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Interactions of *Heterodera glycines*, *Macrophomina phaseolina*, and Mycorrhizal Fungi on Soybean in Kansas[1]

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Abstract: The impact of naturally occurring arbuscular mycorrhizal fungi on soybean growth and their interaction with *Heterodera glycines* were evaluated in nematode-infested and uninfested fields in Kansas. Ten soybean cultivars from Maturity Groups III–V with differential susceptibility to *H. glycines* were treated with the fungicide benomyl to suppress colonization by naturally occurring mycorrhizal fungi and compared with untreated control plots. In *H. glycines*-infested soil, susceptible cultivars exhibited 39% lower yields, 28% lower colonization by mycorrhizal fungi, and an eightfold increase in colonization by the charcoal rot fungus, *Macrophomina phaseolina*, compared with resistant cultivars. In the absence of the nematode, susceptible cultivars exhibited 10% lower yields than resistant cultivars, root colonization of resistant vs. susceptible soybean by mycorrhizal fungi varied with sampling date, and there were no differences in colonization by *M. phaseolina* between resistant and susceptible cultivars. Benomyl application resulted in 19% greater root growth and 9% higher seed yields in *H. glycines*-infested soil, but did not affect soybean growth and yield in the absence of the nematode. Colonization of soybean roots by mycorrhizal fungi was negatively correlated with *H. glycines* population densities due to nematode antagonism to the mycorrhizal fungi rather than suppression of nematode populations. Soybean yields were a function of the pathogenic effects of *H. glycines* and *M. phaseolina*, and, to a lesser degree, the stimulatory effects of mycorrhizal fungi.

Key words: arbuscular mycorrhizal fungi, charcoal rot, endomycorrhizae, *Glycine max*, *Heterodera glycines*, interaction, *Macrophomina phaseolina*, nematode, soybean, soybean cyst nematode, vesicular–arbuscular mycorrhizal fungi.

The soybean cyst nematode, *Heterodera glycines*, is the most serious disease threat to soybean (*Glycine max*) production in the United States. Annual yield losses to this nematode have been estimated at 2.6 and 3.1% across the southern and north central regions of the country, respectively (5,10). In individual fields in Kansas, losses of 35–40% have been observed (22). Damage from *H. glycines* infection results from disruption of root vascular tissues and increased moisture and nutrient stress (6).

Arbuscular mycorrhizal fungi stimulate growth and increase yield of soybean plants (2,17), primarily through enhanced uptake of phosphorus and other soil nutrients (16) and improved water transport (18). Greenhouse studies have shown that mycorrhizal fungi can also increase soybean tolerance to the root-knot nematode, *Meloidogyne incognita* (8,20), and may exhibit some antagonism to *H. glycines* (7,24). Because these fungi are ubiquitous in agricultural soils, it has been suggested that they may represent a possible alternative to standard management tactics for nematode parasites of field crops (8,24). Our objective was to determine the impact of naturally occurring mycorrhizal fungi on the *H. glycines*–soybean interaction under field conditions.

Materials and Methods

Field plots were established at two locations (*H. glycines* race 3-infested and uninfested soils) near Columbus, Kansas, during the summer of 1992. Soils at both locations were silt loams: the infested soil was 36% sand, 40% silt, 24% clay, 1.4% organic matter, pH 7.2; and the uninfested soil was 25% sand, 52% silt, 23% clay, 1.6% organic matter, pH 6.7. Both soils were low in phosphorus content (32–37 kg/ha available P). Ten soybean cultivars from Maturity Groups (MG) III–V and differing in susceptibility to *H. glycines* were planted in eight-row plots 6.1 m long, with 75-cm row spacing on 23 and 25 June. Susceptible cultivars included 'Calland'

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(MG III), 'Clark' (MG IV), 'KS4390' (MG IV), 'Bay' (MG V), 'Essex' (MG V), and 'Hutcheson' (MG V). Resistant cultivars included 'Delsoy 4210' (MG IV), 'Delsoy 4500' (MG IV), 'Forrest' (MG V), and 'Pioneer 9531' (MG V).

The experimental design was a split-plot with cultivars as whole plots. Subplots were untreated or treated with the fungicide benomyl (Benlate 50 WP; E. I. Du Pont de Nemours, Wilmington, DE). Whole plots were arranged in a randomized complete block with four replications, and subplots were randomized within whole plots. Benomyl was applied in 1 liter of water to four rows of each treated plot at a rate of 0.6 g a.i./m^2 2 weeks after planting and at approximately monthly intervals for the remainder of the growing season. Benomyl has been used to vary mycorrhizal activity in field plots (1) and has no direct effect on a wide range of plant species (13). Because suppression of pathogenic fungi can occur from benomyl application, root colonization by the charcoal rot fungus, *Macrophomina phaseolina,* was monitored in addition to mycorrhizal root colonization. Reductions in nematode populations have also been associated with this fungicide (11), but greenhouse applications of benomyl at ×2 field rate to soil infested with *H. glycines* did not show any significant effects on juvenile infectivity or egg production (Winkler, unpubl.).

Plots were treated with the herbicides metolachlor (3.2 kg a.i./ha) and metribuzin (0.8 kg a.i./ha) before cultivation. Soybeans were harvested on 10 November from 4.5 m of each of the two middle rows of each plot.

Soil population densities of *H. glycines* eggs and mycorrhizal spores were determined from a composite sample of four 5-cm-d cores collected 15 cm deep from the two middle rows of each plot at planting and harvest. Cysts were extracted from 100-cm^3 subsamples by a combination of sieving (23) and centrifugal flotation (9). Eggs and second-stage juveniles (J2) were extracted mechanically from cysts (12). Mycorrhizal spores were also extracted from 100-cm^3 subsamples by sieving and centrifugal flotation (4), identified to species (21), and counted. *Glomus ambisporium* and *Glomus mosseae* were the predominant mycorrhizal species present in both fields, averaging 48 and 31 spores/100 cm^3 soil, respectively, across locations. Other species with lower spore densities or which were present at only one location included *Glomus constrictum, Glomus claroideum, Gigaspora margarita, Sclerocystis coremiodes, Glomus etunicatum,* and *Glomus geosporum.* Total spore counts averaged 184 and 106/100 cm^3 soil for *H. glycines*-infested and uninfested locations, respectively.

Root samples were collected 26, 56, and 103 days after planting from four root systems from the two middle rows of each plot. Cysts were dislodged from roots before drying with a high-pressure water spray and collected on a 150-μm pore sieve. Eggs and J2 were extracted from cysts and counted as described above. Soybean roots were dried at 90 C for 48 hours and weights were recorded. The dried roots were subsampled, stained with trypan blue (15), and placed in a petri dish scored in 1-mm squares to determine the percentage of mycorrhizal colonization (3). On the last sampling date, a second set of roots was collected from each plot to determine colonization levels of *M. phaseolina.* These roots were surface sterilized in 0.8% NaOCl, air dried, and ground in a Wiley mill (14). Colonies of *M. phaseolina* were quantified from 100-mg subsamples of milled roots plated on chloroneb-rose bengal agar.

All data were subjected to analysis of variance. *Heterodera glycines* and *M. phaseolina* population data were $\log_{10}(x + 1)$ transformed before analysis. Correlation and multiple regression were used to examine relationships among mycorrhizal colonization, pathogen populations, and soybean seed yield.

Results

Cultivar and benomyl effects on soybean growth and yield: Soybean root growth and

seed yields differed ($P \leq 0.05$) between resistant and susceptible cultivars and among cultivars within *H. glycines* reaction (Tables 1,2). Root weights for resistant cultivars averaged 18% less than root weights for susceptible cultivars across both field locations (Fig. 1A,B). In contrast, seed yields of resistant cultivars were 64% higher than susceptible cultivar yields in the presence of *H. glycines* and 11% higher in the absence of the nematode (Fig. 2A,B). Much of the additional variation in seed yields was attributable to soybean maturity, with later-maturing cultivars outyielding early-maturing cultivars by an average 20% across both locations (Fig. 2A,B).

Benomyl application significantly affected soybean root growth and seed yield only in *H. glycines*-infested soil (Tables 1,2). Root growth of both resistant and susceptible cultivars was 19% greater ($P < 0.01$) when benomyl was applied to soybean grown in the presence of *H. glycines* (Fig. 1A). Seed yields were 9% higher ($P = 0.04$) in benomyl-treated plots than in untreated plots at the nematode-infested location.

Cultivar and benomyl effects on H. glycines, M. phaseolina, and mycorrhizal fungi: *Heterodera glycines* population densities were affected ($P \leq 0.05$) only by the *H. glycines* reaction of the soybean cultivars (Table 1). Egg densities on roots of resistant cultivars averaged only 5% of the densities on susceptible cultivars throughout the growing season (Fig. 3). Final egg densities in the soil were also reduced ($P < 0.001$) in the presence of resistant cultivars.

Soybean cyst nematode reaction of soybean cultivars affected root colonization by mycorrhizal fungi and *M. phaseolina* differentially in the presence of *H. glycines* (Tables 1,2; Figs. 1C,2C). Colonization by mycorrhizal fungi was 28% lower ($P < 0.001$) on susceptible cultivars compared to resistant cultivars (Fig. 1C), whereas there was an eightfold increase ($P < 0.001$) in colonization by *M. phaseolina* on susceptible vs. resistant cultivars (Fig. 2C). Root colonization by mycorrhizal fungi on resistant and susceptible cultivars varied with sampling date in the absence of *H. glycines* (Fig. 1D). There was no effect on *M. phaseolina* populations due to *H. glycines* reaction in uninfested soil (Fig. 2D). Additional variation in root colonization by *M. phaseolina* was

TABLE 1. Analyses of variance for soybean root weight, mycorrhizal root colonization, and *Heterodera glycines* (Hg) egg density in nematode-infested and uninfested fields during 1992.

Source of variation	Mean squares				
	Root weight†		Mycorrhizal colonization‡		Hg egg density‖
	Infested	Uninfested	Infested	Uninfested	
Hg reaction (R)	12.71**	28.36**	2,112.17**	0.18	194.37**
Cultivar (reaction)	9.74**	7.06**	186.66	22.82	3.62
Whole plot error	1.64	1.44	159.92	25.80	1.58
Benomyl (B)	16.77**	1.63	968.02**	68.27	0.09
R × B	0.29	2.96	0.01	0.01	1.45
C(R) × B	3.26	0.90	22.93	38.55	1.04
Subplot error	1.69	0.81	63.95	33.31	0.54
Date (D)	417.07**	357.70**	12,981.62**	4,785.05**	8.63**
R × D	3.31	7.18**	370.29	249.69**	0.00
C(R) × D	4.20**	3.51**	186.01	29.89	1.27
B × D	7.97**	0.30	228.12	39.02	0.39
R × B × D	0.27	0.54	23.54	42.05	0.15
C(R) × B × D	1.97	1.17	68.54	22.76	0.47
Sub-subplot error	1.24	1.03	148.51	39.41	0.66

* $P \leq 0.05$, ** $P \leq 0.01$.
† Soybean root weight measured in grams.
‡ Percentage colonization of soybean roots.
‖ Egg density per g root.

TABLE 2. Analyses of variance for soybean seed yield and *Macrophomina phaseolina* root colonization in *Heterodera glycines* (Hg)-infested and uninfested fields in 1992.

Source of variation	Mean squares			
	Seed yield†		*M. phaseolina* colonization‡	
	Infested	Uninfested	Infested	Uninfested
Hg reaction (R)	2,180.92**	201.04**	10.59**	0.13
Cultivar (reaction)	95.89**	161.81**	10.57**	10.57**
Whole plot error	20.81	22.90	0.47	0.92
Benomyl (B)	66.39*	16.64	3.70	0.92
R × B	0.65	7.62	0.02	0.17
C(R) × B	24.08	9.98	0.28	0.65
Subplot error	14.32	10.62	0.62	0.31

* $P \leq 0.05$, ** $P \leq 0.01$.
† Soybean seed yield measured in kg per ha.
‡ Colonization based on number of propagules per g root.

explained by soybean Maturity Group, with MG V cultivars exhibiting only 1% of the level of colonization found in earlier-maturing cultivars at both field locations (Fig. 2C,D).

Benomyl treatment reduced root colonization by mycorrhizal fungi 20% ($P < 0.001$) at the *H. glycines*-infested location only (Table 1; Fig. 1C). Colonization by *M. phaseolina* was reduced an average 44% ($P \leq 0.10$) across both locations.

Relationships among soybean yield, pathogen populations, and mycorrhizal colonization: Root colonization by mycorrhizal fungi was negatively correlated with *H. glycines* egg densities on roots ($r = -0.21$, $P = 0.06$), whereas root colonization by *M. phaseolina* was positively correlated with egg densities ($r = 0.24$, $P = 0.03$). Soybean seed yield in *H. glycines*-infested soil was positively correlated with mycorrhizal colonization ($r = 0.25$, $P = 0.03$) and negatively correlated with egg densities on roots ($r = -0.65$, $P < 0.001$) and root colonization by *M. phaseolina* ($r = -0.41$, $P < 0.001$). Stepwise regression indicated that variation in seed yield was due to the additive effects of i) late-season nematode densities on roots; ii) the multiplicative interaction of *H. glycines* and *M. phaseolina* root densities; and iii) levels of root colonization by mycorrhizal fungi ($R^2 = 0.65$, $P < 0.001$). Seed yield at the uninfested location was correlated only with colonization levels of *M. phaseolina* ($r = -0.53$, $P < 0.001$).

DISCUSSION

Increases in growth and yield of soybean due to colonization by mycorrhizal fungi, particularly *Glomus* and *Gigaspora* spp., have been reported (2,17). In our study, there appeared to be a beneficial effect on soybean from colonization by naturally occurring mycorrhizal fungi based on the predictive model for yield in nematode-infested soil, but much of this advantage was masked by confounding effects due to *H. glycines* and *M. phaseolina*. For example, benomyl treatment reduced colonization levels of both mycorrhizal fungi and *M. phaseolina* and increased soybean root growth and seed yield in *H. glycines*-infested soil. The stimulatory effect on soybean due to benomyl application indicates that growth and yield suppression from *M. phaseolina* outweighed any beneficial effect from mycorrhizal fungi. We noted, however, that the suppression of mycorrhizal colonization due to benomyl application was not as great as the suppression of *M. phaseolina* colonization. The pathogenic effect of *M. phaseolina* is evident in comparisons of yields and colonization levels among cultivars from different maturity groups. Early-maturing soybean cultivars consistently exhibit higher

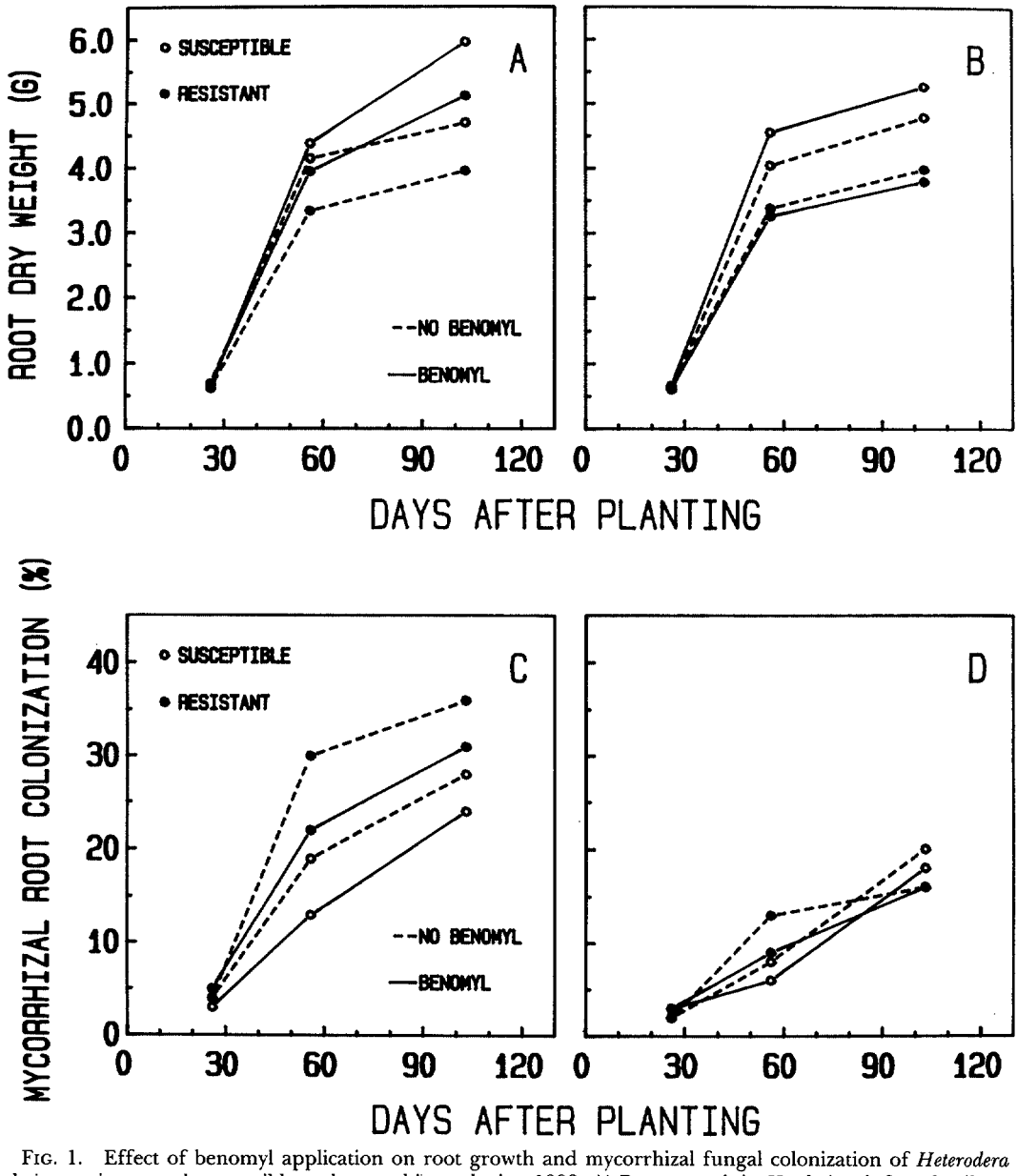

FIG. 1. Effect of benomyl application on root growth and mycorrhizal fungal colonization of *Heterodera glycines*-resistant and susceptible soybean cultivars during 1992. A) Root growth in *H. glycines*-infested soil. B) Root growth in uninfested soil. C) Mycorrhizal colonization in *H. glycines*-infested soil. D) Mycorrhizal colonization in uninfested soil.

colonization rates by *M. phaseolina* under the environmental conditions of southeastern Kansas, even when adjustments are made for plant growth stage (14,22).

Resistant cultivars outyielded susceptible cultivars primarily because of suppression of *H. glycines* reproduction, but a portion of the yield reduction in susceptible cultivars may have resulted from reduced levels of colonization by mycorrhizal fungi. If soybean yield is related to mycorrhizal colonization as suggested by the model, some of the yield benefit typically observed from planting resistant cultivars in *H. glycines*-infested soil is likely due to mycorrhizal symbiosis.

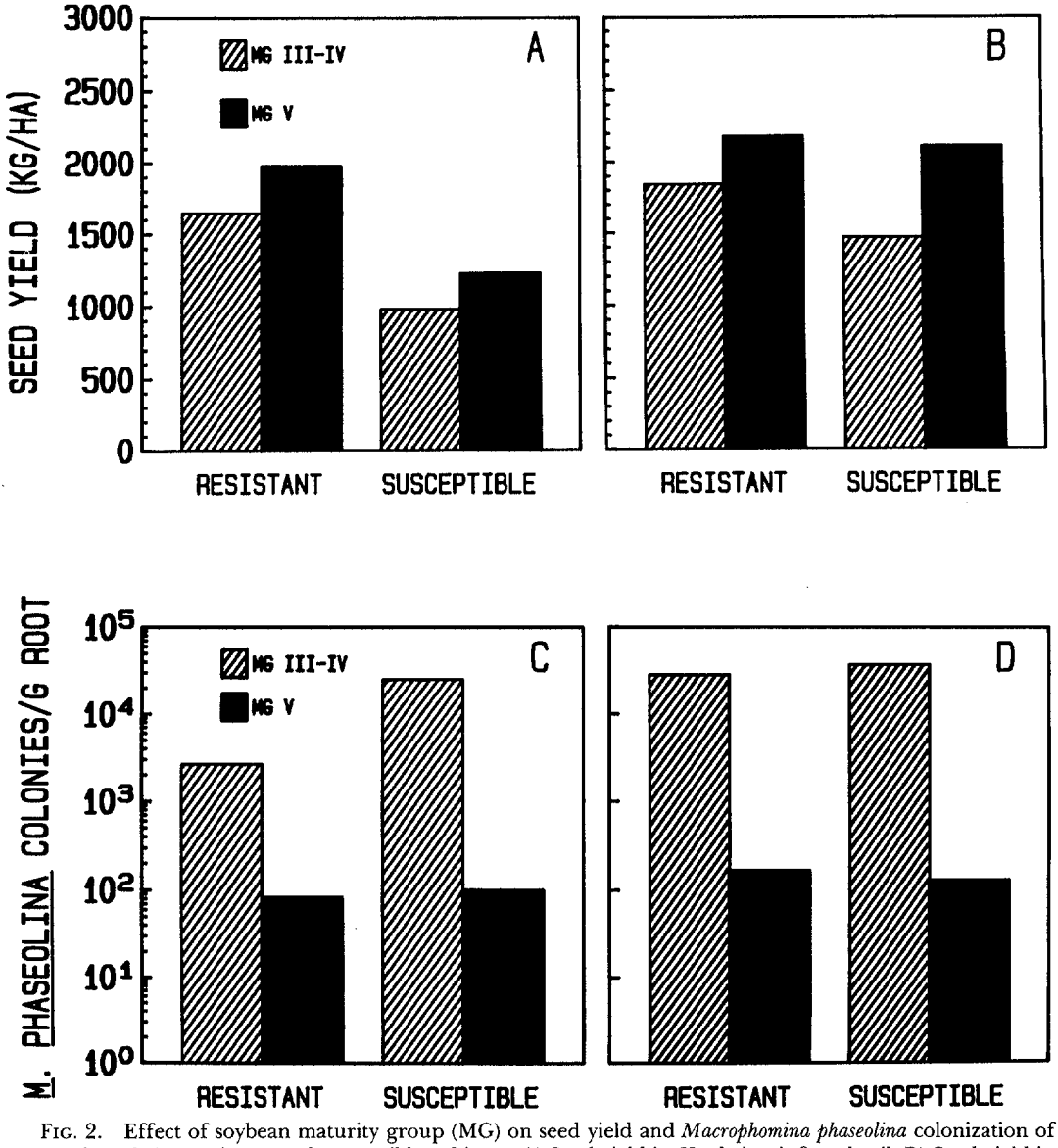

FIG. 2. Effect of soybean maturity group (MG) on seed yield and *Macrophomina phaseolina* colonization of *Heterodera glycines*-resistant and susceptible cultivars. A) Seed yield in *H. glycines*-infested soil. B) Seed yield in uninfested soil. C) *M. phaseolina* colonization in *H. glycines*-infested soil. D) *M. phaseolina* colonization in uninfested soil.

Root colonization by mycorrhizal fungi was suppressed on nematode susceptible cultivars only in the presence of *H. glycines*. Thus, the nematode was antagonistic to the mycorrhizal association. Such suppression from infection by sedentary nematodes has been reported for spore production of mycorrhizal fungi associated with soybean (19). In contrast, root colonization by *M. phaseolina* was enhanced on susceptible cultivars, again only in the presence of *H. glycines*. The interaction of these two pathogens on soybean has been reported (23).

The impact of mycorrhizal fungi on *H. glycines* damage to soybean under field conditions appeared to be minimal in this study. No evidence for nematode suppression by mycorrhizal fungi was observed. To the contrary, *H. glycines* was antagonis-

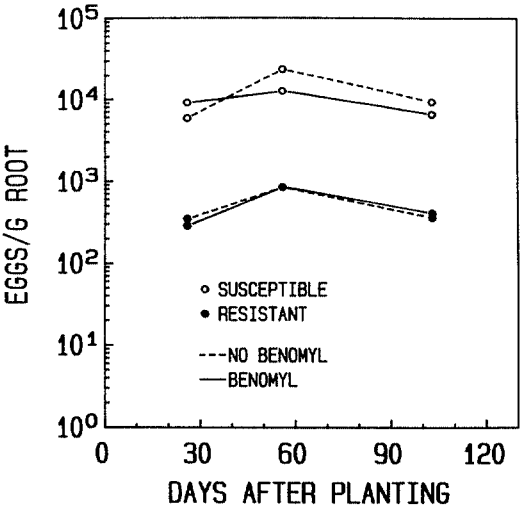

FIG. 3. *Heterodera glycines* egg densities on benomyl-treated and untreated roots of resistant and susceptible soybean cultivars during 1992.

tic to mycorrhizal colonization of soybean roots. Because species of mycorrhizal fungi can differentially influence nematode reproduction (8), fields with higher spore densities or different species combinations of these fungi could yield contrasting results. More selective methods of manipulating the mycorrhizal symbiosis under field conditions must be developed before the role of naturally occurring mycorrhizal fungi in the *H. glycines*–soybean association can be assessed fully.

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