Effects of Herbicide-Induced Stress on Root Colonization of Soybeans by *Macrophomina phaseolina*

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ABSTRACT

Canaday, C. H., Helsel, D. G., and Wyllie, T. D. 1986. Effects of herbicide-induced stress on root colonization of soybeans by *Macrophomina phaseolina*. Plant Disease 70:863-866.

Effects of herbicide-induced stress on root colonization of soybeans by *Macrophomina phaseolina* varied with herbicide. In field studies, stresses induced with chloramben and 2,4-DB significantly increased root colonization by *M. phaseolina*, whereas herbicide stress induced with alachlor significantly reduced root colonization by *M. phaseolina*. Herbicide stresses induced with glyphosate and vernolate had no effect on root colonization by *M. phaseolina*. Trifluralin significantly reduced root colonization by *M. phaseolina* in the absence of significant herbicide stress. With the exception of trifluralin, changes in root colonization by *M. phaseolina* appeared more closely related to root injury than to herbicide stress per se.

Additional key words: acifluorfen, bentazon, *Glycine max*

Charcoal rot, caused by *Macrophomina phaseolina* (Tassi) Goid., is currently one of the most important fungal diseases of soybeans (*Glycine max* (L.) Merr.) in Missouri. In recent surveys, all soybean fields sampled harbored the pathogen (T. D. Wyllie, *unpublished*). Disease development is favored by many conditions that stress the host, including high soil temperature (1,13,15), low soil moisture (6,8), seedling crowding (20), plant wounding (10), and flowering and seed fill (3,22).

The effects of herbicides on plant pathogens and plant diseases have been reviewed (2,11,16). More recently, several disease-herbicide interactions on soybeans involving soilborne pathogens have been reported (5,12,19). The effects of herbicides on inoculum survival of *M. phaseolina* have also been investigated (7). We know of no reports, however, on the effects of herbicides or herbicide-induced stress on charcoal rot of soybeans.

We report the results of preliminary studies on the effects of stresses induced with herbicides on an early stage of disease development of charcoal rot of soybeans, i.e., root colonization by *M. phaseolina* (20).

MATERIALS AND METHODS

Herbicide-induced stresses were evaluated during the summers of 1983 and 1984 in field plots at the Agronomy Research Center of the University of Missouri near Columbia on a Mexico silt loam (fine, montmorillonitic Mesic Udollic Ochraqualf) naturally infested with *M. phaseolina*. Inoculum levels of the fungus, determined as described previously (18), were about 22 ± 9 and 34 ± 10 microsclerotia per gram of soil ($\bar{x} \pm s$) in 1983 ($n = 19$) and 1984 ($n = 24$), respectively.

In 1983 plots were 3 × 16 m and consisted of two rows each of soybean cultivars Williams 79 and Dunfield with rows spaced 76 cm apart. Seed was planted on 6 June. Herbicides were applied as foliar sprays on 7 July, when soybeans were at growth stages V4–V5. Four herbicides plus a water control were evaluated: acifluorfen (Blazer 2S), bentazon (Basagran) plus 2.3 L of crop oil per hectare, glyphosate (Roundup), and 2,4-DB (Butyrac 200). Acifluorfen, bentazon, and 2,4-DB were applied at labeled rates (Table 1). Glyphosate was

Table 1. Effects of herbicides tested in 1983 on plant growth and root colonization by *Macrophomina phaseolina*[a]

			Cultivar			
			Williams 79		Dunfield	
Herbicide	Rate (kg a.i./ha)	Relative root injury[b]	Plant height[c] (cm)	Root colonization[d] (log cfu/g)	Plant height[c] (cm)	Root colonization[e] (log cfu/g)
None (control)	···	0	79	0.845	78	0.858
Acifluorfen	0.56	0	80	0.320	76	0.884
Bentazon	1.12	0	80	0.416	76	1.185
Glyphosate	0.28	0	76*[f]	0.387	66**	1.107
2,4-DB	0.21	VS	73**	1.811*	59***	2.437**

[a] Plots were planted 8 June in a field naturally infested with about 22 microsclerotia of *M. phaseolina* per gram of soil.

[b] Ratings are based on observed root injury of soybeans collected 6 wk after herbicide application: 0 = little or no injury, VS = very severe injury (>95% of plants with root malformation).

[c] Plant heights on 29 September at soybean growth stage R7. Differences in plant heights reflect relative herbicide stress. Herbicide stresses were induced by applying herbicides directly over soybean rows on 6 July when cultivars Williams 79 and Dunfield were at soybean growth stages V5 and V4, respectively.

[d] Roots collected 16 August at stage R5.

[e] Roots collected 19 September at stage R6.5.

[f] Values followed by asterisks differ significantly from the control (* = $P<0.05$, ** = $P<0.01$, and *** = $P<0.001$) according to Fisher's least significant difference.

Journal Series No. 9920 of the Missouri Agricultural Experiment Station Journal, Columbia 65211. Approved by the director.

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Accepted for publication 13 February 1986.

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applied at a sublethal rate. Herbicides were applied directly over soybean rows to induce herbicide stress. Treatments were replicated twice in a completely randomized design. Herbicide injury observations and plant height were recorded, and root colonization by *M. phaseolina* was determined.

In 1984, plots were 3 × 7.6 m and consisted of four rows of cultivar Williams 82 spaced 76 cm apart. Five herbicides were evaluated against a herbicide-free control: alachlor (Lasso II 15G), chloramben (Amiben 10G), trifluralin (Treflan 5G), 2,4-DB, and vernolate (Vernam 7E). Alachlor, chloramben, trifluralin, and vernolate were broadcast over plots at 2.0, 2.0, 2.5, and 1.3 times normal application rates, respectively (Table 2), and preplant-incorporated immediately before planting on 4 June. These rates were chosen after consultation with extension agronomists as likely to stress but not kill soybeans. The 2,4-DB was applied as a directed spray between rows (avoiding soybean contact) on 7 July, when soybeans were at stage V10. Periodic hand-hoeing of weeds during the growing season supplemented all treatments. Soil samples 10 cm deep were collected from plots (12 samples per plot) 5 wk after planting. Samples from each plot were bulked and the number of microsclerotia per gram of *M. phaseolina* determined (18). Root colonization by *M. phaseolina* was determined, and plant stand, plant height, herbicide injury observations, and yield were recorded.

During each growing season, representative plants from each plot were collected to determine the extent of root colonization by *M. phaseolina*. Three (1983) or four (1984) plants were collected from each plot per collection. Taproots (1983) or taproots plus large lateral roots (1984) were carefully excavated from the soil to a depth of 15–20 cm, washed free of adhering soil, and surface-disinfested in 1% sodium hypochlorite for 30 sec. The proximal portions of roots were trimmed to 15 cm long, then air-dried for 2–3 days in paper envelopes. Dried roots were ground in a Wiley mill with either a 0.85-mm (20-mesh) (1983) or 1.7-mm (10-mesh) (1984) screen. A subsample (0.2–0.5 g) of each root was mixed with 100 ml of modified chloroneb-mercuric chloride-rose bengal agar at about 50 C and immediately poured into eight petri dishes (100 × 15 mm). The medium was a modification of that of Meyer et al (14) and contained 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. The medium was without the previously described pH adjustment (14). Dishes were incubated in the dark at 33 ± 1 C for 7–10 days. Colonies of *M. phaseolina* were then counted. Additional root subsamples were dried at 50 ± 5 C for 24–48 hr for moisture determination. The $\log_{10}$ colony-forming units per gram of oven-dry root was used as a quantitative index of relative root colonization (17).

RESULTS

Application of 2,4-DB induced the most severe stress observed in the 1983 study. Soybeans of both Williams 79 and Dunfield appeared wilted for about 2 wk after application, and plant height at growth stage R4 was reduced significantly compared with the herbicide-free controls (Table 1). Symptoms of 2,4-DB injury, e.g., proliferation of numerous adventitious roots 1–5 cm below the soil surface and formation of callus tissue on the lower stem, were observed on nearly all plants. Root colonization by *M. phaseolina* was increased significantly with 2,4-DB when roots were collected 6 and 10½ wk after herbicide application (Table 1).

Severe herbicide stress was also observed with application of glyphosate. Plant heights were reduced significantly compared with the controls, and the youngest one to three trifoliates of soybeans were chlorotic for 1–2 wk after application. Glyphosate-induced stress had no observable effect on root growth or root colonization by *M. phaseolina*.

Slight herbicide stresses were observed with applications of acifluorfen or bentazon. Herbicide injury was limited to necrotic flecking of leaves (herbicide burn) and some downward cupping of leaf margins. Neither acifluorfen nor bentazon had any observable effect on plant height, root growth, or root colonization by *M. phaseolina*.

The effects of the soil-incorporated herbicides in 1984 are summarized in Table 2. The average numbers of colony-forming units per gram of root of *M. phaseolina* for all treatments 8 and 10 wk after planting were 97.9 and 248.8, respectively. Variances of $\log_{10}$ transformed data for the two sampling periods were homogenous according to Hartley's test for homogeneity of population variances, hence data for the two samplings were averaged to obtain grand means.

Applications of alachlor or chloramben induced moderate herbicide stresses in the 1984 study. Both herbicides significantly reduced plant height compared with the control and often caused root injury on seedlings collected 5 wk after planting. Taproot recurvature (curling) in the upper 5–7 cm of the soil was the most common root injury of chloramben-stressed seedlings. Root colonization by *M. phaseolina* was increased significantly with chloramben compared with the control when grand means were calculated (Table 2). A reduction in lateral roots in the upper 5–10 cm of the soil was frequently observed with alachlor-stressed seedlings. Secondary and tertiary roots of these seedlings were often shortened and enlarged (stubby) and were slightly fewer in number than the roots of unstressed seedlings. The leaves of alachlor-stressed seedlings were often distorted and crinkled. Root colonization by *M. phaseolina* was reduced significantly compared with the control when roots were collected from alachlor-treated plots 8 wk after planting but was not affected significantly when roots were collected from these plots 10 wk after planting (Table 2).

Moderate herbicide stress was also induced with application of vernolate. The height of vernolate-stressed plants was reduced significantly compared with the control (Table 2), and seedling leaves with this treatment were occasionally distorted and crinkled. Little or no root injury was observed on seedlings collected from vernolate-treated plots, and no effect was observed on root colonization by *M. phaseolina*.

Slight herbicide stress was observed

Table 2. Effects of herbicides tested in 1984 on plant growth and root colonization by *Macrophomina phaseolina* of cultivar Williams 82[a]

Herbicide	Rate (kg a.i./ha)	Relative root injury[b]	Plant height[c] (cm)	Root colonization (log cfu/g) Stage R3 (30 July)	Stage R4 (13 Aug.)	Grand mean[d]
None (control)	···	0	65	1.991	2.294	2.142
Alachlor	8.4	M	62*[e]	1.500*	2.361	1.931
Chloramben	5.6	M	60***	2.362	2.509	2.435*
Trifluralin	4.2	0	64	1.646	2.065	1.855*
Vernolate	3.5	0	61**	1.824	2.236	2.030
2,4-DB[f]	0.2	0	65	1.745	2.386	2.066

[a]Plots were planted 4 June in a field naturally infested with about 35 microsclerotia of *M. phaseolina* per gram of soil.

[b]Ratings are based on observed root injury of seedlings collected 5 wk after planting; 0 = little or no injury and M = moderate injury (about 40% of plants with root malformation).

[c]Plant heights on 10 August at stage R4. Differences in plant heights reflect relative herbicide stress. Herbicide stresses were induced by preplant-incorporating alachlor, chloramben, trifluralin, and vernolate at 2.0, 2.0, 2.5, and 1.3 times normal application rates, respectively.

[d]Grand mean = ($\log_{10}$ cfu at R3 + $\log_{10}$ cfu at R4)/2.

[e]Values followed by asterisks differ significantly from the control (* = $P<0.05$, ** = $P<0.01$, and *** = $P<0.001$) according to Fisher's least significant difference.

[f]2,4-DB in 1984 was applied between rows on 7 July, avoiding contact with soybeans and therefore inducing no stress.

with application of trifluralin. Some seedling stunting was noted in two of the four trifluralin-treated plots 3 wk after planting, but there was no significant effect on plant height when heights were recorded 6 wk later. No leaf injury was observed, and root injury was rare. Root colonization by *M. phaseolina*, however, was decreased significantly compared with the control when grand means were calculated (Table 2).

When 2,4-DB was applied as a directed spray between rows in 1984, avoiding contact with soybeans, no herbicide stress or injury was observed. Without herbicide stress or injury, 2,4-DB had no significant effect on root colonization by *M. phaseolina* or on plant height.

Analysis of variance of 1984 data indicated that none of the herbicides had a significant effect on plant stand, or the number of microsclerotia of *M. phaseolina* in soil samples collected 5 wk after planting, or on yield ($P = 0.06$, $P = 0.85$, and $P = 0.16$, respectively). Mean plant stands were 90, 82, 79, 84, 92, and 80 plants per 5 m of row for the control, alachlor, chloramben, trifluralin, 2,4-DB and vernolate plots, respectively (Fisher's least significant difference at $P = 0.05$ [FLSD] = 12 plants per 5 m). Soil populations of *M. phaseolina* were 48, 43, 38, 39, 39, and 41 microsclerotia per gram of soil, respectively (FLSD = 18 microsclerotia per gram). Mean yields were 0.58, 0.54, 0.44, 0.47, 0.55, and 0.55 kg of seed (dry wt) per 5 m of row, respectively (FLSD = 0.16 kg/5 m).

Table 3. Summary of observed relationships between herbicide-induced stress, root injury, net change in host root surface area in upper 5–10 cm of soil, and root colonization by *Macrophomina phaseolina*

Herbicide	Herbicide-induced stress	Root Injury	Host root surface area	Colonization by *M. phaseolina*
Acifluorfen	Slight	None	None	None
Alachlor	Moderate	Moderate	Decreased	Decreased
Bentazon	Slight	None	None	None
Chloramben	Moderate	Moderate	Increased	Increased
Glyphosate	Severe	None	None	None
Trifluralin	Slight	None	None	Decreased
2,4-DB[a]	Severe	Severe	Increased	Increased
2,4-DB[b]	None	None	None	None
Vernolate	Moderate	None	None	None

[a] Herbicide applied directly over soybean rows (1983).
[b] Herbicide applied between soybean rows (1984).

DISCUSSION

The observed relationships between herbicide stress, root injury, net change in root surface area caused by root injury, and root colonization by *M. phaseolina* are summarized in Table 3. From these studies, it appears that herbicide stress per se has no general effect on root colonization by *M. phaseolina*. With moderate to severe herbicide stresses, chloramben and 2,4-DB increased root colonization by *M. phaseolina*, alachlor decreased root colonization by *M. phaseolina*, and glyphosate and vernolate had no detectable effect. Most effects on root colonization were, however, associated with moderate to severe root injury. Root injury was observed with three of the four herbicides that affected root colonization by *M. phaseolina*: alachlor, chloramben, and 2,4-DB. When no root injury was observed, root colonization was generally unaffected. The only exception was trifluralin, which significantly decreased root colonization by *M. phaseolina* in the absence of any apparent root injury.

With the exception of trifluralin, there appeared to be a direct relationship between the net change in host root surface area near the soil surface caused by root injury and the net effect on root colonization by *M. phaseolina*. When foliar application of 2,4-DB caused extensive proliferation of adventitious roots in the upper 5 cm of soil, there was a large increase in the $\log_{10}$ colony-forming units of *M. phaseolina* recovered from taproot tissues. Taproot recurvature associated with chloramben root injury increased to a lesser degree the host surface area in the upper 5–7 cm of soil and led to a more modest increase in taproot colonization by *M. phaseolina*. Conversely, the root injury associated with alachlor led to less host surface area exposed to the pathogen in the upper 5–10 cm of soil and less root colonization by *M. phaseolina*. Any changes in host surface area or tissue susceptibility near the soil surface are particularly important with *M. phaseolina*, because disease with this pathogen is favored by high (≥30C) soil temperatures (1,13,15) and highest soil temperatures in the summer are recorded nearest the soil surface (9). On a typical hot (35 C), sunny July afternoon in 1984, for example, we measured soil temperatures of 39, 34, 30, 28, and 26 C at depths of 2.5, 5, 10, 15, and 20 cm, respectively, in the vicinity of the herbicide plots. Many cellular and physiological changes in host root tissues must certainly accompany the root injuries observed and may alter host susceptiblity in ways that lead to differences in root colonization by *M. phaseolina*. However, the changes in host surface area near the warm soil surface (the probable infection court) appear to be of sufficient magnitude to account for most of the differences in root colonization by *M. phaseolina*. More studies are needed, with and without herbicides, on the relationships between host root surface area, root infection, and root colonization to determine if this hypothesis is tenable.

The decrease in root colonization by *M. phaseolina* after application of trifluralin does not follow the pattern described (root injury was uncommon, and no general change in root surface area was noted), suggesting another mechanism must be involved. Filho and Dhingra (7) reported significant decreases in soil populations of *M. phaseolina* after soil incorporation of five herbicides. In our 1984 study, we noted slight decreases in soil populations of *M. phaseolina* with all herbicides. However, the decreases were not significant, and soil populations with trifluralin were comparable to those with other herbicides that did not affect disease, e.g., 2,4-DB and vernolate. A mechanism other than decreased soil population of *M. phaseolina* is needed to explain the root colonization decrease with trifluralin. Low soil concentrations of trifluralin have been reported to enhance chlamydospore production and germination of *Fusarium oxysporum* f. sp. *vasinfectum* (Atk.) Snyd. & Hans. and to increase soil populations of fungi, bacteria, and actinomycetes (21). Accumulated evidence suggests that *M. phaseolina* is a poor competitor, primarily because of its sensitivity to other soil microorganisms (4). Increased activity of other soil microorganisms and competitors may have led to the observed decrease in root colonization with trifluralin.

Our results must be considered preliminary. Additional studies are needed to ascertain the mechanisms behind the observed interactions and to verify herbicide-induced effects over a wider range of growing conditions, soil types, inoculum levels, and soybean cultivars. The effects of different herbicide rates and corresponding stress levels on root colonization by *M. phaseolina* also need additional study. Finally, the herbicides we used represent many chemical groups and modes of action. The relationships between chemical group and mode of action and root colonization by *M. phaseolina* also should be investigated further. These additional supportive studies will be necessary to fully evaluate the potential for alachlor, chloramben, trifluralin, and 2,4-DB to affect root colonization of soybeans by *M. phaseolina*.

ACKNOWLEDGMENTS

This research was supported in part by funds from Monsanto Agricultural Products Co., St. Louis, MO, and by the Missouri Soybean Merchandising Council.

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