Annals of Applied Nematology 1:35–40. 1987.

Effect of *Heterodera glycines* on Charcoal Rot Severity in Soybean Cultivars Resistant and Susceptible to Soybean Cyst Nematode[1]

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Abstract: Field experiments were conducted in two soil types in northeastern Kansas to evaluate the influence of *Heterodera glycines* on the severity of charcoal rot in group III soybean cultivars resistant and susceptible to soybean cyst nematode race 3. Resistant cultivars Asgrow 3307 and Fayette and susceptible cultivars Asgrow 3127, Harper, Pella, Sprite, and Williams 82 were planted in carbofuran-treated and nontreated plots. *Heterodera glycines* and the charcoal rot fungus, *Macrophomina phaseolina,* were suppressed by carbofuran treatment in loamy sand, but not loam soil, and by nematode-resistant cultivars in both soils. Root densities of the fungus were positively correlated with nematode densities and negatively correlated with seed yield at both locations. Results indicate that *H. glycines* infection can increase colonization of soybean roots by *M. phaseolina* which may increase losses due to charcoal rot.

Key words: interaction, *Heterodera glycines,* soybean cyst nematode, *Macrophomina phaseolina,* charcoal rot.

Charcoal rot, caused by the fungus *Macrophomina phaseolina* (Tassi) Goid., is an important disease of soybean (*Glycine max* (L.) Merr.) in Kansas (13). The soybean cyst nematode (SCN) *Heterodera glycines* Ichinohe was recently recovered from several soybean production fields in northeastern Kansas (18). The interaction of *H. glycines* and *M. phaseolina* separately in disease complexes has been documented (1,17), but studies on the concomitant infection of soybean by these pathogens are lacking.

Infection by *H. glycines* results in disruption of vascular tissues and increased host susceptibility to moisture stress (5,16). Severity of charcoal rot, which is frequently related to stress (11,13), may be enhanced subsequently in the presence of the nematode. The objectives of this study were to determine the effect of *H. glycines* on colonization of soybean roots by *M. phaseolina* and the influence of the resulting interaction on soybean yield.

Materials and Methods

Experiments were conducted during 1986 in two commercial soybean fields in northeastern Kansas known to be naturally infested with *H. glycines* race 3 and *M. phaseolina.* Soil types at these locations were Sarpy loamy sand (82% sand, 4% silt, 14% clay, 0.8% organic matter; pH 8.0) and Onawa loam (52% sand, 32% silt, 16% clay, 1.1% organic matter; pH 8.1). Preplant population densities in the loamy sand averaged 1,421 ± 432 *H. glycines* eggs + second-stage juveniles (J2)/100 cm^3 soil and 45 ± 5 propagules of *M. phaseolina*/5 g soil. Average preplant population densities of the nematode and fungus in the loam soil were 1,016 ± 305 eggs + J2/100 cm^3 and 38 ± 7 propagules/5 g, respectively.

The experimental design was split plot with nematicide treatments as whole plots and soybean cultivars a subplots. Carbofuran-treated (22.40 g a.i./100 m in an 18-cm band, equivalent to 2.89 kg a.i./ha overall) and nontreated whole plots were randomized in complete blocks with four replications. Five soybean cultivars susceptible to *H. glycines* (Asgrow 3127, Harper, Pella, Sprite, and Williams 82) and two cultivars resistant to *H. glycines* race 3 (Asgrow 3307 and Fayette) were planted in four-row plots 6.1 m long with 75-cm row spacing. All cultivars were from maturity group III.

Before planting on 15 May, plots at the loamy sand and loam sites were cultivated and treated with the herbicides trifluralin (0.56 kg a.i./ha) and alachlor (2.67 kg a.i./

Received for publication 27 February 1987.

[1] Contribution no. 87-329-J from the Kansas Agricultural Experiment Station, Manhattan KS 66506.

This research was supported in part by a research grant from the Kansas State Board of Agriculture–Soybean Commission.

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TABLE 1. Analysis of variance for *Heterodera glycines* and *Macrophomina phaseolina* root populations, and soybean yield in loamy sand soil.

Source of variation	Mean squares			
	H. glycines		*M. phaseolina*	
	(R2)	(R6)	(R6)	Yield
Block	0.04	0.52*	1.12	28.65
Carbofuran (CA)	8.69*	0.26	11.54*	325.93*
Whole-plot error	0.64	0.04	0.45	29.14
Cultivar (CU)	4.53*	3.51*	3.03*	362.25*
CA × CU	0.46	0.20	0.81	53.23
Subplot error	0.35	0.26	0.41	26.35
CV(%)	30.4	27.1	36.9	12.0

Growth stages from Fehr's scale: R2 = flowering, R6 = completed seed development (7).
* $P < 0.05$.

ha), respectively. Carbofuran 15G was applied postplant to all four rows of treated plots with a Model 901-3JRKLT Gandy applicator (Gandy Co., Owatonna, MN). The chemical was manually incorporated into the top 2–3 cm. Plots were hand weeded as necessary.

Soil population densities of *H. glycines* were determined at planting and at harvest (7–8 October). Four 5-cm-d soil cores were collected to a depth of 15 cm from the two center rows of each plot. Soil cores were mixed and 100-cm^3 subsamples were processed for vermiform nematodes using a modified Christie-Perry technique (4). Cysts were collected on a 150-μm-pore sieve and counted. To obtain estimates of preplant egg populations, cysts and root residues were macerated in a blender for 2–3 minutes. The resulting mixture was poured through a 180-μm-pore sieve to remove broken cysts and debris. Eggs and J2 from sievings were concentrated by centrifugal-flotation (8). Initial soil densities of *M. phaseolina* were estimated from 5-g subsamples as described by Mihail and Alcorn (10).

Root samples for nematode and fungus assays were collected from eight plants selected at random from the outer two rows of each subplot at soybean growth stages R2 (flowering), R4 (2 cm pod length), and R6 (seed development completed) (7). Cysts and females of *H. glycines* were obtained from half of the root samples of each cultivar by spraying the roots with pressurized water over a 150-μm-pore sieve. Eggs and J2 were extracted by maceration and centrifugal-flotation as previously described to enhance detection of differences in nematode populations (2). Numbers of eggs and J2 were adjusted for extraction efficiency based on the observed contents of 6–10 randomly selected cysts from each cultivar. *Macrophomina phaseolina* was quantified from the remaining four root samples from each subplot using the milled-root technique described by Pearson et al. (13).

Soybeans were harvested on 7–8 October from 4.5 m of each of the inner two plot rows. Analyses of variance were performed on all response variables and linear relationships were examined through correlation. Nematode and fungal population data were log-transformed [$\log_{10}$ (x + 1)] prior to analysis.

In a separate study, the influence of carbofuran on the growth of *M. phaseolina* was examined in vitro. Technical grade (97.8%) carbofuran (FMC Corporation, Princeton, NJ) was added to a defined minimal salts medium (14) at concentrations of 0, 50, 75, 100, 250, and 500 ppm. Three replicate agar plates of each concentration were inoculated with 1-mm agar plugs of mycelium from the advancing margin of a 2-day-old culture of *M. phaseolina*. Plates were incubated at 30 C in the dark and colony diameters were measured after 28 and 56 hours to determine growth rates.

RESULTS

In loamy sand, carbofuran treatment affected ($P < 0.05$) early season (R2) *H. glycines* root levels, late season (R6) *M. phaseolina* root levels, and soybean yield (Table 1, Fig. 1). Carbofuran reduced early season nematode (egg + J2) populations/g root an average of 74% compared with the nontreated controls (Fig. 1A). Nematode densities in carbofuran-treated plots recovered and were not different from nontreated plots by 16 weeks after planting

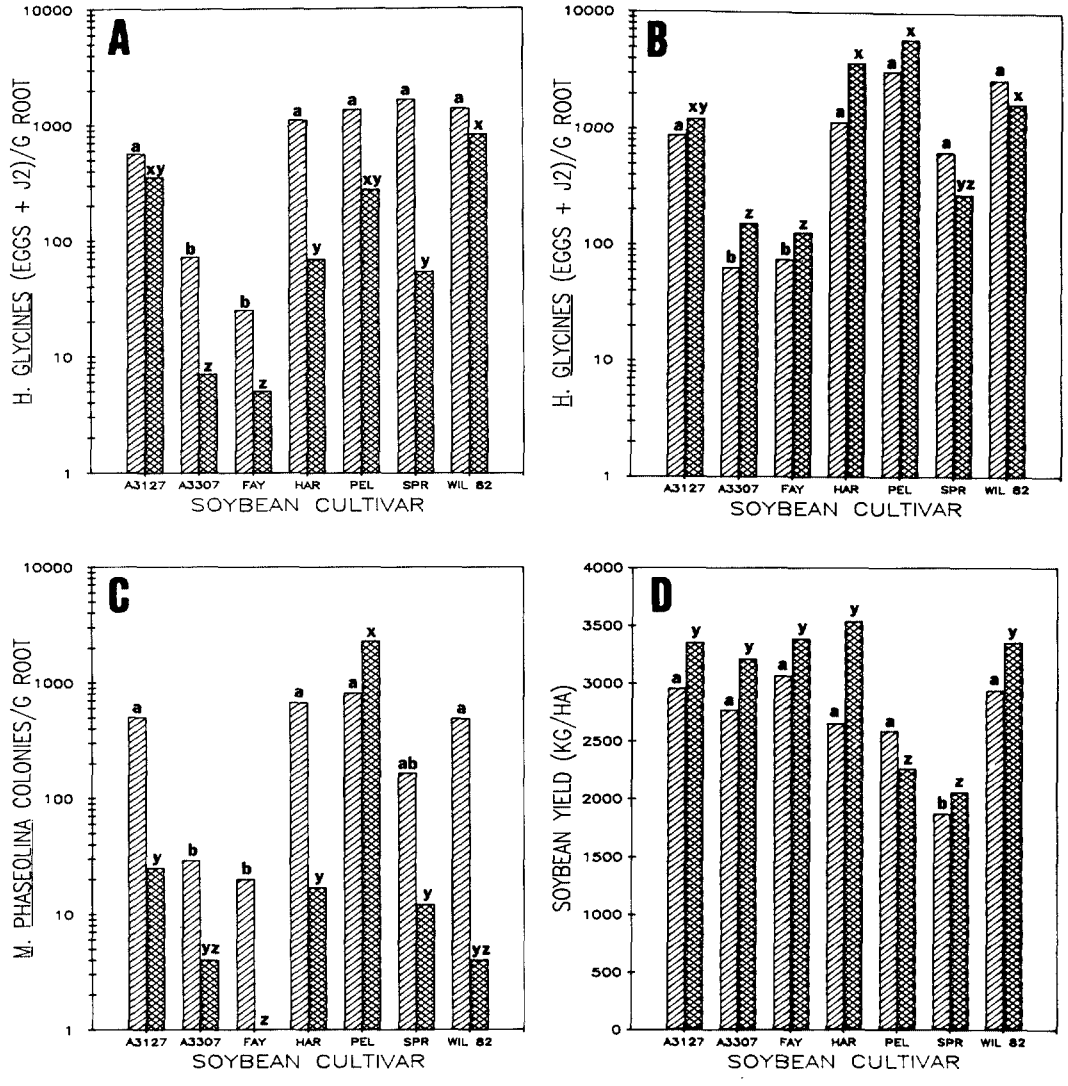

FIG. 1. Effect of carbofuran and soybean cultivar on *Heterodera glycines* and *Macrophomina phaseolina* root populations, and soybean yield in Sarpy loamy sand. Bars with diagonal lines = nontreated. Bars with cross-hatching = carbofuran-treated. Cultivars within a whole-plot treatment with the same letter are not significantly different based on LSD comparisons ($P < 0.05$); A3127 = Asgrow 3127, A3307 = Asgrow 3307, FAY = Fayette, HAR = Harper, PEL = Pella, SPR = Sprite, WIL 82 = Williams 82. A) Root populations of *H. glycines* eggs and second-stage juveniles (J2) at soybean growth stage R2 (flowering). B) Root populations of *H. glycines* eggs and J2 at soybean growth stage R6 (seed development complete). C) Root populations of *M. phaseolina* at soybean growth stage R6. D) Soybean yield.

(R6) (Fig. 1B). Late season fungal root populations in carbofuran-treated plots were, on average, 96% lower than populations in nontreated plots (Fig. 1C). Seed production by carbofuran-treated soybeans increased an average of 12% over that by nontreated soybeans (Fig. 1D).

Cultivar differences ($P < 0.05$) were observed for both early and late season nematode root populations, R6 fungal populations and soybean yield (Table 1, Fig. 1). Egg and juvenile production by *H. glycines* was similar among susceptible cultivars throughout the season but lower among the resistant cultivars Asgrow 3307 and Fayette (Fig. 1A, B). In general, nematode-resistant cultivars also had lower *M. phaseolina* densities (Fig. 1C). In nontreated

TABLE 2. Analysis of variance for *Heterodera glycines* and *Macrophomina phaseolina* root populations, and soybean yield in loam soil.

Source of variation	Mean squares			
	H. glycines		*M. phaseolina*	
	(R2)	(R6)	(R6)	Yield
Block	0.24	0.39	0.32	104.57
Carbofuran (CA)	0.32	0.15	0.43	244.75
Whole-plot error	0.11	0.12	0.42	26.16
Cultivar (CU)	3.50*	3.39*	3.23*	156.53
CA × CU	0.03	0.22	0.60	70.56
Subplot error	0.12	0.26	0.33	77.52
CV (%)	17.1	37.9	63.6	21.4

Growth stages from Fehr's scale: R2 = flowering, R6 = completed seed development (7).
* $P < 0.05$.

plots, however, fungal populations in SCN-resistant cultivars were not significantly different from those in the susceptible cultivar Sprite. Cultivars yielded similarly, except for Pella (treated plots) and Sprite which had lower yields (Fig. 1D).

In the loam soil, nematode and fungal densities were affected by cultivar ($P < 0.05$) but not by nematicide application (Table 2, Fig. 2). Densities of both organisms were generally lower than those observed at the sandier location. Root populations of *H. glycines* were relatively high and generally similar among susceptible cultivars throughout the season and relatively low on the resistant cultivars Asgrow 3307 and Fayette (Fig. 2A, B). Cultivars differed in the level of root colonization by *M. phaseolina*, with Pella exhibiting higher fungal concentrations than the other SCN-susceptible lines (Fig. 2C). Fungal densities in Asgrow 3127, Sprite, and Williams 82 were not different from those in SCN-resistant cultivars. Soybean yield was not different among cultivars (Table 2, Fig. 2D).

In both soil types, root populations of *M. phaseolina* at soybean growth stage R6 were positively correlated ($P < 0.05$) with the number of *H. glycines* eggs + J2 recovered per gram root. In loamy sand, fungal populations were better correlated with early season ($r = 0.73$) than with late season ($r = 0.60$) nematode densities; in loam soil this correlation was observed only with late season nematode densities ($r = 0.80$). Seed yield was inversely related to R6 populations of the fungus ($r = -0.82$ in loamy sand; $r = -0.80$ in loam soil) but was not significantly correlated with nematode populations.

Carbofuran reduced ($P < 0.05$) growth of *M. phaseolina* in vitro at concentrations of 100 ppm. A 15% reduction in growth rate occurred at 100 ppm, but further inhibition was not observed at 250 ppm or at 500 ppm. Nematicide concentrations below 100 ppm did not reduce growth of the fungus.

DISCUSSION

Macrophomina phaseolina root colonization was reduced when *H. glcyines* was controlled by nematicide application or resistant cultivars. Carbofuran probably did not directly reduce fungal densities, since the highest concentration of this nematicide failed to severely inhibit fungal growth in vitro. Reduced plant stress from nematode control, however, may have influenced root colonization by the fungus. Plant stress is important in the development of charcoal rot (11,13). With above average rainfall and moderate temperatures, nematode infection appeared to provide the stress necessary to increase charcoal rot incidence, even in the absence of extensive drouth stress. The observation that *M. phaseolina* root colonization was generally low at the loam soil site compared to the loamy sand site corresponds to a reduced level of stress.

Reduction of fungal root populations by the application of carbofuran on SCN-resistant cultivars further adds support that initial infection by the nematode may produce enough stress to enhance fungal colonization. Juveniles of *H. glycines* readily penetrate the roots of resistant cultivars and incite limited histological changes (6,16). The resulting hypersensitive response by the host may weaken the plant's defenses against other invading pathogens.

The severity of charcoal rot of sorghum

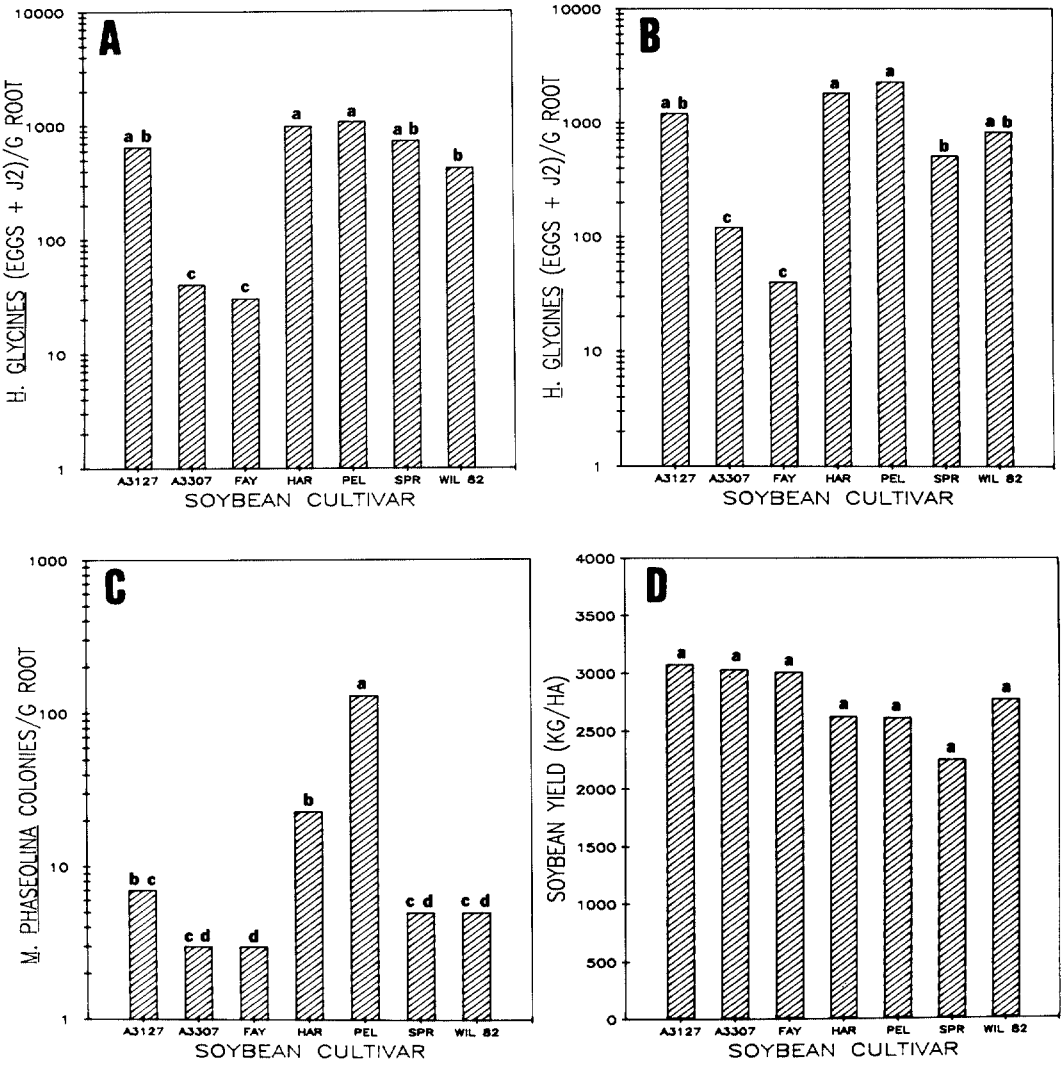

FIG. 2. Effect of soybean cultivar on *Heterodera glycines* and *Macrophomina phaseolina* root populations, and soybean yield in Onawa loam. Data from carbofuran-treated and nontreated plots are combined. Cultivars with the same letter are not significantly different based on LSD comparisons ($P < 0.05$); A3127 = Asgrow 3127, A3307 = Asgrow 3307, FAY = Fayette, HAR = Harper, PEL = Pella, SPR = Sprite, WIL 82 = Williams 82. A) Root populations of *H. glycines* eggs and second-stage juveniles (J2) at soybean growth stage R2 (flowering). B) Root populations of *H. glycines* eggs and J2 at soybean growth stage R6 (seed development complete). C) Root populations of *M. phaseolina* at soybean growth stage R6. D) Soybean yield.

has been reported to be unaffected by *Pratylenchus hexincisus,* presumably because the nematode does not invade the vascular tissue and interfere with water translocation (12). Conversely, severity of *M. phaseolina* root-rot of French bean was reported to be increased by concomitant infection with *Meloidogyne incognita* (1). *Heterodera glycines,* like species of *Meloidogyne,* causes considerable disruption of vascular tissues (5,16), which could enhance the severity of charcoal rot as observed in this study. Additionally, it has been suggested that physiological changes in nematode-infected root tissues may provide a more favorable substrate for fungal development (3,9). One such alteration may result from the accumulation of nitrogenous compounds as occurs in environmentally stressed plants (19). Evidence that isolates of *M. phaseolina* ef-

ficiently utilize stress-related nitrogenous compounds, such as asparagine and proline (14), could explain the strong positive relationship between populations of the fungus and *H. glycines*.

Powell (15) reported that populations of sedentary nematodes are generally suppressed as a result of interaction with fungi. This suppressive effect was not observed in our study, however, since late season *H. glycines* population densities were positively related to root densities of *M. phaseolina* in both soil types. The cultivar Pella consistently supported high populations of both the nematode and the fungus. Conversely, the SCN-susceptible cultivar Sprite, which exhibits restricted root colonization by *M. phaseolina* (13), was generally associated with lower late season nematode densities. Under greenhouse conditions, *H. glycines* populations have been observed to be higher in pots infested with the charcoal-rot fungus (Todd and Pearson, unpubl.). Thus, reproduction of *H. glycines* may be favored by concomitant *M. phaseolina* infection.

Differences in yield between carbofuran-treated and nontreated plots, or among soybean cultivars resistant and susceptible to SCN, were more closely related to root population densities of *M. phaseolina* than to *H. glycines* populations. With the exception of Sprite, soybean yields were inversely correlated with fungal populations at R6. The aberrant response of Sprite can be explained as resulting from a suboptimum seeding rate. Sprite is a short-statured cultivar that requires a heavy seeding rate to attain optimum yield potential.

Evidence from this study indicates that an important disease interaction exists between *H. glycines* and *M. phaseolina* and that the role of *H. glycines* in this interaction may be of greater importance to soybean production in certain areas than the effect of the nematode alone.

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