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Early Growth of Soybean as Altered by *Heterodera glycines*, Phenamiphos and/or Alachlor[1]

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Abstract: Greenhouse and field experiments were conducted to determine the effects of phenamiphos and/or alachlor on early growth of soybean, root morphology, and infection and resurgence of *Heterodera glycines* (race 1). All tests were planted to 'Ransom' soybeans. In greenhouse experiments without nematodes, root growth was inhibited at 5 days by alachlor treatments and at 10 days by phenamiphos treatments; with nematodes, phenamiphos treatments enhanced root growth. Phenamiphos also suppressed early penetration of soybean roots by *H. glycines* in the greenhouse. Early soybean growth parameters among treatments were generally similar in the field. Nematode penetration was limited with treatments containing phenamiphos at one location. Plants treated with only alachlor had less nematode infection than did the control; however, plants treated with herbicide/nematicide combinations had more nematode penetration than did plants treated with phenamiphos alone. Alterations of root growth and interference with the efficacy of phenamiphos are two processes by which alachlor may enhance soybean susceptibility or suitability to *H. glycines*.
Key words: pesticide interaction, soybean cyst nematode, *Glycine max*, herbicide, nematicide.

Pesticides are often applied in combination by growers and commercial applicators. Research on the interaction of pesticides has been limited primarily to the effects on crop plants (24) and on soilborne fungi (11,27). The relative lack of pesticide specificity may cause numerous effects on nontarget organisms (3,15,24).

Herbicides may affect nematodes in four general ways. Some have a negligible effect (13,14); others indirectly reduce nematode populations through control of various weed hosts (9,16,19); a few are somewhat nematicidal (10,21,22,25); and some herbicides enhance nematode reproduction (2,9,10,17,25,26). Herbicide application in combination with another pesticide can have a major impact on the activity of one or both compounds and effects on the target organism (24).

Herbicides applied in combination with nematicides may (5,17,23,28) or may not (1,20) alter the efficacy of the nematicide. The herbicide cycloate applied with aldicarb to *Heterodera schachtii* Schmidt infested fields of *Beta* spp. had no impact on the nematicide or the nematode (1). Sodium azide applied alone was slightly nematicidal but when applied with carbofuran reduced the efficacy of the nematicide (23). Applications of the herbicides vernolate, trifluralin, and metribuzin with aldicarb enhanced control of *Heterodera glycines* Ichinohe (17). Alachlor used in combination with several nematicides resulted in greater numbers of nematodes than did the nematicides alone (5,28).

The objective of this research was to determine the effects of alachlor and phenamiphos, applied alone and in combination, on early growth of soybean, root morphology, and early infection of soybean roots by *H. glycines*.

Materials and Methods

Studies were conducted in the greenhouse and in two naturally infested *H. glycines* (race 1) field sites in North Carolina. All experiments were factorial and included the following treatments: untreated control, alachlor, phenamiphos, and phenamiphos + alachlor. Separate greenhouse tests were conducted with and without *H. glycines* to characterize soybean response to the pesticides alone and to pesticide/nematode interactions.

Greenhouse experiments: Two experiments were conducted to determine the effects of phenamiphos and/or alachlor on soybean root morphology with and without *H. glycines*. Phenamiphos and/or alachlor (20 ml of a 25 μg/ml aqueous solution) were added to 500 cm^3 steam sterilized

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loamy sand soil (85% sand, 3% clay, 12% silt, and 1% organic matter) to give final soil concentrations of 1.0 $\mu g/cm^3$. The soil and nematicide solution were vigorously mixed in a polyethylene bag for 30 seconds and then placed in 10-cm-d clay pots.

The first experiment was conducted without nematodes. Following nematicide incorporation, one *Glycine max* (L.) Merr. 'Ransom' seed was planted 2 cm deep. After planting, 20 ml of 25 $\mu g/ml$ alachlor solution were pipetted over the soil surface. Water (20 ml) was applied to the soil in the same manner as the pesticides in treatments not receiving the compound(s).

The treatments were replicated 16 times and randomized in complete blocks. At 5 and 10 days after planting, eight replicates were harvested and plant growth data collected. Fresh weights of shoots and roots (primary and secondary) were determined. Number of secondary roots, primary root length, and primary root diameter were measured at each harvest. Primary root diameter was determined with a micrometer at the mid-length of the root. Ratios and percentages were computed from these basic measurements.

Heterodera glycines was included in the second experiment to assess the influence of nematode/pesticide interaction on root morphology. Mature cysts of *H. glycines* (race 1) cultured in the greenhouse on 'Lee 68' soybean were collected and then crushed with a glass tissue homogenizer to release the eggs. Inoculum of 3,000 eggs per pot was placed via 10-ml volumes of water into a 2-cm deep hole in the soil and seeded as in the first experiment. The four treatments were randomized in 32 complete blocks. Eight replicates were harvested 3, 6, 9, and 12 days after planting. In addition to growth parameters listed for the first test, primary and secondary roots were stained using a modification of the NaOCl-acid fuchsin staining technique (7) and the number of infections determined.

Field experiments: Experiments were conducted in fields of the coastal plains naturally infested with *H. glycines* (race 1) near Smithfield, Johnston County, and the Upper Coastal Plains Research Station near Rocky Mount, Edgecombe County, North Carolina. Initial densities of *H. glycines* were 385 and 455 eggs/500 cm^3 soil at Smithfield and Rocky Mount, respectively. Treatments were the same as those used in the greenhouse experiments.

Phenamiphos (2.24 kg a.i./ha) was applied in a 30-cm wide band and incorporated with a rolling tine attached to the front of the planter. The nematicide was applied and Ransom soybeans planted on 26 May 1982 in Smithfield and 24 June 1982 in Rocky Mount. The herbicide alachlor was sprayed broadcast onto designated plots (2.24 kg a.i./ha) on 27 May 1982 in Smithfield and immediately after planting in Rocky Mount.

Plots were four rows wide (0.92-m row spacing) and 12 m long. Treatments were replicated six times and data collected from the two center rows. Preplant soil samples were taken by compositing 10–12 soil cores (2.5-cm-d) taken to a depth of 15–20 cm. Aliquants of 500-cm^3 soil were processed by elutriation (8) and centrifugal-flotation (12). Plant samples were taken at 4 or 5, 8, and 12 days after planting. Four plants were removed from each plot and fresh shoot and root weights determined; roots were stained and nematodes counted as described for the greenhouse experiment. Analysis of variance was performed on all data. Orthogonal contrasts were calculated for the comparisons: phenamiphos vs. no phenamiphos, phenamiphos vs. phenamiphos + alachlor, and alachlor vs. the untreated control.

RESULTS

Effects of phenamiphos and alachlor on soybean growth and root morphology—greenhouse experiment: The herbicide alachlor alone and in combination with phenamiphos limited root growth at 5 days. Root weight ($P = 0.10$), primary root length ($P = 0.05$), secondary root weight ($P = 0.05$), and number of secondary roots ($P = 0.05$), were lower in treatments having alachlor (Table 1). Shoot/root ratio was higher with alachlor than with the untreated control. Phenamiphos alone and in combination with alachlor limited primary root weight; shoot/root ratios were greater with these treatments.

Shoot weight, primary root length, and secondary root weight at 10 days were less ($P = 0.05$) in treatments having phenamiphos (Table 1). Shoot weight was higher

TABLE 1. Effects of alachlor and/or phenamiphos on early growth of soybean in the greenhouse.

		Soybean growth parameters*						
Days after planting	Treatment	Shoot wt (g)	Root wt (g)	Primary root wt (g)	Primary root length (mm)	Secondary root wt (g)	Secondary root number†	Shoot/root ratio
5	Untreated control	0.74	0.42	0.30	165	0.12	47	1.8
	Alachlor	0.76	0.31	0.26	104	0.05	26	2.4
	Phenamiphos	0.88	0.38	0.26	144	0.12	44	2.3
	Phenamiphos + alachlor	0.73	0.28	0.22	102	0.06	28	2.7
	Orthogonal contrasts‡	NS	b,c	a	B,C	B,C	B,C	A,B
10	Untreated control	0.78	0.47	0.22	328	0.25		1.7
	Alachlor	0.88	0.52	0.28	148	0.24		1.7
	Phenamiphos	0.61	0.39	0.21	186	0.18		1.5
	Phenamiphos + alachlor	0.78	0.46	0.31	154	0.16		1.7
	Orthogonal contrasts‡	A,C	NS	B,C	A,B	A		NS

* All data are means of eight replicates; pesticides were applied at the rate of 1.0 $\mu g/cm^3$ soil each.
† Secondary roots were too numerous to count at 10 days.
‡ Letters are used to designate differences as determined by orthogonal contrasts: A = presence vs. absence of phenamiphos, B = alachlor alone vs. untreated control, and C = phenamiphos vs. phenamiphos + alachlor; capital letters indicate significance at $P = 0.05$, lower case at $P = 0.10$; NS = no significant difference.

($P = 0.05$) with phenamiphos + alachlor treatments than with the nematicide alone. Primary root weight was more ($P = 0.05$) in treatments containing alachlor; primary root length, however, was less ($P = 0.05$) with alachlor alone treatments than with the untreated control. Generally, all pesticide treatments restricted soybean growth at 10 days compared to the untreated control.

Influence of phenamiphos and/or alachlor and H. glycines on soybean growth and root morphology—greenhouse experiment: At 3 days after planting, the radicals had emerged slightly, but the shoots had not emerged above the soil. Root weight was greater ($P = 0.10$) with alachlor alone treatments than with the untreated control. At 6 days, phenamiphos + alachlor treatments had greater shoot ($P = 0.10$) and primary root weights ($P = 0.05$) than did the nematicide alone treatment (Table 2). Plants grown in soil treated with phenamiphos had greater shoot weight ($P = 0.05$), root weight ($P = 0.05$), primary root length ($P = 0.05$), secondary root weight ($P = 0.05$), and number of secondary roots ($P = 0.10$). Primary root weight at 9 days was higher ($P = 0.10$) in the combination treatment than with phenamiphos alone (Table 2). All treatments having phenamiphos showed greater shoot weights ($P = 0.10$), root weights ($P = 0.05$), and secondary root weights ($P = 0.05$). At 12 days, all pesticide treatments had higher ($P = 0.10$) root weights than did the untreated control.

Total soybean cyst nematode (SCN) penetration and SCN/g root were lower ($P = 0.01$) in treatments having phenamiphos at 3 days (Table 3). Phenamiphos alone and in combination with alachlor limited ($P = 0.01$) primary, secondary, and total root penetration and penetration/g root by SCN at 6, 9, and 12 days.

Effects of phenamiphos, alachlor, and H. glycines on soybean growth—field experiments: Treatments containing phenamiphos had lower root weights ($P = 0.05$; phenamiphos—0.47 g, no phenamiphos—0.55 g) at Smithfield at 5 days. Differences at Smithfield were not significant at 8 days; however, phenamiphos alone and in combination with alachlor limited shoot weights ($P = 0.01$; phenamiphos—1.33 g, no phenamiphos—1.60 g) at 12 days. No significant differences were present at any of the sampling dates at Rocky Mount.

SCN infection of soybean was similar for all treatments on all sampling dates at Smithfield (Table 4). At Rocky Mount, secondary root penetration ($P = 0.05$), to-

TABLE 2. Early growth of soybean as affected by alachlor, phenamiphos, and *Heterodera glycines* in the greenhouse.

		Soybean growth parameters*						
Days after planting	Treatment	Shoot wt (g)	Root wt (g)	Primary root wt (g)	Primary root length (mm)	Secondary root wt (g)	Secondary root number†	Shoot/root ratio
6	Untreated control	0.85	0.39	0.25	160	0.14	47	2.3
	Alachlor	0.80	0.38	0.28	161	0.10	38	2.1
	Phenamiphos	0.87	0.46	0.25	183	0.21	53	1.9
	Phenamiphos + alachlor	1.02	0.46	0.30	184	0.16	51	2.3
	Orthogonal contrasts‡	A,c	A	C	A	A	a	NS
9	Untreated control	1.12	1.01	0.32	236	0.69		1.1
	Alachlor	1.11	1.10	0.32	216	0.78		1.0
	Phenamiphos	1.27	1.16	0.31	259	0.85		1.1
	Phenamiphos + alachlor	1.24	1.25	0.36	253	0.89		1.0
	Orthogonal contrasts‡	a	A	c	NS	A		NS

* All data are means of eight replicates; pesticides were applied at the rate of 1.0 $\mu g/cm^3$ soil each; 3,000 *H. glycines* eggs/10-cm-d clay pot were used as inoculum.

† Secondary roots were too numerous to count at 9 days.

‡ Letters are used to designate differences as determined by orthogonal contrasts: A = presence vs. absence of phenamiphos and C = phenamiphos vs. phenamiphos + alachlor; capital letters indicate significance at $P = 0.05$, lower case at $P = 0.10$; NS = no significant differences.

tal penetration ($P = 0.10$), and penetrations/g root ($P = 0.10$) were lower with treatments containing phenamiphos at 4 days. Total SCN root penetration ($P = 0.05$) and SCN/g root ($P = 0.10$) were also suppressed by phenamiphos at 8 days. Phenamiphos + alachlor treatments had higher total SCN penetration ($P = 0.10$) at 8 days than did phenamiphos alone. At 12 days, treatments having phenamiphos showed suppressed SCN penetration of primary and secondary roots ($P = 0.01$). Alachlor alone treatments had lower SCN penetration of primary and secondary roots ($P = 0.01$) than did the untreated control.

DISCUSSION

The effects of the herbicide alachlor on soybeans were readily apparent in the absence of *H. glycines.* Root-growth parameters were lower at 5 days in the greenhouse with alachlor. Alachlor is an anilide herbicide applied preemergence for grass and small seeded broadleaf weed control. The herbicide is absorbed by the emerging coleoptile and interferes with protein synthesis (6). The principle effects may be seen through suppressed root growth and subsequent disruption of nutrient uptake (6). These effects account for the observed inhibition of growth present in soybeans exposed to this herbicide. Alachlor-treated plants appeared to be 1 or 2 days behind in growth when compared with plants not receiving the pesticide.

Phenamiphos, in the absence of SCN, seemed primarily responsible for the suppressed growth of soybean observed at 10 days; however, plant growth responses in the phenamiphos + alachlor treatments showed similar trends to those of alachlor alone in the absence of nematodes.

With the introduction of *H. glycines* into the pesticide/plant interaction, main effects were associated with the presence or absence of the nematicide. Enhanced soybean growth was consistently observed in the greenhouse with phenamiphos treatments. This response reflects the protection from nematodes afforded the plant by phenamiphos and the resulting suppression of SCN infection. When *H. glycines* was present, the plants treated with phenamiphos + alachlor tended to respond like those receiving only phenamiphos, particularly at days 9 and 12. Numbers of SCN penetrating the roots were negatively correlated with plant growth.

TABLE 3. Effects of alachlor and/or phenamiphos on early penetration of soybean roots by *Heterodera glycines* in the greenhouse.

Treatment	Number of *H. glycines* penetrating roots*							
	Primary root	Secondary root†	Total	Per g root	Primary root	Secondary root	Total	Per g root
	3 days				6 days			
Untreated control	4		4	31	13	33	45	112
Alachlor	5		5	23	15	33	48	134
Phenamiphos	0		0	1	1	6	7	14
Phenamiphos + alachlor	0		0	1	2	7	8	18
Orthogonal contrasts‡	A		A	A	A	A	A	A
	9 days				12 days			
Untreated control	12	86	98	102	20	111	131	111
Alachlor	16	92	108	101	21	118	138	92
Phenamiphos	2	27	29	25	3	32	35	22
Phenamiphos + alachlor	4	29	33	27	2	31	33	21
Orthogonal contrasts‡	A	A	A	A	A	A	A	A

* All data are means of eight replicates; pesticides were applied at the rate of 1.0 $\mu g/cm^3$ soil each; 3,000 *H. glycines* eggs/10-cm-d clay pot were used as inoculum.
† No secondary roots were present at 3 days.
‡ The letter indicates differences as determined by orthogonal contrasts: A = presence vs. absence of phenamiphos, P = 0.01.

Plant weights from the two field sites demonstrated few differences among treatments. The results from Smithfield conflicted with those of the greenhouse experiments. Plant weights were greater with treatments having no phenamiphos. Plant-growth parameters at Rocky Mount showed trends similar to the greenhouse experiment but lacked statistical significance. The late planting date could have played a role in this plant-response suppression.

In greenhouse studies, treatments containing phenamiphos restricted SCN penetration at all sampling dates. Although the precise mode of action of this compound is not clearly understood, phenamiphos disrupts nematode behavior, possibly by impairing nervous-system coordination (29). Other research indicates phenamiphos tends to affect physiological systems other than the neuromuscular coordinating system (18). The nematicide apparently interferes with the nematode's ability to locate host roots. Nematode penetration differences began to decline by 12 days after inoculation, perhaps indicative of nematicide dissipation and a resultant decrease in plant protection.

Nematode penetration of roots was apparently unaffected by phenamiphos at Smithfield. Suppressed SCN penetration of the host was consistently associated with treatments having phenamiphos at Rocky Mount, however. Nematode penetration at Rocky Mount was lower with alachlor alone than with the untreated control. Alachlor may be somewhat nematicidal as reported for several herbicides (10,21,22,25). These nematicidal effects may be associated with altered soybean growth and root morphology, as in vitro experiments with alachlor and SCN eggs have failed to demonstrate any toxic effects (4). The reduced penetration of roots by SCN may be the result of alachlor's inhibition of root growth (6) and simply a product of fewer available sites for nematode penetration. Alachlor, however, had no impact on *H. glycines* in Illinois (17).

Nematode penetration in phenamiphos + alachlor treatments was consistently higher than with the nematicide alone. Although alachlor alone can apparently limit SCN penetration, the addition of the herbicide to phenamiphos may result in an antagonistic interaction, decreas-

TABLE 4. Early season penetration of soybean roots by *Heterodera glycines* as affected by alachlor and/or phenamiphos at two field sites in North Carolina.

		Number of *H. glycines* juveniles penetrating roots*											
		4 days†				8 days				12 days			
Location	Treatment	Primary root	Secondary root	Total	Per g root	Primary root	Secondary root	Total	Per g root	Primary root	Secondary root	Total	Per g root
Smithfield	Untreated control	66	15	81	39	93	83	176	275	145	183	328	457
	Alachlor	53	7	61	28	94	100	193	284	113	163	276	348
	Phenamiphos	48	9	57	31	151	97	248	392	153	139	291	462
	Phenamiphos + alachlor	62	11	73	41	105	88	193	295	172	144	316	515
	Orthogonal contrasts‡	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Rocky Mount	Untreated control	105	11	117	333	184	70	253	323	729	932	1,660	2,162
	Alachlor	101	11	112	310	165	54	219	271	353	387	740	959
	Phenamiphos	53	5	58	158	71	32	103	141	193	237	430	545
	Phenamiphos + alachlor	78	7	85	239	138	63	201	240	241	299	540	759
	Orthogonal contrasts‡	NS	A	a	a	NS	NS	A,c	a	A,B	A,B	A,B	A,B

* All data are means of six replicates; pesticides were applied at recommended rates.

† Samples were collected at 5 days at Smithfield.

‡ The letters are used to designate differences as determined by orthogonal contrasts: A = presence vs. absence of phenamiphos, B = alachlor alone vs. untreated control, and C = phenamiphos vs. phenamiphos + alachlor; capital letters indicate significance at $P = 0.05$, lower case at $P = 0.10$; NS = no significant difference.

ing the efficacy of the nematicide. A similar situation has been reported for sodium azide and carbofuran (23). Other nematicide/herbicide combinations have been more effective in control of SCN than the nematicide alone (17). An increase in SCN penetration with phenamiphos + alachlor treatments could play a key role in the enhanced mid- to late-season resurgence phenomenon previously noted with use of this pesticide combination (5,28). Greater early season SCN infection should translate into higher numbers of this pest at harvest. An understanding of the mechanism of alachlor's effects on phenamiphos and/or SCN is necessary to avoid any potential problems that may arise from this herbicide's use in infested fields.

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