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In Vitro Hatch and Survival of *Heterodera glycines* as Affected by Alachlor and Phenamiphos[1]

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Abstract: *Heterodera glycines* (race 1) eggs were exposed to aqueous solutions of selected concentrations of the herbicide alachlor and the organophosphate nematicide phenamiphos alone and in herbicide-nematicide combinations. Phenamiphos (0.5 μg/ml) + alachlor (0.063, 0.125, or 1.0 μg/ml) treatments increased the incidence of juvenile hatch over that of untreated controls at 18 days. At 18 and 25 days, phenamiphos (0.5 μg/ml) treatments contained more juveniles than did phenamiphos at 1.0 μg/ml. Phenamiphos (1.0 μg/ml) alone and in combination with alachlor (1.0 μg/ml) suppressed hatch for 21 days and juvenile survival for more than 21 days. Alachlor treatments enhanced juvenile survival compared to the untreated control at 14 and 21 days. Technical alachlor gave results similar to those of the formulated product.

Key words: pesticide interaction, soybean cyst nematode, nematicide, herbicide.

Late season resurgence of plant-parasitic nematodes commonly occurs after soil treatments with a nematicide. This resurgence frequently results in greater final population densities of nematodes than in untreated soils and is usually attributed to increased root growth. Resurgence may be enhanced with organophosphate nematicides and herbicide combinations (5,14). Many such interactions may be related to the compounds' lack of specificity which can result in numerous effects on nontarget organisms (9,13). Research on herbicide-nematicide interactions has been limited primarily to their impact on the crop plant (13); effects on nematodes are not well understood (1,5–7,10–12,14). Frequently, these pesticide interactions alter nematode control compared to nematicides alone (5–7,10,12,14). Sodium azide applied alone was somewhat nematicidal, but when applied in combination with carbofuran, reduced the efficacy of the nematicide (12). Alachlor in combination with several nematicides resulted in increased numbers of nematodes compared with the nematicide alone treatments (5,14). Specific effects of herbicide-nematicide interactions on nematode hatch, survival, root penetration, reproduction, and general behavior have received little attention. Lower population levels of *Heterodera glycines* Ichinohe juveniles often occur in soils treated with aldicarb in combination with vernolate, trifluralin, or metribuzin than in soils treated with the nematicide alone (10); however, the specific processes involved are unknown.

The objective of this research was to determine the effects of alachlor and phenamiphos alone and in herbicide-nematicide combinations on the hatch and survival of *H. glycines.*

Materials and Methods

Five randomized complete block design experiments were conducted in the laboratory to determine the effects of alachlor and phenamiphos alone and in herbicide-nematicide combinations on hatch and survival of the soybean cyst nematode (SCN), *H. glycines* (race 1). Eggs of SCN were exposed to aqueous solutions of the pesticide treatments in vitro in closed 60 × 15-mm glass petri dishes maintained in an incubator at 29 C. The petri dishes were observed periodically and the numbers of juveniles counted.

Stock solutions of 1.0 mg/ml were prepared for alachlor and phenamiphos from the formulated products. Dilutions were made to concentrations four times that required for each treatment. Alachlor solutions (4×) prepared included 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, and 16.0 μg/ml; nematicide solutions (4×) consisted of 2.0, 4.0, and 8.0 μg/ml.

Mature cysts were collected from established greenhouse stock soybean cultures

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by elutriation (8) and then crushed with a glass tissue homogenizer to release the eggs. The homogenate was passed through nested 65- and 26-μm screens with the eggs retained on the latter. The eggs were resuspended in water, quantified, and pipetted into the petri dishes in two 5-ml volumes. Treatments were replicated four times unless otherwise indicated.

Both living and dead juveniles present for any particular time period were included in the hatch counts. Survival counts differentiated between living and dead juveniles and were expressed as percent survival of the original hatch. Juveniles with transparent esophagi were considered living, whereas those with an opaque or granulated esophagus were classified as dead (body contents not extruded upon cuticular puncture). Following the period of time allowed for hatch, the eggs were removed and the living juveniles counted every 7 days for 21 days to determine percent survival. All experiments were repeated a minimum of two times.

Experiments 1 and 2: Alachlor concentrations of 0.063, 0.125, 0.25, 0.5, 1.0, 2.0, and 4.0 μg/ml were used in the first experiment; 5 ml of the appropriate 4× alachlor solution were added to 5 ml tap water in a petri dish. The egg suspension (150 eggs in 10 ml water) was then added giving a final volume of 20 ml/dish and diluting the herbicide solution to the desired concentration. The control treatment consisted of tap water only. Petri dishes were observed and the hatched nematodes recorded every 2 days for 10 days.

The second experiment included three concentrations (0.5, 1.0, and 2.0 μg/ml) of phenamiphos. The study was set up as previously described for the first experiment.

Experiment 3: A 3 × 2 factorial experiment with herbicide-nematicide combinations was conducted with three concentrations of alachlor (0.063, 0.125, and 1.0 μg/ml), two of phenamiphos (0.5 and 1.0 μg/ml), and a water control. Treatments were prepared as before except the number of eggs was increased to 1,000/petri dish. Juvenile counts were made every 3 days for a 12-day period and every 7 days thereafter for an additional 21 days.

Experiment 4: Treatments consisted of alachlor (1.0 μg/ml) and/or phenamiphos (1.0 μg/ml) and an untreated control (water). To separate the processes of hatch and survival, 26-μm nylon and plastic screens were constructed and placed in each petri dish. The 26-μm nylon screen was selected because it retained nematode eggs and dead juveniles, yet allowed live juveniles to pass through. These screens simplified counting of juveniles (by eliminating eggs and debris), permitted study of hatch over time, allowed for determination of survival rates for particular groups of juveniles, and provided separation of such processes as continuing hatch and rapid degradation vs. initial hatch and prolonged survival.

The experiment was set up as experiments 1–3, except the egg suspension (5,000/petri dish) was pipetted onto the nylon screen. After 7 days, each screen (containing unhatched eggs) was transferred intact to another petri dish containing fresh solution. This procedure was repeated twice resulting in three 7-day groups of hatched juveniles. The eggs were discarded after 21 days, but each group of juveniles was observed every 7 days for an additional 21 days, and percent survival determined. Treatments were replicated five times.

Experiment 5: Technical alachlor (1.0 μg/ml) alone and in combination with phenamiphos (1.0 μg/ml) and the treatments indicated for experiment 4 were included in this study. Treatments were prepared, set up, and observed in the same manner as the fourth experiment, except 1,000 eggs/petri dish were used.

Statistical analysis: All data were subjected to analysis of variance, and least significant differences (LSD) were calculated if the F value was significant. Orthogonal contrasts were calculated for the following comparisons: presence vs. absence of nematicides, phenamiphos vs. phenamiphos + alachlor, and alachlor vs. the control. In the fifth experiment, additional contrasts were computed: phenamiphos vs. phenamiphos + technical alachlor, and technical alachlor vs. the control.

Results and Discussion

Experiments 1 and 2: Hatch of SCN was very low in both experiments. Treatment means for the 4-day counts ranged from four to eight juveniles, whereas the 8-day

TABLE 1. In vitro hatch of *Heterodera glycines* in aqueous solutions of phenamiphos and/or alachlor at selected concentrations.

Treatment	Pesticide concentration (μg/ml)	Numbers of *H. glycines* juveniles per petri dish*		
		12 days	18 days†	25 days†
Untreated control	1.0	98	108	124
Alachlor	1.0	90	130	139
Alachlor	0.125	119	130	134
Alachlor	0.063	115	116	124
Phenamiphos	1.0	105	117	132
Phenamiphos	0.5	104	119	129
Phenamiphos + alachlor (P + A)	1.0 + 1.0	107	115	131
P + A	1.0 + 0.125	126	122	140
P + A	1.0 + 0.063	99	91	116
P + A	0.5 + 1.0	108	139	150
P + A	0.5 + 0.125	133	147	157
P + A	0.5 + 0.063	120	139	164
LSD 0.05		28	34	
0.10		24	29	NS

* All data are the means of four replications; 1,000 *H. glycines* eggs/petri dish, maintained at 29 C.
† Orthogonal contrasts indicate the numbers of juveniles at 18 and 25 days are higher ($P = 0.01$ and 0.05, respectively) with 0.5 μg/ml phenamiphos-alachlor treatments than with 1.0 μg/ml phenamiphos-alachlor treatments.

counts varied from five to ten. The 2-, 6-, and 10-day counts showed even smaller differences. Consequently, adequate separation of treatment effects was not achieved. Subsequent experiments were conducted with a minimum of 1,000 eggs/petri dish.

Experiment 3: Hatch of SCN was not different among treatments at 3-, 6-, or 9-day counts; however, at 12 days, phenamiphos (0.5 and 1.0 μg/ml) + alachlor (0.125 μg/mg) treatments contained greater ($P = 0.05$) numbers of juveniles than did the control (Table 1). Phenamiphos (0.5 μg/ml) + alachlor (0.063, 0.125, and 1.0 μg/ml) treatments had more ($P = 0.10$) juveniles than did the control at 18 days. Numbers of juveniles at 18 and 25 days were higher in phenamiphos (0.5 μg/ml) + alachlor combinations than in the high rate of phenamiphos (1.0 μg/ml) + alachlor treatments.

Greater numbers of juveniles were generally associated with treatments having 0.125 μg/ml alachlor. This effect was maximal with the low level of phenamiphos-herbicide combination. Alachlor has been shown to be somewhat nematicidal at recommended rates (2,3); however, levels of 0.125 μg/ml may have been too low to exert nematicidal effects. Phenamiphos at 0.5 μg/ml was less toxic than at 1.0 μg/ml, accounting for the differences in the numbers of juveniles. Alachlor (0.063 μg/ml) was probably too low a concentration to significantly alter the effects of phenamiphos (1.0 μg/ml) when used in combination. Hatch of SCN in the untreated control was consistently lower than in any other treatment. This response indicated that some stimulation of hatch occurred with the pesticides. This effect was greatest with 0.5 μg/ml phenamiphos + 0.125 μg/ml alachlor at 12 and 18 days.

Experiments 4 and 5: Hatch of SCN was suppressed ($P = 0.05$) in treatments containing phenamiphos (Table 2). Inhibition of hatch has also been demonstrated for *Heterodera schachtii* Schmidt when exposed to aldicarb (15). Phenamiphos may damage or kill some juveniles within eggs and reduce hatch. Any such effective reduction of nematodes in the field at planting would provide soybeans early season protection and allow development of more root biomass. Plants receiving early season protection from nematodes would be capable of supporting larger nematode populations later in the growing season. In addition, if reduced hatching was due to inhibition, then more eggs would be available to hatch when the root system is relatively large.

Survival was strongly related to the presence or absence of the nematicide, as phenamiphos treatments sharply limited ($P = 0.01$) juvenile survival (Table 3). More ($P = 0.05$) juveniles survived in alachlor treat-

TABLE 2. Hatch of *Heterodera glycines* in vitro in aqueous solutions of phenamiphos and/or alachlor at 7-day intervals for 21 days.

Treatment	Pesticide concentration (μg/ml)	Numbers of *H. glycines* juveniles per petri dish*			
		0–7 days	7–14 days	14–21 days	Total
Untreated control		1,789	981	43	2,813
Alachlor	1.0	1,945	982	44	2,971
Phenamiphos	1.0	1,378	847	16	2,241
Phenamiphos + alachlor	1.0 + 1.0	1,466	800	12	2,278
Orthogonal contrasts†		A	a	A	A

* All data are the means of five replications; 5,000 *H. glycines* eggs/petri dish, maintained at 29 C.
† Orthogonal contrasts indicate lower numbers of juveniles present with the nematicide treatments than with treatments having no nematicide; capital letter indicates significance at $P = 0.01$, lower case at $P = 0.05$.

TABLE 3. In vitro survival of *Heterodera glycines* in aqueous solutions of phenamiphos and/or alachlor 21 days after hatch.

Treatment	Pesticide concentration (μg/ml)	Percentage of *H. glycines* surviving 21 days after hatch*			
		0–7 days†	7–14 days	14–21 days	Total
Untreated control		39.6	1.8	13.7	26.1
Alachlor	1.0	46.6	2.8	28.9	31.6
Phenamiphos	1.0	5.4	0.4	0.0	3.4
Phenamiphos + alachlor	1.0 + 1.0	4.0	0.3	5.0	2.7
Orthogonal contrasts‡		A	A,b	A,b	A,b

* All data are the means of five replications; maintained at 29 C.
† Percent survival of original juveniles hatching for each 7-day period.
‡ Letters are used to designate differences as determined by orthogonal contrasts: A = presence vs. absence of phenamiphos ($P = 0.01$) and b = alachlor vs. the untreated control ($P = 0.05$).

ments than in the untreated control at 21 days after hatch. This increased survival in the alachlor treatments apparently is of little advantage to SCN, since host infection was suppressed by the herbicide (2). Hence, surviving juveniles may not be infective. Greater survival could result from the herbicide effects on other micro-organisms such as bacteria or fungi. Alachlor could be toxic to these micro-organisms and thus protect dying or dead SCN juveniles from degradation. Juvenile deterioration occurred more rapidly in the untreated controls, lending credence to this conclusion. This hypothesis could conflict with a report of increased seedborne fungi on soybean seed harvested from alachlor-treated plots (4).

Results from the fifth experiment were similar to those from the fourth experiment. Technical alachlor appeared to be slightly more toxic than the formulated product at similar concentrations.

Although hatch and survival of SCN differed among treatments, the occurrence of these effects depended primarily upon the presence or absence of the nematicide. The failure of these experiments to demonstrate differences between phenamiphos and phenamiphos + alachlor treatments indicates that hatch and survival dissimilarities probably are subtle, not significant, or function over a long period of time and could not be determined in short-term experiments. However, the mode of action in vitro may also be different than that occurring in the soil.

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