Journal of Nematology 30(3):347–352. 1998.
© The Society of Nematologists 1998.

Influence of Herbicide Application to Soybeans on Soybean Cyst Nematode Egg Hatching[1]

BRIAN C. LEVENE,[2] MICHEAL D. K. OWEN,[2] AND GREGORY L. TYLKA[3]

Abstract: The hatching of *Heterodera glycines* eggs in soybean root exudates collected after postemergence application of three herbicides, and the hatching potential of *H. glycines* eggs from females feeding on herbicide-treated plants, were measured in vitro. Hatching in all root exudate solutions (RES) was greater than in deionized water but less than in 0.003 M $ZnSO_4$ solution. Filtering RES with a 0.22-µm-filter increased *H. glycines* hatching in RES. Application of acifluorfen, bentazon, and lactofen to foliage of soybean plants inhibited hatching of *H. glycines* eggs from the same plants. Hatching in RES from the different herbicide-treated soybeans was similar. Application of crop oil concentrate and non-ionic surfactant adjuvant to foliage did not affect hatching of *H. glycines* eggs from soybean plants.

Key words: Glycine max, hatching, herbicide, *Heterodera glycines*, nematode, postemergence, reproduction, root exudates, SCN, soybean, soybean cyst nematode.

Herbicides may reduce nematode densities by controlling weed hosts, affecting nematodes directly or altering the host plant. Possible direct effects of herbicides on nematodes include inhibiting egg hatch, restricting the migration of juveniles to host plants, and inhibiting the development of nematodes within host plant roots. Several soil-applied herbicides have inhibited nematode hatching in vitro at concentrations similar to those found in spray tank solutions, but effects of the herbicide were markedly reduced when tested at concentrations typically found in soil (Perry and Beane, 1989). Fedorko et al., (1977) reported 70% nematode mortality after exposure to several levels of technical-grade monolinuron [N-(4-chlorophenyl)-N-methoxy-N-methylurea] for 48 hours.

Nematicide-herbicide combinations generally are successful at controlling their targeted pests, although preplant herbicides may reduce or enhance nematicide efficacy (Payan et al., 1987). Antagonism between alachlor [2-chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl) acetamide] and fenamiphos [ethyl 3-methyl-4-(methylthio)phenyl(1-methylethyl)phosphoramidate] may explain late-season *H. glycines* population resurgence observed with field applications of these pesticides (Bostian et al., 1986; Sipes and Schmitt, 1989).

Three weeks after a 28-day exposure of *Globodera rostochiensis* and *Heterodera schachtii* to field-use rates of several thiocarbamate herbicides, virtually no nematode egg hatch was observed (Perry and Beane, 1989). However, the effect of these herbicides on nematode hatching was decreased at reduced concentrations of the pesticide. Wong et al. (1993) reported significant inhibition of *H. glycines* egg hatch when free eggs were incubated in solutions of acifluorfen [5-[2-chloro-4-(trifluromethyl)phenoxy]-2-nitrobenzoic acid]. Application of trifluralin, monolinuron, and the nematicides fenamiphos and aldicarb [2-methyl-2-(methylthio)propionaldehyde-0-(methylcarbomyl)oxime], alone or in mixtures, to soybean field plots at recommended field application rates resulted in lower *H. glycines* population densities than in the untreated control plots (Oji et al., 1988). Twice the normal rate of trifluralin and pendimethalin reduced *H. glycines* second-stage juvenile (J2) population densities without affecting soybean yields (Youmans, 1986).

Postemergence herbicides also may have adverse effects on nematodes. *Heterodera glycines*-infested soybean plots treated postemergence with acifluorfen plus bentazon [3-(1-methylethyl)-(1H)-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide] had significantly lower late-season nematode population den-

Received for publication 16 May 1997.

[1] Journal Paper No. *J-17413* of the Iowa Agricultural and Home Economics Experiment Station, Ames, IA 50011. Project Nos. 2062 and 3288.

[2] Department of Agronomy, 2104 Agronomy Hall, Iowa State University, Ames, IA 50011.

[3] Department of Plant Pathology, 351 Bessey Hall, Iowa State University, Ames, IA 50011.

E-mail: mdowen@iastate.edu

sities than hand-weeded plots (Browde et al., 1994). Postemergence applications of acifluorfen, bentazon, lactofen [(±)-2-ethoxy-1-methyl-2-oxoethyl 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate], crop oil concentrate (COC) (paraffin base petroleum oil, polyol fatty acid esters, and their polyethoxylated derivatives [83:17 by vol]), and NIS (a mixture of alkylarylpolyoxyethylene, glycols, free fatty acids, and isopropanol) reduced *H. glycines* population densities at 4 and 8 weeks after application (WAA) (Levene et al., 1998). The mechanism by which these herbicides were effective against *H. glycines* development was proposed to be plant-mediated.

The objectives of this research were to determine if root exudates from herbicide-treated soybean plants affected the rate of hatching of *H. glycines,* and to measure the hatching potential of *H. glycines* eggs that developed on herbicide-treated soybean plants.

Materials and Methods

Collection of root exudates: Concurrent studies were conducted with pasteurized and *H. glycines*-infested potting material to determine *H. glycines*- and herbicide-induced changes in root exudate solutions (RES), as exhibited by changes in *H. glycines* hatching. The studies were conducted in the greenhouse with 'Corsoy 79' soybean, an *H. glycines*-susceptible cultivar, planted into 15-cm-diam. plastic pots. Each pot contained 2,500 cm^3 of a potting material consisting of equal parts of sand and Canisteo clay loam soil (fine loamy, mixed [calcareous], mesic Typic Haplaquolls). The *H. glycines*-infested potting material contained approximately 5,000 *H. glycines* race 3 eggs/100 cm^3. At 1 week after planting, all pots were thinned to six soybean plants at the VC stage of development (Fehr et al., 1971). Plants were watered daily and fertilized weekly with a commercial 20-20-20 (N-P-K) fertilizer for optimal soybean growth and development. High-pressure sodium lights supplemented natural lighting with approximately 300 $\mu M \cdot s^{-1} \cdot m^{-2}$ photosynthetic photon flux density for a photoperiod of 15 hours.

Herbicides and adjuvants were applied to foliage at normal field application rates 5 weeks after planting, when soybean plants were at the V5 stage of development. Foliar treatments included: i) the herbicide acifluorfen at a rate equivalent to 0.56 kg/ha plus the nonionic surfactant X-77 (Valent U.S.A., Walnut Creek, CA), ii) the herbicide bentazon at 1.12 kg/ha plus Prime Oil (COC) (Riverside/Terra, Sioux City, IA), iii) the herbicide lactofen at 0.46 kg/ha plus COC, iv) COC alone, v) X-77 alone, vi) no herbicide or adjuvant. Whenever used, COC and X-77 were applied at rates equivalent to 1.34 and 0.24 liters/ha, respectively. The application timing was chosen because RES from 5-week-old soybean plants has enhanced *H. glycines* hatch (Schmitt and Riggs, 1991). The herbicide and adjuvant treatments used are known to reduce *H. glycines* reproduction on soybeans (Levene et al., 1998). All treatments were applied in a CO_2-powered spray chamber delivering 234 liters/ha at 245 kPa. After approximately 1 hour, when the treatments had dried on the leaves, soybean plants were carefully removed from the potting material and the roots were washed in tap water. Plants from the untreated controls were handled similarly. Three intact plants were placed into a 250-ml flask containing 200 ml of deionized water and returned to the greenhouse for 48 hours. Flasks were covered with aluminum foil to limit root exposure to sunlight. The RES was passed through four layers of cheesecloth to remove particulate matter, RES volume was adjusted to 210 ml with deionized water, and 20 ml of the solution was used to establish an *H. glycines* hatching bioassay. The remaining RES was frozen until needed, based on the knowledge that freezing preserves effects of RES in juvenile behavior assays (Papademetriou and Bone, 1983).

Hatching of H. glycines eggs in root exudates: Hatching bioassays were established on the same day as collection of RES. Eggs were collected from adult *H. glycines* females and cysts on roots of untreated, 30-day-old soybeans (Niblack et al., 1993) and surface-sterilized with 0.5% chlorhexidine diacetate

for 15 minutes, then rinsed repeatedly with sterile water (Acedo and Dropkin, 1982). Each bioassay consisted of 8,000 eggs placed on a 2-cm circular nylon screen (38-μm pores) (Wong et al., 1993); the screen was placed into a 3- × 8- × 1-cm plastic tray that contained 10 ml of fresh RES or a control solution. The control solutions were deionized water and 0.003 M $ZnSO_4$, both adjusted to pH 7.0. Deionized water permits and $ZnSO_4$ solution stimulates egg hatch of *H. glycines* (Clarke and Shepherd, 1966). Trays for each replication were placed into a single plastic storage box, and all storage boxes were incubated at 25 °C in complete darkness. The hatching screens were transferred to trays containing identical fresh solutions every 3 days over a 21-day period. The J2 that hatched and passed through the nylon screen into the solution in the tray were counted following each transfer. After 21 days, the remaining unhatched eggs and J2 in each sieve were counted and percent hatch was calculated. Each RES was tested as nonsterile extracts and also was filtered through a 0.22-μm-pore filter. Fresh RES was thawed from frozen samples 24 hours before use. Screens, trays, and storage boxes were washed and surface-sterilized with ultraviolet light before use.

Concurrent RES studies were conducted with a randomized split-plot design with *H. glycines* presence or absence in the potting material as the main plot and herbicide treatment and exudate filtration as the split treatments. All treatments were replicated five times, the studies were repeated, and the data were combined for analysis. Data were subjected to an analysis of variance (ANOVA). When significant differences among treatments were detected with ANOVA ($P \leq 0.05$), means were separated with Fisher's least-significant-difference (LSD) test ($P = 0.05$) (SAS Institute, Cary, NC).

Hatching of H. glycines eggs from herbicide-treated plants: Soybean plants were grown in *H. glycines*-infested potting material as described previously. The same herbicide treatments described for the RES experiments were applied 3 weeks after planting, and soybeans were maintained in the greenhouse 1 more week. Plants then were removed from the potting material, and a stream of water was used to dislodge newly developed *H. glycines* females from the plant roots. The females were collected on a 250-μm-pore sieve and combined by treatment; their eggs were extracted (Niblack et al., 1993), surface-sterilized (Acedo and Dropkin, 1982), and used to establish a hatching bioassay. Eggs from each treatment were incubated in deionized water or 0.003 M $ZnSO_4$ during a 21-day incubation period, and egg hatching was assessed as described above. Viability of unhatched eggs at the end of the incubation period was not determined. Experiments were conducted as randomized, complete-block designs. All treatments were replicated five times, the study was repeated, and data were combined for analysis. Data were analyzed as described above.

Results and Discussion

Hatching of H. glycines eggs in root exudates: RES from soybean plants infected and uninfected with *H. glycines* stimulated egg hatching similarly, 17.4 and 20.0%, respectively, after 21 days (LSD = 2.8%). Therefore, the data were combined and analyzed as a randomized complete block, with herbicide treatment and exudate sterilization comprising the treatments.

Filtered RES had greater *H. glycines* hatch than the unfiltered RES over the 21-day incubation period (Fig. 1). Since filtering at 0.22 μm presumably removed bacteria and fungi, the hatch-stimulating factor in RES likely was not from bacterial or fungal contaminants. It is possible that there was no stimulatory effect of the RES and that contaminants in the unfiltered RES may have inhibited hatch.

The rate of *H. glycines* hatching was greatest during the initial 15 days of incubation for all solutions tested (Fig. 2). After 6 days, all RES stimulated hatching more than deionized water, but less than the $ZnSO_4$ solution. However, for all herbicide-RES treatments, *H. glycines* hatching was similar

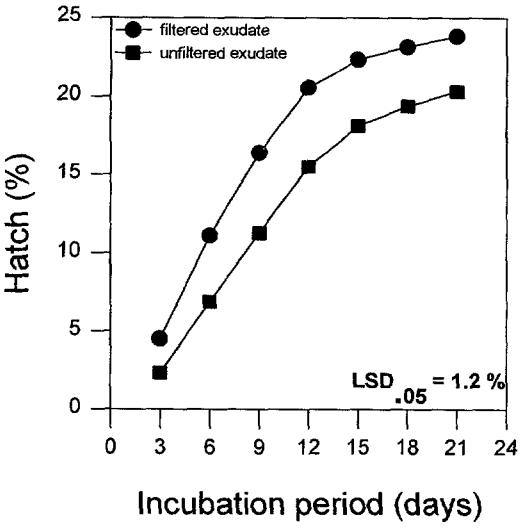

FIG. 1. Effects of filtering (0.22 μm) of soybean root exudate solution (RES) on hatch of *Heterodera glycines*. RES was collected 35 days after planting. Each data point is the overall mean of 120 observations from five replications of six herbicide and two nematode inoculation treatments in each of two complete factorial experiments. The LSD is for comparison of means between incubation periods for either filtered or unfiltered RES or for comparison between filtered and unfiltered RES solution means for any incubation period ($P = 0.05$).

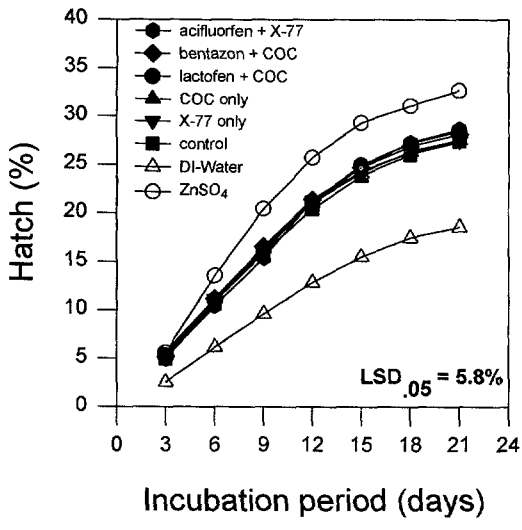

FIG. 2. Effects of incubation solutions on hatch of *Heterodera glycines*. Eggs were incubated in deionized water (DI-water), 0.003 M $ZnSO_4$, or one of the six combinations of three herbicides (acifluorfen, bentazon, and lactofen) and two adjuvants (crop oil concentrate, COC, or the nonionic surfactant X-77). Each data point is the overall mean of 40 observations from five replications of two root exudate solution filtration and two nematode inoculation treatments in each of two complete factorial experiments. The LSD is for comparison of means between incubation periods for either incubation solution or for comparison of incubation solution means for any incubation period ($P = 0.05$).

over the 21-day period of incubation, and the number of J2 remaining on the screen at 21 days was similar. Therefore, no treatment reduced *H. glycines* hatching relative to deionized water. This result suggests that the reduction in *H. glycines* reproduction on herbicide-treated soybeans observed by Levene et al. (1998) was not due to changes in root exudates following herbicide application. The parent molecules of the herbicide formulations included in the experiments are not highly mobile in plants, making any direct effect on nematodes unlikely (Humburg, 1989). However, RES were collected for only 2 days after application; movement of herbicide metabolites or production of natural compounds requiring more than 2 days to accumulate might have reduced *H. glycines* reproduction if RES had been collected for a longer period of time.

Hatching of H. glycines eggs from herbicide-treated plants: Hatching of *H. glycines* eggs in $ZnSO_4$ solution was greater than in deionized water, when averaged across herbicide treatments (Fig. 3A). Some herbicide treatments also influenced *H. glycines* hatching when data for both incubation solutions were averaged (Fig. 3B). No interaction between herbicide treatment and incubation solution was detected. A lower percentage of eggs from acifluorfen- and bentazon-treated plants than from control plants hatched when averaged for both incubation solutions. Browde et al. (1994) reported lower *H. glycines* population densities in field plots treated with a mixture of acifluorfen and bentazon than in hand-weeded control plots. Eggs from nonionic surfactant- and lactofen-treated soybean plants had lower hatching percentages than those from control plants after 9 days. Eggs from crop oil concentrate-treated plants had greater hatch than those from control and bentazon-treated plants initially; however, values were similar after 6 days. Incubating eggs from acifluorfen- and lactofen-treated plants

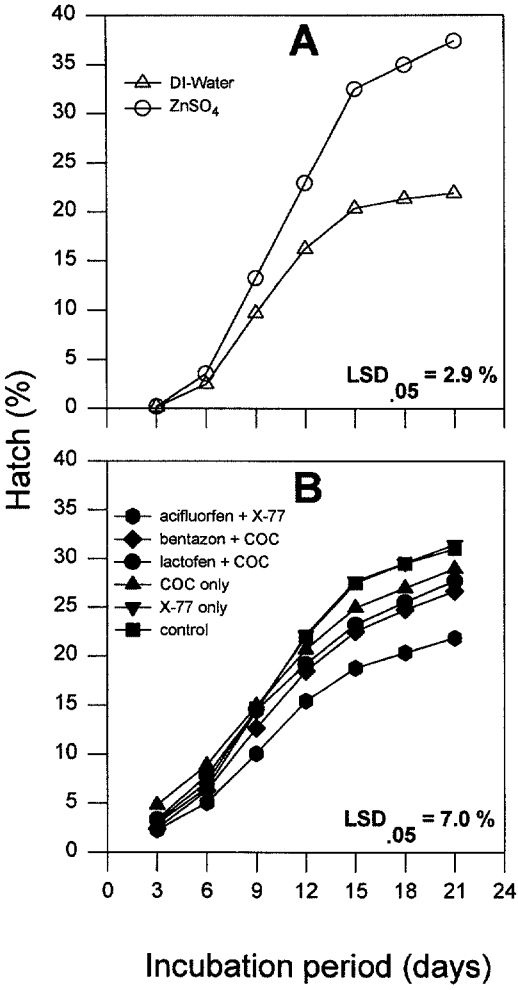

FIG. 3. Effect of application of three herbicides (acifluorfen, bentazon, and lactofen) and two adjuvants (crop oil concentrate, COC, or the nonionic surfactant X-77) on hatch of *Heterodera glycines* eggs collected from females and cysts developing on treated plants. Eggs were collected 28 days after planting and 7 days after herbicide application and were incubated in either deionized water (DI-water) or 0.003 M $ZnSO_4$. A) Effect of incubation solution on hatch. Each data point is the mean of 60 observations from five replications of the six herbicide treatment levels in two experiments. B) Effect of herbicide treatment on hatch. Each data point is the mean of 20 observations from five replications of both incubation solutions in two experiments. The LSD is for comparison of means between incubation periods for any incubation solution (A) or herbicide treatment (B) or for comparison of means between incubation solutions (A) or herbicide treatments (B) for any individual incubation period ($P = 0.05$).

in $ZnSO_4$ solution did not improve hatching to a level similar to the untreated control.

The RES from herbicide-treated and untreated soybeans in our study stimulated *H. glycines* hatching similarly. Furthermore, movement of J2 in RES probably was not affected by the herbicide treatments; the numbers of J2 remaining on the hatching screens at the end of the experiments were an indirect measure of J2 movement. Hatching potential, however, was reduced when plants on which eggs developed were treated with herbicides. Therefore, the herbicide treatments tested did not appear to inhibit egg hatching or restrict J2 movement. Nematode development within the roots of herbicide-treated soybean plants may have been affected, but the cause of lowered hatching potential was not determined. The active herbicide ingredient, metabolites, formulation products in the herbicide, and secondary plant metabolites formed after herbicide application each could have affected the hatching potential of *H. glycines* eggs collected from treated soybean plants.

LITERATURE CITED

Acedo, J. R., and V. H. Dropkin. 1982. Techniques for obtaining eggs and juveniles of *Heterodera glycines*. Journal of Nematology 14:418–420.

Bostian, A. L., D. P. Schmitt, and K. R. Barker. 1986. Population changes of *Heterodera glycines* and soybean yields resulting from soil treatment with alachlor, fenamiphos, and ethoprop. Journal of Nematology 18: 458–463.

Browde, J. A., G. L. Tylka, L. P. Pedigo, and M. D. K. Owen. 1994. Response of *Heterodera glycines* populations to a postemergence herbicide mixture and simulated insect defoliation. Journal of Nematology 26:498–504.

Clarke, A. J., and A. M. Shepherd. 1966. Inorganic ions and the hatching of *Heterodera* spp. Annals of Applied Biology 58:497–508.

Fedorko, A., M. Kamionek, J. Kozlowska, and E. Mianowska. 1977. The effects of some carbamide herbicides on nematodes from different ecological groups. Polish Ecological Studies 3:23–28.

Fehr, W. R., C. E. Caviness, D. T. Burmood, and J. S. Pennington. 1971. Stages of development descriptions for soybean, *Glycine max* (L.) Merrill. Crop Science 11: 929–931.

Humburg, N. E., ed. 1989. Herbicide handbook of the Weed Science Society of America. Champaign, IL: Weed Science Society of America.

Levene, B. C., M. D. K. Owen, and G. L. Tylka. 1998. Response of soybean cyst nematode to herbicides on soybeans. Weed Science 46:264–270.

Niblack, T. L., R. D. Heinz, G. S. Smith, and P. A. Donald. 1993. Distribution, density, and diversity of *Het-*

erodera glycines in Missouri. Supplement to the Journal of Nematology 25:880–886.

Oji, O., V. T. Sapra, and R. P. Pacumbaba. 1988. Effects of nematicide and herbicide interactions on field populations of cyst nematode and agronomic characters of soybean. Journal of Agronomy and Crop Science 160:309–313.

Papademetriou, M. K., and L. W. Bone. 1983. Chemotaxis of larval soybean cyst nematode, *Heterodera glycines* race 3, to root leachates and ions. Journal of Chemical Ecology 9:387–396.

Payan, L. A., A. W. Johnson, and R. H. Littrell. 1987. Effects of nematicides and herbicides alone or combined on *Meloidogyne incognita* egg hatch and development. Annals of Applied Nematology (Supplement to the Journal of Nematology 19) 1:67–70.

Perry, R. N., and J. Beane. 1989. Effects of certain herbicides on the in vitro hatch of *Globodera rostochiensis* and *Heterodera schachtii*. Revue de Nématologie 12:191–196.

Schmitt, D. P., and R. D. Riggs. 1991. Influence of selected plant species on hatching of eggs and development of juveniles of *Heterodera glycines*. Journal of Nematology 23:1–6.

Sipes, B. S., and D. P. Schmitt. 1989. Development of *Heterodera glycines* as affected by alachlor and fenamiphos. Journal of Nematology 21:24–32.

Wong, A. T. S., G. L. Tylka, and R. G. Hartzler, 1993. Effect of eight herbicides on the in vitro hatching of *Heterodera glycines*. Journal of Nematology 25:578–584.

Youmans, C. D. 1986. Herbicidal effects on the soybean cyst nematode (*Heterodera glycines*). Ph.D. dissertation, University of Arkansas, Fayetteville, AR.