and rotation crops were planted annually in mid to late May. Soybean seed yield data were collected by harvesting 10.1-m lengths of the middle two rows. Seed yields were corrected to a 13% moisture content basis.

A bulk soil sample for the *M.*

Table 1. Temporal arrangement of alternate crops in rotation with soybean

Rotation	1980	1981	1982	1983	1984
Monoculture	Soybean <sup>z</sup>	Soybean	Soybean	Soybean	Soybean
2 Year	Alternate	Soybean	Alternate	Soybean	Alternate
	Soybean	Alternate	Soybean	Alternate	Soybean
3 Year	Alternate	Alternate	Soybean	Alternate	Alternate
	Alternate	Soybean	Alternate	Alternate	Soybean
	Soybean	Alternate	Alternate	Soybean	Alternate
4 Year	Alternate	Alternate	Alternate	Soybean	Alternate
	Alternate	Alternate	Soybean	Alternate	Alternate
	Alternate	Soybean	Alternate	Alternate	Alternate
	Soybean	Alternate	Alternate	Alternate	Soybean

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*phaseolina* assay was composed of 20 soil cores 19 mm in diameter × 15 cm deep taken in the middle two rows. Soil samples were collected in late March or early April, in May at planting, and in October or November at harvest (hereafter referred to as overwinter, planting, and harvest samples, respectively). Planting samples were taken after primary cultivation and seedbed preparation that incorporated crop residues from the previous year, harvest samples were collected from soil that had been disturbed since planting only by shallow cultivations for weed control, and overwinter samples were drawn from plots undisturbed since harvest. A portion of the soil samples was assayed for *H. glycines*, as previously described (5). The overwinter sample in 1983 was not assayed for microsclerotia. Bulk samples for assay of *M. phaseolina* were thoroughly mixed and 5-g wet weight subsamples were randomly drawn. Soil was thoroughly mixed with modified chloroneb-mercuric chloride-rose bengal agar at about 50 C and immediately poured into eight petri dishes (100 × 15 mm). The medium has a modification of that of Meyer et al (8), and contained 20 g of agar, 15 g of rice, 75 mg of chloroneb, 90 mg of rose bengal, 40 mg of streptomycin sulfate, 60 mg of potassium penicillin, and 1.7 mg of mercuric chloride per liter of distilled water. The

medium was without the previously described pH adjustment (8). Dishes were incubated in the dark at 33 C for 7–10 days. Colonies of *M. phaseolina* were then counted.

Agronomic practices common to the area's agriculture were followed. Corn and grain sorghum were side-dressed annually with 112 kg/ha N and had a preemergence application of atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) at 2.5 kg a.i./ha. Cotton was side-dressed with 55 kg/ha N and had a preemergence application of fluometuron (1,1-dimethyl-3-[ $\alpha\alpha\alpha$ -trifluoro-*m*-tolyl]urea) at 1.1 kg a.i./ha. Soybeans received a preemergence application of alachlor (2-chloro-2'-6'-diethyl-*N*-[methoxy-methyl]acetanilide) at 3.4 kg a.i./ha and linuron (3-[3,4-dichlorophenyl]-1-methoxy-1-methylurea) at 0.4 kg a.i./ha from 1980 to 1982. Only alachlor, at 2.2 kg a.i./ha, was applied in subsequent years. All crops were fertilized in 1983 with 135 kg/ha of K. Five centimeters of water was applied by an overhead irrigation system annually in mid to late July.

The experimental design was a randomized complete block with four replications. Rotations were arranged so that soybeans could be grown each year regardless of rotation scheme, giving a total of 28 treatments (Table 1). Some rotation schemes were identical for

varying lengths of time during the experiment. These identities were considered as multiple observations nested within a single rotation scheme. Because this introduced an unbalanced number of observations for response variables, the experiment was analyzed by the general linear model procedure of the Statistical Analysis System (11) and least squares means were separated by a test of least significant differences.

Soybean seed yield response to cyst nematode density was previously reported (5). The relationship between density of *M. phaseolina* in soil and yield was explored with regression models (9,11). Regression analysis and frequency tables were used to determine if there was an interaction between *M. phaseolina* and *H. glycines* with respect to yield. Frequency tables of soybean yield, classified by densities of *M. phaseolina* and *H. glycines*, were constructed using known threshold responses of yield to *H. glycines* (5) and high and low levels of *M. phaseolina*, divided at the median.

## RESULTS

Population levels of *M. phaseolina* were significantly affected ( $P < 0.05$ ) by rotation crops and lengths at all sample times (Table 2). Higher densities of *M. phaseolina* generally were found as soybeans appeared more frequently in rotations, regardless of whether soybeans

**Table 2.** Population levels of *Macrophomina phaseolina* at planting, harvest, and in early spring as affected by soybean monoculture and soybeans in rotation with corn, cotton, or grain sorghum

Rotation scheme (1980-1981-1982- 1983-1984) <sup>x</sup>	1982		1983		1984		1985	
	Harvest	Planting	Harvest	Overwinter	Planting	Harvest	Overwinter	Planting
Sb-Sb-Sb-Sb-Sb	18 a <sup>y</sup>	18 ab	18 b	35 b	30 a	28 abcde	35 a	30 ab
Sb-Cr-Sb-Cr-Sb	12 bc	15 bc	18 b	26 bcde	27 ab	31 abc	35 a	26 bc
Cr-Sb-Cr-Sb-Cr	11 bc	12 bcd	29 a	17 cdefg	17 bcde	33 ab	25 abcd	31 ab
Sb-Cr-Cr-Sb-Cr	12 bc	10 cd	12 bcd	38 b	26 ab	37 a	33 a	30 ab
Cr-Sb-Cr-Cr-Sb <sup>z</sup>			11 bcd	12 efg	12 de	17 defgh	21 bcde	20 cde
Cr-Cr-Sb-Cr-Cr	6 de	9 cd	17 b	19 cdef	18 bcde	26 bcdef	19 cdef	17 def
Sb-Cr-Cr-Cr-Sb			16 bc	11 efg	15 bcdef	22 bcdefg	28 ab	15 def
Cr-Sb-Cr-Cr-Cr						16 efg	13 efg	18 cdef
Cr-Cr-Cr-Sb-Cr	2 e	4 d	10 bcd	23 bcdef	25 ab	30 abcd	22 bcde	26 bc
Sb-Ct-Sb-Ct-Sb	13 ab	12 bcd	10 bcd	11 efg	13 cdef	16 efg	17 cdefg	13 ef
Ct-Sb-Ct-Sb-Ct	5 e	7 d	8 bcd	57 a	13 cdef	38 a	20 bcdef	26 bc
Sb-Ct-Ct-Sb-Ct	6 de	8 cd	14 bcd	35 b	24 abc	30 abcd	26 abc	33 ab
Ct-Sb-Ct-Ct-Sb			5 d	6 g	7 f	14 fgh	11 fgh	15 def
Ct-Ct-Sb-Ct-Ct	6 de	11 bcd	11 bcd	12 efg	9 ef	10 h	10 gh	11 f
Sb-Ct-Ct-Ct-Sb			5 d	7 fg	8 ef	14 fgh	18 cdefg	18 cdef
Ct-Sb-Ct-Ct-Ct						8 h	6 h	10 f
Ct-Ct-Ct-Sb-Ct	4 e	5 d	6 cd	34 bc	20 abcde	30 abcd	26 abc	24 bcd
Sb-Gs-Sb-Gs-Sb	10 bcd	25 a	14 bcd	16 defg	16 bcdef	26 abcdef	25 abcd	19 cde
Gs-Sb-Gs-Sb-Gs	8 cde	12 bcd	15 bcd	24 bcde	21 abcd	38 a	26 abc	20 cde
Sb-Gs-Gs-Sb-Gs	10 bcd	8 cd	11 bcd	42 ab	20 abcde	32 abc	34 a	38 a
Gs-Sb-Gs-Gs-Sb			7 cd	12 efg	10 ef	14 fgh	15 defgh	15 def
Gs-Gs-Sb-Gs-Gs	6 de	11 bcd	14 bcd	16 defg	16 bcde	20 defg	18 cdef	15 ef
Sb-Gs-Gs-Gs-Sb			5 cd	11 efg	9 ef	20 cdefgh	22 bcde	19 cdef
Gs-Sb-Gs-Gs-Gs						11 gh	15 efg	11 ef
Gs-Gs-Gs-Sb-Gs	4 e	4 d	11 bcd	29 bcd	22 abcd	39 a	35 a	27 bc

<sup>x</sup> Crops listed in temporal sequence over 5-yr period. Sb = soybean cultivar Forrest, Cr = corn, Ct = cotton, Gs = grain sorghum.

<sup>y</sup> Means (cfu/5 g of soil) within a column followed by the same letter are not significantly different at  $P = 0.05$ , according to a protected test of least significant differences.

<sup>z</sup> Some rotation schemes were identical to others for varying lengths of time and results were combined for analysis.

were rotated with corn, cotton, or grain sorghum. Numbers of colony-forming units (cfu) formed under soybean monoculture were sufficiently high to place that treatment in the highest group of means according to a test of least significant differences ( $P < 0.05$ ) in six of eight sampling times. Soybean monoculture fell into the second highest group of means at harvest of 1983 and overwinter of 1984. Nonsoybean crops often had significantly less microsclerotia in the second year after soybeans than in the first year. After 2–3 yr of corn, cotton, and grain sorghum, colony counts generally were in the lowest group. There were few significant differences between the second and third year out of soybeans. The numbers of microsclerotia in soil rebounded to higher densities after a soybean crop was grown in rotation. These higher densities sometimes were not noted until the overwinter or the planting sample was determined.

Microsclerotial densities for each sampling time were statistically contrasted after grouping the treatments. First, rotations that did not have soybeans grown in the season preceding the sample period were grouped by crop species (Table 3). Fewer colonies were found for cotton-soybean rotations than for corn-

soybean rotations in seven of eight sample periods. Cotton and grain sorghum contrasts produced only one significant difference, when colonies recovered were lower for cotton than sorghum in March of 1985. Corn-soybean rotations resulted in significantly more microsclerotia than sorghum soybean rotations on two occasions.

The effect of the length of time between soybean crops on reproduction of *M. phaseolina* on soybeans was also examined with contrasts between treatment groups. In this case, soybeans were grown in the season preceding the sampling period and corn, cotton, and sorghum were grouped together (Table 4). Groups were separated based on the number of years between soybean plantings. Soybean monoculture led to the production of significantly more microsclerotia than the 2-yr rotation in four of eight sample periods. Microsclerotia counts for 2-yr rotations were also significantly higher than those from 3-yr rotations on four occasions, with harvest of 1982 and overwinter of 1985 held in common with the previous contrast. Four-year rotations were significantly higher than 3-yr rotations in one case, and were significantly lower than monoculture in three of six comparisons.

A downward trend in population level with time was evident for some sampling times but not for others.

Population dynamics of *M. phaseolina* were probably affected by soil moisture supply during the growing season. Mid-to late-season precipitation is typically sporadic in southeast Missouri. Total precipitation during July and August was about 116, 30, and 96 mm in 1982, 1983, and 1984, respectively. Highest overall numbers of microsclerotia were recovered from the March 1984 to May 1985 period (Table 2).

The relationships between *M. phaseolina*, *H. glycines*, and soybean seed yield were explored by linear regression analysis and frequency tables of yield classified by soil infestation levels of *M. phaseolina* and *H. glycines*. Population density of *H. glycines* was quantitatively related to soybean yield reduction in 1982 and 1984 (5), but there was no test for the relationship between yield in 1982 and density of *M. phaseolina* because the fungus was not assayed until harvest of 1982. There was no significant soybean yield reduction in 1983 due to a dose response to population levels of *M. phaseolina* ( $0.2 > P > 0.1$ ), but there was a reduction in 1984 ( $P < 0.05$ ). The regression equation

**Table 3.** Population densities of *Macrophomina phaseolina* contrasted to indicate influence of three alternate crops grown in rotation with soybean for one or more seasons prior to sampling time

Previous crop/ contrast	Sampling time								
	1982		1983		1984			1985	
	Harvest	Planting	Harvest	Overwinter	Planting	Harvest	Overwinter	Planting	
Corn/ cotton	10 <sup>y</sup> * <sup>z</sup>	10 ****	15 **	17 *	17 **	28 *	22 *	23 *	
Cotton/ sorghum	5 ****	7 ****	8 ****	9 ****	9 ****	21 ****	16 ***	19 ****	
Sorghum/ corn	8 ****	9 ****	10 *	14 ****	13 ****	20 *	24 ****	21 ****	

<sup>y</sup> Density as cfu/5 g of soil.

<sup>z</sup> Probability that contrasted means within a sampling time are equal: \* < 0.05, \*\* < 0.01, \*\*\* < 0.001, \*\*\*\* > 0.05.

**Table 4.** Effect of length of time between soybean crops on population densities of *Macrophomina phaseolina* after growing soybeans in the season prior to the sampling time

Rotation cycle/ contrast	Sampling time								
	1982		1983		1984			1985	
	Harvest	Planting	Harvest	Overwinter	Planting	Harvest	Overwinter	Planting	
Soybean monoculture/ 2 year	18 <sup>x</sup> ** <sup>y</sup>	18 ****	18 ****	35 ****	30 **	28 ****	35 *	30 ***	
2 year/ 3 year	12 ***	17 **	17 ****	33 ****	17 ****	24 **	26 ***	19 ****	
3 year/ 4 year	6 ****	7 ****	12 ****	38 ****	23 ****	15 ****	16 *	17 ****	
4 year/ monoculture	... <sup>z</sup>	...	9 *	29 ****	22 ****	19 ****	23 ***	17 ***	

<sup>x</sup> Density as cfu/5 g of soil.

<sup>y</sup> Probability that contrasted means within a sampling time are equal: \* < 0.05, \*\* < 0.01, \*\*\* < 0.001, \*\*\*\* > 0.05.

<sup>z</sup> Soybeans after 3 yr of an alternate crop did not appear as a treatment until 1983.

that best explained yield reduction in 1984 due to fungus and nematode was: expected yield ( $\text{g}/\text{m}^2$ ) =  $212 - 6.5(\text{nematode eggs}/\text{g of soil at planting}) - 1.2(\text{cfu}/5 \text{ g of soil at planting})$ . The  $t$  values for coefficients of both independent variables were significantly different from zero at  $P < 0.05$ , the adjusted  $r^2$  was 42%, and there was no discernible pattern to residual values. Natural logs of microsclerotial density at harvest of 1983 and nematode density at planting of 1984 also could be related to yield in 1984, but this resulted in a marginally poorer adjusted  $r^2$  of 41%. Fungal density in the overwinter of 1984 sample could not be related to soybean yield in 1984. Regression models did not suggest an interaction, either positive or negative, between the two organisms. None of the tested interaction terms (products of pathogen densities and their log transforms) contributed significantly to models.

The frequency tables also suggested that there was no interaction between fungus and nematode. In 1983, a year in which no yield reduction could be attributed to *H. glycines* (5), yields consistently were reduced when density of *M. phaseolina* increased from 0–7 cfu/5 g to >7 cfu/5 g (Table 5). However, as nematode densities increased, yield in 1983 also increased. In 1984, there was a significant yield reduction with increasing densities of *H. glycines* (5). When yields from 1984 were tabled with 0–9 cfu/5 g and >9 cfu/5 g (number of observations = 20 in each class), there was actually slightly more yield at the highest levels of fungus and nematode than at high nematode and low fungus levels.

## DISCUSSION

Cotton-soybean rotations consistently reduced populations of *M. phaseolina* more than corn and grain sorghum rotations. Grain sorghum rotations were slightly better than corn rotations in lowering fungal density. Parenthetically, growers in southeast Missouri tend to avoid rotating cotton with soybeans because residual N from the soybean crop makes it difficult to manage the nitrogen supply to the following cotton

crop. Too much N can produce rank vegetative growth and fewer bolls, resulting in lower lint yield.

Higher counts of microsclerotia were found for soybean monoculture than when soybeans were grown every other year, significantly so in four sample periods representing three growing seasons. Soybeans every 2 yr resulted in more microsclerotia than soybeans every 3 yr in four instances representative of two growing seasons. Thereafter, however, the decline of microsclerotia under the influence of a poor host appears to be very slow or, in some cases, absent entirely. For example, in the Ct-Ct-S-Ct-Ct rotation, 11 cfu/5 g of soil were found both at planting and harvest in 1983, the year after soybeans were grown. At the end of the study in May 1985, the density was still 11 cfu/5 g. These results suggest that only longer-term rotations of soybeans with alternate crops would be effective in decreasing levels of microsclerotia to acceptable, nondamaging levels, if, in fact, all microsclerotia are pathogenic and equally virulent. Crop rotation among hosts of *M. phaseolina* appears to be a viable option in the management of charcoal rot.

Although sampling did not include preharvest of 1982 periods, it appeared that pre-1982 seasons were not conducive to the production of a great many microsclerotia. This was not the case for 1983 and 1984, dry growing seasons, that led to the recovery, beginning in March 1984, of the highest fungal counts during the experiment. Peak population levels found at harvest of 1984 were very likely due to the joint contributions of 1983 and 1984 soybean crops. The effectiveness of crop rotations with soybean in reducing population density of *M. phaseolina* will be strongly influenced by environment.

Adequate sampling accuracy is a problem in studying soilborne organisms, including *M. phaseolina* (4). However, replication (number of replicates = four in this study) tends to alleviate the effects of divergent outliers, and the analysis performed here included multiple observations within replicates for some rotations (Table 2), further increasing the level of precision. Also, the time of

sampling appeared to have an influence on the population levels of *M. phaseolina* detected. After a soybean crop, higher numbers were sometimes not found until the overwinter or planting sample. Occasional high counts in the overwinter sample could have been due to the inclusion in the bulk sample of small pieces of soybean stem debris that had been left on the soil surface since harvesting. As a worst case example, 57 cfu/5 g of soil were recorded in the spring of 1984 for the cotton-soybean rotation, compared with 18 cfu/5 g at soybean harvest in 1983 (Table 2). A decrease to 13 cfu/5 g at cotton planting in 1984 was followed by a measurement of 38 cfu/5 g at harvest. Thus, it was difficult to discern whether we were dealing with a high or low population in this case. Crop residue, with the exception of basal portions of cotton stems, was not noticeable after 1 yr.

There was no demonstration of either decreased or increased soybean yield loss due to an interaction between *M. phaseolina* and *H. glycines*. Although the relationship between the respective population dynamics of the fungus and nematode was not examined critically, populations appeared to reproduce most under different environmental conditions. Highest populations of *M. phaseolina* on soybean occurred during the driest years of the test (1983 and 1984), while nematode populations in 1983 just maintained their preplant levels (5). Interaction effects could not be analyzed rigorously because formal statistical tests require a more structured experimental design (3). Factorial experiments should be conducted with controlled infestation levels before a lack of interaction is accepted conclusively.

Management of *H. glycines* by crop rotation of soybeans with nonhosts may have the added benefit of reduced risk of charcoal rot. The data indicate significant effects of crop species and length of rotation on population dynamics of *M. phaseolina*. Future work should include pathogenicity studies of isolates of *M. phaseolina*.

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**Table 5.** Soybean seed yield ( $\text{g}/\text{m}^2$ ) in 1983 and 1984 classified by soil population levels of *Macrophomina phaseolina* and *Heterodera glycines*<sup>a</sup>

<i>H. glycines</i> (eggs/g)	<i>M. phaseolina</i> (cfu/5 g)					
	1983			1984		
	<7	>7	$\bar{x}$	<9	>9	$\bar{x}$
<0.47	202	164	196	203	189	198
0.48–2.5	218	184	193	216	171	194
>2.5	241	197	206	153	164	160
$\bar{x}$	208	186		194	173	

<sup>a</sup>Nematode density classes from yield reduction model (4): less than yield reduction threshold, 0–10% yield reduction, and >10% yield reduction. Fungal density classes were formed by dividing the observations ( $n = 40$  in each year) into two groups at the median.

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