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Effects of Eight Herbicides on In Vitro Hatching of *Heterodera glycines*[1]

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Abstract: Laboratory studies were conducted to evaluate effects of selected herbicides on hatching of free eggs of the soybean cyst nematode, *Heterodera glycines*. The herbicides used were Atrazine (atrazine), Basagran (bentazon), Bladex (cyanazine), Blazer (acifluorfen), Command (clomazone), Lasso (alachlor), Sonalan (ethalfluralin), and Treflan (trifluralin). Treatments comprised two concentrations of commercial herbicide formulations and deionized water and 3.14 mM zinc sulfate as negative and positive controls, respectively. Eggs were extracted from females and cysts, surface disinfested, and incubated in herbicide or control solutions at 25 ± 2 C in darkness. Hatched second-stage juveniles were counted every other day for 24 days. Hatching of *H. glycines* eggs in 50 and 500 µg/ml Blazer was 42 to 67% less than that in deionized water and 61 to 78% less than that in zinc sulfate solution. Zinc sulfate significantly increased hatching activity in 50 µg/ml but not 500 µg/ml Blazer. The other herbicides tested at various concentrations had no significant effect on egg hatching. The specific component of Blazer inhibiting egg hatching is unknown. Suppression of hatching by Blazer indicates that this postemergence soybean herbicide may have a potential role in managing *H. glycines*.

Key words: acifluorfen, alachlor, atrazine, bentazon, clomazone, cyanazine, ethalfluralin, *Glycine max*, hatching, herbicide, *Heterodera glycines*, nematode, soybean cyst nematode, trifluralin.

Pesticides, especially herbicides, are common agricultural inputs that could influence the biology and population dynamics of plant-parasitic nematodes. The effects of herbicides, alone or in combination with nematicides, on cyst nematodes have been investigated extensively. Herbicides may stimulate, inhibit, or have no effect on hatching of cyst nematode eggs. Kraus and Sikora (9) reported that the herbicide diallate increased hatching from cysts of *Heterodera schachtii* Schmidt in laboratory and greenhouse experiments. Similarly, they found increased invasion of sugarbeet roots by *H. schachtii* following treatment with diallate under field conditions, but treatments of diallate and the nematicide aldicarb reduced *H. schachtii* population densities (10). In vitro incubation of *Globodera rostochiensis* Wollenweber and *H. schachtii* cysts in the herbicides cycloate, pebulate, vernolate, and triallate at medium field application rates inhibited subsequent egg hatching in root diffusate, whereas incubation in the herbicides chloridazon, metribuzin, and lenacil had slight or no adverse effects (11). Chloridazon and triallate also reduced hatching from cysts of *G. rostochiensis* in vitro (2). In greenhouse studies, however, hatching from cysts of *G. rostochiensis* and *G. pallida* Stone was unaffected by chloridazon and triallate, although reduced host invasion by *G. rostochiensis* and increased host invasion by *H. schachtii* were observed in pots treated with triallate (2).

Most prior investigations of the effects of herbicides on *Heterodera glycines* Ichinohe were conducted in field plots or greenhouse pots. Population densities of *H. glycines* were greater in soils treated with trifluralin relative to untreated soil in field and greenhouse studies in Arkansas and Illinois (8,12). Field plots treated with the herbicides metribuzin, alachlor, or linuron in addition to aldicarb had lower final population densities of *H. glycines* than plots treated with aldicarb alone (15). In contrast, *H. glycines* population densities were greater in plots treated with a combination of alachlor and the nematicide fenamiphos

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than in plots with the nematicide alone (16). Greater numbers of *H. glycines* penetrated the roots of plants treated with alachlor and fenamiphos than plants treated with fenamiphos alone, but fewer nematodes infected alachlor-treated plants than untreated plants (5). Plots treated with alachlor and the nematicide fensulfothion also had greater population densities of *H. glycines* than plots treated with the nematicide alone (14).

Few herbicides have been evaluated in vitro for effects on hatching of *H. glycines* eggs. In one such study, a solution of alachlor and fenamiphos increased hatching of *H. glycines* compared to hatching in tap water, and alachlor enhanced the survival of second-stage juveniles (J2) relative to tap water (4). Herbicides that stimulate hatching of *H. glycines* eggs could be employed to induce egg hatching in the absence of soybeans, as in fields planted to corn. Conversely, herbicides that inhibit hatching of *H. glycines* eggs could be used advantageously to reduce infection when soybeans are grown. The purpose of this study was to determine the effects of selected corn and soybean herbicides on hatching of free *H. glycines* eggs in vitro.

Materials and Methods

Commercial formulations of herbicides were used in these experiments instead of technical grade material because commercial products are readily available and results would have greater applicability to agronomic practices. Trade names are used in presentation and discussion of results because the commercial formulations were used. The following herbicides were tested: Atrazine 4L (atrazine; 6-chloro-N-ethyl-N′-[1-methylethyl]-1,3,5-triazine-2,4-diamine), Basagran 4S (bentazon; 3-[1-methylethyl]-[1H]-2,1,3-benzothiadiazin-4[3H]-one 2,2-dioxide), Bladex 4L (cyanazine; 2-[[4-chloro-6-(ethylamino)-1,3,5-triazin-2-yl]amino]-2-methylpropanenitrile), Blazer 2L (acifluorfen; 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid), Command 4EC (clomazone; 2-[(2-chlorophenyl)methyl]-4,4-dimethyl-3-isoxazolidinone), Lasso 4EC (alachlor; 2-chloro-N-[2,6-diethylphenyl]-N-[methoxymethyl]acetamide), Sonalan 3EC (ethalfluralin; N-ethyl-N-[2-methyl-2-propenyl]-2,6-dinitro-4-[trifluoromethyl]benzenamine), and Treflan 4EC (trifluralin; 2,6-dinitro-N,N-dipropyl-4-[trifluoromethyl]benzenamine). Blazer, Command, Sonalan, and Treflan are commonly used in soybean fields; Atrazine and Bladex are corn herbicides; Basagran and Lasso are used in both corn and soybean production. Aqueous solutions of the commercial herbicide formulations were prepared using deionized water previously adjusted to pH 7.0 with 1.0 M HCl and 1.0 M KOH and were evaluated at two concentrations: 3 and 33 μg/ml Atrazine, 50 and 500 μg/ml Basagran, 17 and 170 μg/ml Bladex, 50 and 500 μg/ml Blazer, 50 and 500 μg/ml Command, 24 and 240 μg/ml Lasso, 1 and 10 μg/ml Sonalan, and 1 and 10 μg/ml Treflan. Atrazine, Bladex, Lasso, Sonalan, and Treflan were evaluated at concentrations that approximated the maximum and one-tenth of the maximum solubility of the herbicide formulations in aqueous solution. Basagran, Blazer, and Command are much more soluble in aqueous solution than the aforementioned herbicide formulations, but were tested at the arbitrarily selected concentrations of 50 and 500 μg/ml.

An Iowa population of race 3 *H. glycines* was cultured on *Glycine max* (L.) Merrill 'Corsoy 79' in the greenhouse. Immediately before experimentation, adult females and cysts were removed from 30-day-old soybean roots on a 850-μm-pore sieve with a high-pressure stream of water. The females and cysts were collected on a 250-μm pore sieve and subsequently separated from soil and root debris by sucrose centrifugation (7). When appropriate, females and cysts were crushed with a motorized pestle to release the eggs (3). Females and cysts or eggs were surface disinfested with 0.5% chlorhexidine diacetate for 15 minutes and rinsed repeatedly with sterile water (1).

Individual hatching units consisted of a

microsieve, tray, and eggs or females and cysts. The microsieves were constructed from circles of nylon monofilament screen (38-μm pores) stretched over the end of an 18-mm-d cylinder and inserted into a 20-mm-d cylinder; cylinders were polypropylene test-tube caps with the ends removed. The microsieves were placed into 32-mm-wide × 72-mm-long × 14-mm-deep rectangular polystyrene trays with grid patterns inscribed on the bottom to facilitate counting of hatched J2.

Effects on hatching of free eggs: At the beginning of each experiment, the trays and sieves were exposed to radiation from a 30 W germicidal UV light for at least 30 minutes. A minimum of 5,000 eggs were dispensed onto each microsieve, which was placed immediately into the tray containing 12 ml of a herbicide solution or a control solution. Deionized water, adjusted to pH 7.0 with 1.0 M HCl and 1.0 M KOH, and 3.14 mM zinc sulfate, a *H. glycines* egg hatching stimulant (6), were negative and positive controls, respectively. Zinc sulfate and the herbicide solutions were prepared with deionized water adjusted to pH 7.0. The hatching units were placed in 20-cm-wide × 27-cm-long × 9.5-cm-deep polystyrene containers and were randomized within complete blocks (each container contained one replication of each treatment), with five replicates per treatment. The eggs were incubated in darkness at 25 ± 2 C, except when the hatching units were removed and the microsieves were transferred to new trays. Every other day for 24 days, the microsieves and eggs were transferred to freshly sterilized trays filled with fresh solution, and the J2 remaining in the old trays were counted. At the end of each experiment, the number of unhatched eggs remaining on each microsieve was determined. The individual counts of J2 were converted into percentages of the total eggs.

At selected times throughout the experiments, cumulative percentage hatching for the treatments was subjected to analysis of variance ($P \leq 0.05$), followed by Fisher's least significant difference test ($P = 0.05$) if significant treatment effects were detected (13). All experiments were conducted two or more times.

Influence of zinc sulfate on hatching in Blazer: Blazer was tested to examine the effect of the formulated herbicide compound in the presence of zinc sulfate on hatching of *H. glycines* eggs. Treatments consisted of 50 and 500 μg/ml Blazer with and without 3.14 mM zinc sulfate. Deionized water and 3.14 mM zinc sulfate were the negative and positive controls, respectively. All other aspects of the experiment were as described above. The experiment was conducted three times.

Effects on hatching of eggs within females and cysts: The effects of Blazer, Lasso, and Treflan on hatching of eggs within *H. glycines* adult females and cysts were determined in another experiment. For each treatment, five groups of 20 surface-disinfested adult females and cysts were used. The adult females and cysts were placed onto each microsieve and incubated in aqueous solutions of herbicide, zinc sulfate, or deionized water. The total number of eggs hatching over a 24-day period was determined. At the end of the experiment, the adult females and cysts from each microsieve were broken, and the J2 and unhatched eggs contained within were counted. All other aspects of the experiment were identical to the aforementioned studies on effects of herbicides on hatching of free eggs. The experiment was repeated once.

Results

All hatching experiments were conducted two or more times, and the significant differences detected between treatments were consistent among replicate experiments. The results presented herein are from initial experiments for each herbicide.

Effects on hatching of free eggs: In all experiments, eggs hatched primarily during the first 2 weeks of incubation, regardless of treatment. Blazer inhibited hatching of *H. glycines* eggs. The percentage of

hatched eggs in 50 and 500 μg/ml Blazer was less ($P \leq 0.05$) than that in deionized water or zinc sulfate solution (Fig. 1). Fewer ($P \leq 0.05$) eggs hatched in 500 μg/ml Blazer than in 50 μg/ml Blazer. Inhibition of hatching by the two concentrations of Blazer occurred by 4 days of incubation and was consistent throughout the experiment. At the end of the experiment, hatching in 50 μg/ml and 500 μg/ml Blazer was 42 and 67%, respectively, of that in deionized water. Significant differences in percentage of hatched eggs between zinc sulfate and the other treatments were detected after 2 days of incubation, and the differences increased throughout the experiment.

All other herbicides tested had no effect on hatching of *H. glycines* eggs. Throughout all experiments, hatching in solutions of Atrazine, Basagran, Bladex, Command, Lasso, Sonalan, and Treflan was not significantly different from that in deionized water, regardless of herbicide concentration (Table 1). The differences between hatching in the herbicide solutions and that in deionized water usually increased with time, but the differences never became significant. Hatching in the herbicide solutions was less ($P \leq 0.05$) than hatching in zinc sulfate solution beginning after 2 to 6 days of incubation and lasting throughout the remainder of the experiments (Table 1).

Influence of zinc sulfate on hatching in Blazer: Treatments of 50 and 500 μg/ml Blazer suppressed ($P \leq 0.05$) hatching of *H. glycines* eggs by 33 and 60%, respectively, compared to deionized water at day 24 (Fig. 2). Significant differences between hatching in these two concentrations of Blazer and hatching in deionized water were first detected after 6 days of incubation and persisted throughout the experiment. Similarly, hatching in 500 μg/ml Blazer plus zinc sulfate was less ($P \leq 0.05$) than that in deionized water beginning at 2 days of incubation, and the difference lasted throughout the remainder of the experiment. In contrast, hatching of *H. glycines* eggs incubated in 50 μg/ml Blazer plus zinc sulfate was greater ($P \leq 0.05$) than hatching in deionized water or in the other herbicide treatments after 10 days of incubation, but less ($P \leq 0.05$) than hatching in zinc sulfate solution. More ($P \leq 0.05$) *H. glycines* eggs hatched in zinc sulfate solution than in all other treatments beginning day 2.

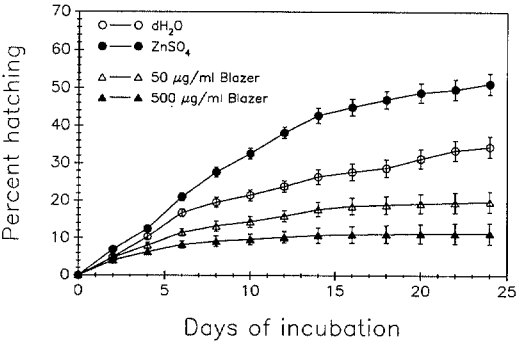

FIG. 1. Cumulative percentage hatching of free *Heterodera glycines* eggs in deionized water (dH_2O), 3.14 mM zinc sulfate ($ZnSO_4$), and two concentrations of Blazer herbicide. Error bars represent least significant difference ($P = 0.05$).

Effects on hatching of eggs within females and cysts: When Blazer, Lasso, and Treflan were evaluated using groups of 20 females and cysts, zinc sulfate elicited a maximum of 8% hatching, and 14% hatching occurred in deionized water at day 24 (data not shown). When the experiment was repeated, hatching was considerably less in the second experiment, with maximum cumulative percentage hatching between 1–2% in the herbicide solutions, which was not significantly different from the 1% hatch in deionized water (data not shown). Differences ($P \leq 0.05$) between the percentage of hatched eggs in zinc sulfate and the other treatments were not found until day 24. Maximum hatching in zinc sulfate was approximately 5% in the second experiment.

DISCUSSION

In the research reported herein, commercially formulated products of herbicides were used. In addition to active ingredients, formulated herbicides contain inert ingredients, carriers, emulsifiers, wetting agents, and other components. No

TABLE 1. Hatching of *Heterodera glycines* eggs after 12 and 24 days of incubation in seven herbicides relative to hatching in deionized water and 3.14 mM zinc sulfate.

Herbicide	Concentration (µg/ml)	Percentage difference from: Deionized water, Day 12	Deionized water, Day 24	Zinc sulfate, Day 12	Zinc sulfate, Day 24
Atrazine	3	−2.9	−4.4	−17.2*	−21.4*
	33	−1.3	−3.5	−15.6*	−21.2*
Basagran	50	+2.7	+2.4	−15.8*	−28.9*
	500	+2.1	+1.6	−16.4*	−29.8*
Bladex	17	+0.9	+0.7	−17.6*	−30.6*
	170	−0.3	−1.2	−18.8*	−32.5*
Command	50	+2.1	+1.6	−16.4*	−29.7*
	500	+1.2	+2.2	−17.3*	−29.2*
Lasso	24	−0.6	−2.0	−14.8*	−18.7*
	240	−1.2	−4.3	−15.4*	−21.5*
Sonalan	1	+0.3	−1.1	−36.0*	−44.3*
	10	−0.9	−2.0	−37.2*	−45.2*
Treflan	1	−0.8	−2.8	−16.4*	−26.1*
	10	−0.6	−2.3	−16.3*	−25.6*

Data presented are means of five replications per treatment. Numbers followed by asterisks are significantly different ($P \leq 0.05$) from deionized water or zinc sulfate.

attempts were made to determine whether observed effects were due to active herbicide ingredients or other components in the formulations. Other researchers have reported trade names and common names of the herbicides, often synonymously, in reports of studies of herbicide effects on *H. glycines*. Many of the earlier reports do not indicate whether purified technical grade active ingredients or commercial formulations were utilized.

The percentage of hatched eggs in the concentrations of Atrazine, Basagran, Bladex, Command, Lasso, Sonalan, and Treflan we tested did not differ significantly from corresponding percentages in the deionized water controls. This result contrasts with previous reports that alachlor and trifluralin, the respective active ingredients in Lasso and Treflan, stimulate hatching of *H. glycines* in infested soils in the greenhouse and field (8,12,14,16). Differences in the herbicide formulations or the type of experimental system (soil vs. in vitro) used could explain the discrepancy between our results with Lasso and Treflan and prior reports with alachlor and trifluralin.

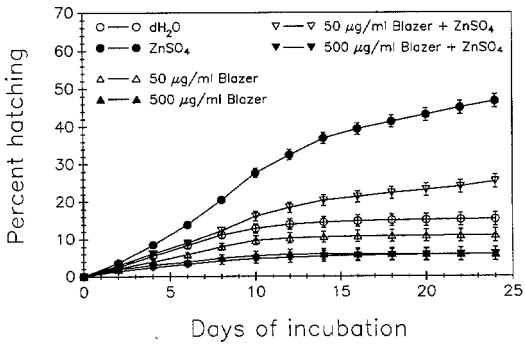

FIG. 2. Cumulative percentage hatching of free *Heterodera glycines* eggs in deionized water (dH_2O), 3.14 mM zinc sulfate ($ZnSO_4$), two concentrations of Blazer herbicide, and two concentrations of the herbicide Blazer plus 3.14 mM zinc sulfate ($ZnSO_4$). Error bars represent least significant difference ($P = 0.05$).

Our results also indicate that Blazer had a consistently negative effect on *H. glycines* egg hatching. Furthermore, concentrations of Blazer at 500 µg/ml, but not 50 µg/ml, in the presence of 3.14 mM zinc sulfate inhibited hatching of *H. glycines* eggs.

The effects of Blazer, Lasso, and Treflan on *H. glycines* eggs contained within intact females and cysts were also examined. These eggs are more representative of eggs that survive for years under natural field conditions. The cyst wall may act as a barrier that could prevent herbicides from reaching the eggs within. When adult females and cysts were exposed to aqueous

solutions of the selected herbicides, results differed from those previously reported (8,12,14,16). Also, 3.0-mM concentrations of zinc chloride and zinc sulfate have been reported to stimulate hatching of *H. glycines* eggs within intact cysts (6), but this effect was not observed in our experiments. Hatching of *H. glycines* eggs was low in our experiments with females and cysts, and hatching in zinc sulfate treatments never exceeded 8%. Although unknown, the cause of the poor hatching of eggs within females and cysts in our experiments could be due to the use of young adult females and cysts in the assays. A mixture of younger and older females and cysts was used in the first experiment, and a larger percentage of eggs hatched than when primarily young females and cysts were used in the second assay. Measures were not taken to precisely standardize the age of the females and cysts used in these experiments. Also, females and cysts used in our experiments were surface disinfested, unlike prior experiments (6). Consequently, another possible reason for the low percentage of hatching of eggs within females and cysts, although unlikely, could be the detrimental effects of surface disinfestation with chlorhexidine diacetate. Insufficient rinsing or binding of the disinfestant to the body wall of the females and cysts could have had an adverse effect on the eggs within.

The results of our in vitro egg assays reveal that Blazer, a postemergence herbicide for soybeans, was effective in inhibiting egg hatching and, thus, may have potential for a role in management of *H. glycines* if future research reveals a similar effect on eggs within intact cysts. Such a product would be useful in reducing initial infections by J2 and thus subsequently reducing the magnitude of population density increases throughout a growing season when a susceptible soybean crop is planted. One aspect that needs to be clarified is identification of the actual component of the formulated product that inhibits its hatching. Because we used commercial formulations of all herbicides in our studies, the specific components of Blazer responsible for this inhibition are unknown. Such information is needed to establish optimum doses of the inhibitory component and to avoid phytotoxic effects of increased concentrations of the active herbicide ingredient. Also, additional work is needed to determine the effects of Blazer on *H. glycines* eggs within females and cysts. Finally, effects of Blazer on hatching of *H. glycines* should be studied in soil under greenhouse and field conditions. If consistent inhibition of egg hatch by Blazer can be documented in natural soils, a novel means of managing *H. glycines* could be developed.

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