Colonization of Soybean Roots by *Macrophomina phaseolina*

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ABSTRACT

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Nine soybean cultivars, representing maturity groups III, IV, and V, were monitored for root colonization by *Macrophomina phaseolina* in naturally infested, fumigated, and fumigated-infested soils. Fumigation reduced initial soil populations by 80% but did not significantly reduce subsequent disease incidence. Cultivars differed in the rates at which they were colonized. On the basis of colonization rates calculated using time after planting and host ontogeny, cultivars can be better evaluated at specific growth stages than at specific times after planting. Bay, Essex, and Forrest (group V cultivars) had the slowest rates of colonization. Sprite (a group III cultivar) had a rate similar to the group V cultivars and yielded like Essex. Fungal population in root systems of soybean at growth stage R8 (harvest maturity) was negatively correlated with yield.

Macrophomina phaseolina (Tassi) Goid. causes charcoal rot of more than 500 plant species worldwide (7). Under hot, dry environmental conditions, many economically important crops, including soybean (*Glycine max* (L.) Merr.), suffer significant yield losses from this disease (5). Suggested cultural means of control, such as irrigation or fertilization (7), are often impractical or ineffective when environmental conditions are favorable for disease development. Developing resistant cultivars would be an effective alternative control of the problem, but extensive screening has indicated that all cultivars may become infected (1,6). The objectives of this study were to determine if soybean cultivars differ in their rate of colonization by *M. phaseolina* and, if so, what effect this has on yield.

MATERIALS AND METHODS

Design. A split-plot design was used with randomized complete blocking of whole plots on soil known to be infested with *M. phaseolina*. Whole-plot treatments included naturally infested soil, fumigated soil, and fumigated soil with the fungus reintroduced at planting. For fumigation, methyl bromide at 224 kg/ha was pressure-injected 20 cm deep behind seven shanks, each spaced 30.5 cm apart. To impede loss of fumigant, a 1-mil tarp was laid after injection and removed 48 hr later. Subplot treatments included three cultivars from each of maturity groups III, IV, and V, each with four rows 76 cm wide and 9.1 m long. The experiment was replicated three times.

Sclerotial inoculum was prepared by inoculating moist, autoclaved oat kernels with an isolate obtained the previous year from a plant in a field adjacent to the experimental site. Oats were incubated at 30 C for 6 wk, then air-dried and packaged in a 1:1 (v/v) ratio with the soybean seed. At packaging, all seed received twice the recommended rate of *Rhizobium japonicum* as dry Nitragin (The Nitragin Co., Milwaukee, WI). Soybeans were planted on 13 June 1983, 10 days after fumigation.

Sampling procedure. At 3-wk intervals throughout the growing season, two randomly selected plants from each of the outer rows of the subplots were sampled and used to determine the amount of fungus present in host root systems. Yield determinations came from the two end-trimmed center rows 4.6 m long. Growth stage evaluations were made as described by Fehr et al (2), from V1 (vegetative development) through R1 (early reproduction, ie, flowering) to R8 (harvest maturity). A technique similar to that described by Short et al (6) was used to quantify *M. phaseolina* as fungus per gram of root (dry weight). Differentially heated samples yielded estimates of sclerotial and mycelial propagules (6).

Root systems from sampled plants excised at the cotyledonary node were washed in tap water. After removing nodules, roots were surface-sterilized in 0.8% sodium hypochlorite for 1 min, then blotted dry with paper toweling. The four root systems from a single subplot were placed in a paper bag and dried in a forced-air oven at 28 C for 15 hr. Roots were ground in a Wiley mill through 850-μm (20-mesh) and 370-μm (40-mesh) screens. Three subsamples (50–100 mg) from each subplot were used. One was mixed with 100 ml of PDA (Difco) amended as described by McCain (3) and poured into five petri dishes (100 × 15 mm). The second was placed in the amended PDA, heated 20 min at 50 C, and plated. The third sample was used for moisture determination. Petri dishes were incubated at 30 C for 2–4 days and *M. phaseolina* colonies were counted.

RESULTS AND DISCUSSION

Soil samples taken at planting were plated in chloroneb-mercury-rose bengal agar (4) for population determinations. Fumigated and nonfumigated soils contained 17.8 ± 1.8 and 93.8 ± 3.5 propagules of *M. phaseolina* per gram of soil, respectively. Neither fumigation nor infestation with colonized oats significantly changed disease incidences from those of naturally infested soil; cultivar differences, however, were observed (Table 1). Both internal and external reddish brown lesions were observed on the lower stems of plants throughout the growing season. Disease development, however, became most pronounced late in the season as plants approached maturity (R7–R8). At this time, both internal and external sclerotia were observed on the lower stems of plants. Other diseases were not observed, perhaps partly because of hot,

Table 1. Analysis of variance for the total propagule population of *Macrophomina phaseolina* in soybean roots of nine cultivars with respect to time after planting and host ontogeny

Source of variation	Mean squares
Time after planting[x]	
Fumigation (F)	0.05
F × block (B)	0.92
Cultivar (C)	1.48*[y]
F × C	0.19
F × C × B	0.36
Week (W)	268.00*
W × C	2.93*
Error	0.36
Host ontogeny[z]	
Fumigation (F)	0.18
F × block (B)	0.92
Cultivar (C)	3.93*
F × C	0.24
F × C × B	0.36
Growth stage × F × C	13.32*
Error	0.35

[x] Plant samples were taken at 3-wk intervals throughout the growing season.
[y] * = Significant at $P < 0.05$.
[z] Soybean development was rated using Fehr's growth stage key (2).

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Table 2. Colonization of nine soybean cultivars by *Macrophomina phaseolina* with respect to time and host development

	Time (wk)[a]																			
	6				9				12				15				18			
Cultivar	GS[b]	T[c]	S	M	GS	T	S	M	GS	T	S	M	GS	T	S	M	GS	T	S	M
Harper	R2	14.7	4.9	10.8	R5	654.7	246.4	408.3	R7	1,639.6	1,449.2	190.5	...[d]	...	...	...	...	...	...	...
Sprite	R2	34.9	18.7	15.6	R5	98.0	20.1	77.9	R7	1,592.7	1,623.9	−29.2	...	...	...	...	...	...	...	...
Williams 82	R1	1.2	1.2	0.0	R5	167.9	22.8	145.1	R6	1,430.7	1,253.7	176.9	...	...	...	...	...	...	...	...
DeSoto	R2	8.7	0.0	8.7	R5	239.5	73.5	166.0	R6	1,499.5	1,370.5	129.0	...	...	...	...	...	...	...	...
Douglas	R2	2.5	1.3	1.2	R4	290.0	122.5	167.4	R6	1,743.0	1,614.6	158.4	R8	3,506.4	3,433.7	72.7	...	...	...	...
Crawford	V6	20.9	12.2	8.6	R4	95.3	9.7	85.5	R6	1,216.6	1,044.8	171.8	R7	3,052.2	2,715.2	337.2	...	...	...	...
Essex	V4	7.3	3.6	3.5	R3	115.5	10.4	105.1	R5	843.3	634.7	208.6	R7	1,676.4	1,007.1	669.3	R8	4,257.7	4,467.3	−207.6
Forrest	V4	8.5	2.4	6.1	R2	99.1	21.4	77.8	R5	252.2	106.7	145.5	R6	270.4	37.2	233.2	R8	5,080.7	3,252.2	1,828.4
Bay	V4	1.2	0.0	1.2	R3	39.3	29.8	9.5	R5	129.8	69.2	60.6	R6	237.2	99.6	137.5	R8	2,988.4	2,201.3	787.2

[a] Time after planting.
[b] Fehr's growth stage key (2).
[c] Means of three replicates. T = total fungus propagules, S = sclerotial propagules, and M = mycelial propagules (in units of propagules per gram root dry weight).
[d] Samples were not obtained at these time periods because the genotypes had reached R8 and were harvested.

dry environmental conditions.

Fungal populations in root systems increased exponentially (Table 2). Heated and unheated samples were used to estimate sclerotial (S) and total (T) propagules, respectively. Mycelial (M) propagules were calculated by subtraction. Analyses of $\log_{10}$ transformed data indicated that propagule populations T and S were better fitted by a linear model than was propagule population M. Multiple correlation coefficients were 0.83, 0.76, and 0.60, respectively.

Linear regression was used to model root colonization by *M. phaseolina* with respect to time after planting (Fig. 1) and reproductive host ontogeny (Fig. 2). Group V cultivars tended to have smaller slopes under both time regimes (Table 3).

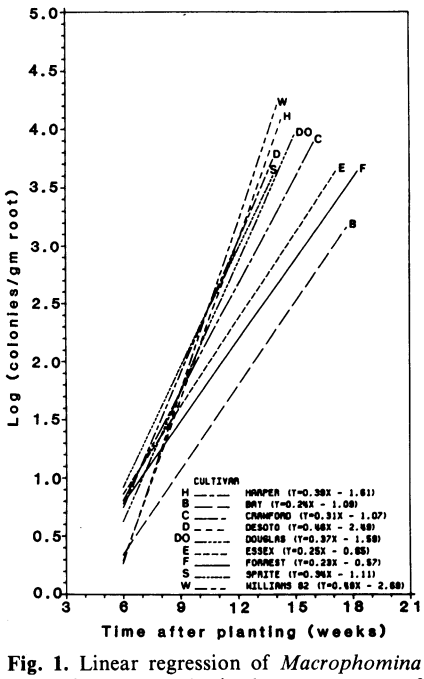

Fig. 1. Linear regression of *Macrophomina phaseolina* propagules in the root systems of nine soybean genotypes, calculated with respect to time after planting. Cultivars of groups III, IV, and V reached physiological maturity at about 12, 15, and 18 wk, respectively. Disease evaluation of genotypes under this system would suggest that early-maturing cultivars are most susceptible to charcoal rot.

Our data indicate that soybeans should be rated for charcoal rot more than once during a growing season and that rating of diverse maturities should be done with respect to host development rather than at arbitrary times during the growing season. Rating cultivars at 3-wk intervals and disregarding host ontogeny would suggest that Harper (A79-336014), Sprite, Williams 82, and DeSoto (the earlier-maturing cultivars) have higher T populations than do Douglas, Crawford, Bay, Forrest, or Essex (Fig. 1). When host development was considered, the relative positions of the regression lines were shifted (Fig. 2). Statistical analysis of the colonization rates was more conservative under the host ontogeny time regime (Table 3). Significant differences among genotypes were observed for T populations at growth stage R8; yield was negatively correlated to these populations (Table 4).

Stress plays an important role in charcoal rot development and maturity group selection may influence the amount of stress a cultivar undergoes at a given location. Growers in southeastern

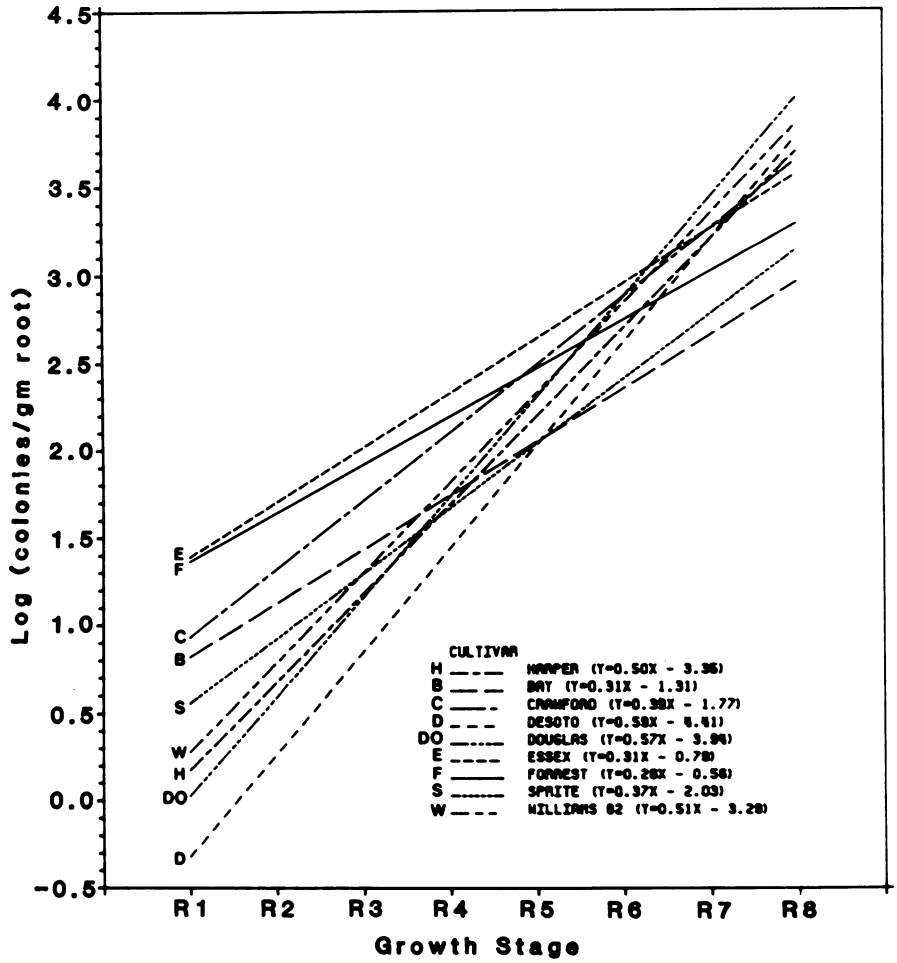

Fig. 2. Linear regression of *Macrophomina phaseolina* propagules in the root systems of nine soybean genotypes, calculated with respect to host reproductive development. Sprite, Bay, and Forrest had lower colonization rates than other test cultivars.

Kansas commonly grow group IV and V cultivars; group III cultivars are often used for double-cropping. These shorter-season cultivars tend to flower when environmental conditions are hot and dry. Long-season cultivars may escape some of this stress by flowering later in the season when temperatures are lower and rainfall is less sporadic. Cultivar response to drought may affect the rate of colonization by *M. phaseolina*; however, it is interesting to note that Sprite differed significantly from the other group III cultivars in root colonization. Its colonization rate was similar to group V genotypes and it yielded much like Essex. Douglas (a group IV cultivar) had the highest colonization rate and lowest yield.

Development of resistant cultivars to control plant disease requires a screening program that will properly evaluate germ plasm. With charcoal rot of soybeans, later-maturing cultivars have an inherent advantage if host development is not considered as a covariate in such evaluations. Bay, Forrest, and Sprite are three cultivars in which *M. phaseolina* root colonization is restricted. The described method of comparing cultivars is being used to identify tolerance and/or resistance mechanisms in soybeans to *M. phaseolina*.

Table 3. Colonization rates by *Macrophomina phaseolina* in nine soybean cultivars with respect to time after planting and host ontogeny

Time after planting[w]			Host ontogeny[x]	
Cultivar	Maturity group	Rate[y]	Cultivar	Rate
Williams 82	III	0.49 a[z]	DeSoto	0.59 a
DeSoto	IV	0.46 ab	Douglas	0.58 a
Harper	III	0.39 abc	Williams 82	0.51 a
Douglas	IV	0.37 bc	Harper	0.50 ab
Sprite	III	0.34 bcd	Crawford	0.39 bc
Crawford	IV	0.31 cd	Sprite	0.37 bcd
Essex	V	0.25 de	Essex	0.31 cd
Bay	V	0.24 de	Bay	0.31 cd
Forrest	V	0.23 e	Forrest	0.28 d

[w] Samples taken at 3-wk intervals throughout the growing season.
[x] Host development was rated using Fehr's growth stage key (2).
[y] Regression slopes of total fungal population with respect to time.
[z] Values followed by the same letter are not significantly different ($P = 0.05$) using a paired t test.

Table 4. Correlation of yield and estimated final population of *Macrophomina phaseolina* in root tissue of nine soybean cultivars

Cultivar	Maturity[v]	Population[w]	Yield (bu/acre)
Bay	27	2.96 A[x]	20.01 a[y]
Forrest	31	3.29 ABC	19.34 a
Essex	23	3.57 BCD	11.18 b
Sprite	0	3.14 AB	10.36 bc
DeSoto	3	3.78 CDE	9.05 bcd
Williams 82	2	3.85 DE	8.00 cd
Crawford	15	3.64 BCDE	7.88 cd
Harper	0	3.70 BCDE	7.21 de
Douglas	8	3.99 E	5.27 e
		$r = -0.82$[z]	

[v] Days to maturity after Harper, which reached R8 on 17 September.
[w] Log (total *M. phaseolina* population per gram of root) at maturity (R8) from regression.
[x] Values followed by the same capital letter are not significantly different ($P < 0.05$) using a paired t test.
[y] Means followed by the same lowercase letter are not significantly different ($P < 0.05$) according to Duncan's multiple range test.
[z] Pearson's correlation coefficient for population vs. yield ($P = 0.006$).

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