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Responses of *Heterodera glycines* Populations to a Postemergence Herbicide Mixture and Simulated Insect Defoliation[1]

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Abstract: Effects of a mixture of the postemergence herbicides acifluorfen and bentazon, and simulated defoliation expected from green cloverworm on population densities of *Heterodera glycines* were determined in field plots in Iowa. The herbicide mixture and defoliation each suppressed soybean growth. Population densities of *H. glycines* were generally lower in herbicide-treated than untreated plots. Population densities of the nematode were unaffected by defoliation in 1988 and 1990–91, but were increased by the treatment in 1989.
Key words: acifluorfen, bentazon, defoliation, *Glycine max*, green cloverworm, herbicide, *Heterodera glycines*, nematode, *Plathypena scabra*, postemergence herbicides, soybean, soybean cyst nematode.

The soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is an important pest of soybean, *Glycine max* (L.) Merr., in the United States. The nematode occurs throughout most of the major soybean producing states of the United States and is becoming an increasing problem (14,32). Soybean yield losses due to SCN can be extensive, with losses negatively correlated with population densities in soil at planting (7,19,27). Additional factors may impact the yield–loss relationships, particularly SCN race, edaphic factors, and other pests (2,23,27).

Numerous abiotic and biotic environmental variables affect SCN population dynamics. Important abiotic factors include soil moisture and temperature (25). The most frequently studied biotic factors are host characteristics, including root growth, developmental stage, and susceptibility to injury (20,25).

Recent studies have investigated interactions among SCN and other soybean pests. Alston et al. (2) and Russin et al. (23) found that SCN population densities increased after insect defoliation of soybean. In contrast, population densities decreased after soybean infection with the stem canker fungus, *Diaporthe phaseolorum* (Cke and Ell.) Sac. var. *caulivora* Athow and Caldwell (23). Weed-stressed soybean harbored fewer SCN than weed-free plants in some fields in eastern North Carolina (2).

Agricultural chemicals can influence SCN population dynamics. Nitrate fertilizer is toxic to SCN at application rates higher than those used in crop production (21). At agriculturally acceptable rates of fertilization, however, population densities generally increase more rapidly than those in unfertilized soil. This stimulative effect on SCN populations is likely indirect, probably related to enhanced root growth following fertilization (12,21).

Pesticides may impact SCN population densities. Nematicides reduce early season numbers of nematodes in soybean by delaying root infection or inhibiting nematode development after infection (29,33). Nontarget effects of soil-applied herbicides on SCN have been noted. Preplant applications of alachlor (4) or trifluralin (18) increase SCN population densities. Antagonism between alachlor and the nematicide fenamiphos reduces fenamiphos efficacy (28), while metribuzin enhances control by aldicarb (26).

Weeds and defoliating insects can limit soybean production. Although weed man-

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agement programs for soybean may include several control tactics, herbicides remain the key one (13). Postemergence applications of acifluorfen plus bentazon herbicides are used commonly for controlling broadleaf weeds in soybean (30). In Iowa (16), applications are recommended during early soybean development, specifically between V3 (third trifoliate leaf) and V6 (sixth trifoliate leaf) growth stages (6). Soybean can sustain considerable injury from these herbicides (16). Both acifluorfen and bentazon disrupt cell membranes, leading to foliar wilting, chlorosis, and necrosis (30). Additionally, several defoliating insects of soybean in the midwestern United States, particularly the green cloverworm (GCW), *Plathypena scabra* F., can cause extensive injury (8). Economically damaging populations of GCW occur during years of excessive spring moth migration from southern states (17).

Determining the effects of multiple stress factors, including pest management tactics, on target and nontarget pest populations is important for understanding pest population dynamics and for improving pest management decisionmaking and practices. Therefore, research was conducted during 1988–91 to quantify the effects of an acifluorfen and bentazon herbicide mixture and simulated GCW defoliation on SCN population densities.

Materials and Methods

Plot establishment and experimental design: In 1988 and 1989, plots were established in a field (cropped to soybean from 1985–87) at the Iowa State Agronomy Farm on a Harps soil (fine-loamy, mesic Typic Calciaquolls; OM = 5.6%; pH = 7.9) in Boone County, Iowa. In 1990 and 1991, plots were located in fields at the Johnson Farm (leased by Iowa State University) on Webster soils (fine-loamy, mixed, mesic Typic Haplaquolls; OM = 4.5%; pH = 7.2) in Story County, Iowa. The field used in 1990 was cropped to corn in 1989 and soybean in 1988. Another field was used in 1991; its crop history was corn in 1990 and soybean in 1989. Soybean cultivars susceptible to SCN were planted all years in rows spaced 0.76 m apart at 85 kg of seed/ha. Soybean 'BSR 201' was planted on 26 May 1988 and 'Asgrow 2187' on 17 May 1989, 31 May 1990, and 7 June 1991. After the seedlings emerged, they were thinned to 25 plants/m row.

Methods for establishing SCN population densities changed during this study; consequently, three experimental designs were used. Randomized complete block (four blocks) and completely random (four replicates) designs were used in 1988 and 1989, respectively. In 1990 and 1991, a randomized complete block with split-plots design (four blocks) was used. Treatments were combinations of two population densities of SCN race 3, three (1988 and 1989) or two (1990 and 1991) rates of acifluorfen plus bentazon, and four levels of simulated GCW defoliation. Treatments were factorially arranged in 1988 and 1989. SCN population density constituted whole-plot treatments in 1990 and 1991. Subplots were assigned factorial combinations of herbicide rate and defoliation level. In 1988 and 1989, plots were four rows wide and 5 m long (1988) or 4 m long (1989). For both 1990 and 1991, subplots were four rows wide and 3 m long. Data were collected from the two center rows. In 1990 and 1991, the eight subplots within a whole plot were separated from the adjacent whole plot by four border rows.

Nematode treatments: Two levels of SCN population density were desired each year. Aldicarb was applied at 3.4 kg a.i./ha in 1988 in an effort to achieve a low Pi. Since the desired levels were not attained, the procedure to obtain distinct population densities was changed in 1989. Five days after planting (22 May), the field was divided into a matrix of 216, 4-row wide × 4-m-long plots. Ten 2.5-cm-d, 20-cm-deep soil cores were collected systematically from the two middle rows of each plot. The 10 soil cores were combined and mixed. Cysts were extracted by sieving (31) from a 100-cm^3 aliquot of soil. These cysts were crushed to release eggs (3). Plots then were categorized (1), and 48 medium Pi plots with 1,006 eggs (SD = 358) and 48 high Pi plots with 4,333 eggs (SD = 1,121) were selected for the experiment. For the 1990 and 1991 studies, 184 eggs/100 cm^3 soil (SD = 136) and 100 eggs/100 cm^3 soil (SD = 105), respectively, were added to the plots (5).

Herbicide treatments: All plots were treated with either a preplant-incorporated application of 1.12 kg a.i. trifluralin/ha (1988) or 2.8 kg a.i. metolachlor/ha (preplant-incorporated in 1989, preemergence in 1990 and 1991) to control weeds. Weeds that emerged after planting were removed by hand. The treatments consisting of acifluorfen plus bentazon (kg a.i./ha) were as follows: 1988—1) 0.14 + 0.84, 2) 0.56 + 0.84, and 3) 0 (applied 27 days after soybean emergence); 1989—1) 0.28 + 0.56, 2) 0.56 + 0.84, and 3) 0 (applied 38 days after soybean emergence); 1990–1991—1) 0.56 + 0.84, and 2) 0 (applied 39 days after soybean emergence in 1990 and 29 days after soybean emergence in 1991). A nonionic surfactant (X-77; Ortho Chemical) was included in all applications (0.5% v/v).

Defoliation treatments: Numbers of soybean leaflets removed to simulate GCW feeding rates were determined with a computer program (L. G. Higley, unpubl.) based on inputs of daily low and high temperatures. The program incorporated models for GCW consumption (9) and development (10). Defoliation treatments were initiated at the R2 growth-stage (full bloom) and concluded at the R4 growth-stage (full pod) (6), coinciding with the characteristic phenology of first-generation GCW larvae in Iowa (17). Specific defoliation periods were 18 to 29 July 1988, 17 to 28 July 1989, 23 July to 3 August 1990, and 22 July to 2 August 1991. Feeding by GCW was simulated spatially by removing leaflets by hand at daily intervals from upper canopy strata (15) of the two middle rows. On days 8 and 12 of defoliation, the two border rows of plants were defoliated by beating them with a strong upward stroking motion of the hands to approximate defoliation levels in middle rows. The desired simulated injury levels corresponded to that expected for 0, 6, 12, and 24 GCW/m row in 1988 and 0, 9, 18, and 36 in 1989. One GCW larva will consume about 54 cm^2 (9) during development. The range of simulated injury was increased in 1990 and 1991 to 0, 18, 72, and 144 GCW/m row. Plant handling and plot traffic in nondefoliated and defoliated plots were similar. No efforts were made to control natural defoliators because they occurred in low population densities and because of potential interference with treatments.

Nematode and soybean responses: Population densities of SCN egg (3) and second-stage juvenile (J2) (11) were sampled at 33 and 69 days after planting (DAP) in 1988; 5, 37, and 82 DAP in 1989; 4, 46, 90, and 123 DAP in 1990; and 1, 33, 76, and 117 DAP in 1991. Soybean responses to herbicides and defoliation also were assessed. Evaluations of crop injury from herbicides were made 7 (12 July 1989 and 18 July 1991) or 8 days (11 July 1988 and 25 July 1990) after applications. A visual rating (0 to 100% scale; 0 = no symptoms, 100 = plant death) of foliar chlorosis and necrosis was made for each plot. Leaf area index (LAI; ratio of leaf to ground area) was assessed immediately after defoliation in 1989, 1990, and 1991. Leaf area indices were calculated from four (1989) or three (1990 and 1991) plants from the two middle rows of each plot on 31 July 1989, 6 August 1990, and 6 August 1991.

Data analysis: Data for 1988 and 1989 were analyzed by year because of unique levels and arrangements of treatments. In contrast, identical herbicide rates and defoliation levels were applied to plots with artificially infested SCN populations in 1990 and 1991. Data for these years were pooled for analysis because no treatment interactions with year were detected. Egg and J2 counts of SCN were transformed to $\log_{10}(x + 1)$ before analysis (x = number of eggs or J2). Data were subjected to analyses of variance (24) for partitioning sums of squares among factors and interactions, and for testing effects having single degrees of freedom for significance ($P \leqslant$

0.05). Significant effects of other factors and interactions were isolated by orthogonal comparisons. Because no interaction terms were significant for SCN counts, data for nematode, herbicide, and defoliation treatments were averaged across levels of other treatments.

Results

Nematode treatments: In 1988, differences in SCN population densities between plots treated and untreated with aldicarb were not significant for numbers of eggs or J2 at 33 DAP or for eggs at 69 DAP. Second-stage juvenile population densities were greater for aldicarb-treated plots at 69 DAP. In 1989, egg and J2 population densities were lower in medium compared to high Pi plots at 5 and 37 DAP, although differences were not significant at 82 DAP. Low population densities of SCN were detected in 23% of noninfested plots by soybean harvest in 1990–91 (5). However, egg and J2 densities were higher in infested plots at each sampling date.

Herbicide treatments: Applications of acifluorfen plus bentazon resulted in soybean injury (Tables 1,2). Marked gradients in crop injury among herbicide treatments occurred in all years.

Herbicide treatments, regardless of rate, were associated with lower SCN population densities in soil than in untreated plots (Tables 1–2). Reductions in eggs were significant at 69 DAP ($P = 0.03$; 35% reduction) in 1988 and at soybean harvest ($P = 0.05$; 11% reduction) for 1990 (123 DAP) and 1991 (117 DAP) pooled data. Herbicide applications caused a 33% reduction ($P = 0.03$) in J2 densities at 69 DAP in 1988 and a 17% reduction ($P = 0.06$) at 82 DAP in 1989.

Defoliation treatments: Increasing levels of defoliation caused linear reductions in leaf area indices (LAI) (Tables 1,2). Averages

TABLE 1. Effects of acifluorfen plus bentazon (Herb) and simulated green cloverworm defoliation (Defol) on *Heterodera glycines* egg and second-stage juvenile (J2) population densities/100 cm^3 soil for 1988 and 1989.†

Treatment	Crop response‡ 1988	Crop response‡ 1989	Egg 1988	Egg 1989	J2 1988	J2 1989
Herb§						
None	0%	0%	1,438	5,188	83	256
Low	10%	9%	861	4,472	52	196
High	14%	19%	1,006	4,841	59	227
F (df)	9.34 (1,57)	67.06 (1,48)	2.63 (2,68)	0.38 (2,72)	2.64 (2,65)	2.49 (2,72)
$P > F$	0.00	0.00	0.08	0.69	0.08	0.09
Contrast F, $P > F$						
None vs. low + high			4.75, 0.03	0.74, 0.39	4.78, 0.03	3.54, 0.06
Low vs. high	9.34, 0.00	67.06, 0.00	0.55, 0.46	0.01, 0.91	0.57, 0.45	1.44, 0.23
Defol‖						
None		3.57	1,035	3,933	70	224
Low		3.08	1,250	4,817	67	234
Med		2.97	1,038	5,625	57	216
High		2.95	1,092	4,958	65	231
F (df)		3.60 (3,72)	0.54 (3,68)	1.72 (3,72)	0.73 (3,65)	0.19 (3,72)
$P > F$		0.02	0.66	0.17	0.54	0.90
Contrast F, $P > F$						
Linear		6.82, 0.02	0.04, 0.84	0.64, 0.42	0.15, 0.70	0.00, 0.94
Quadratic		3.63, 0.06	0.06, 0.81	4.22, 0.04	1.74, 0.19	0.00, 0.95

† Data were $\log_{10}(x + 1)$ transformed for analyses, but untransformed means are reported. Soils were sampled at 69 days after planting, 31 days after herbicide application, and 5 days after defoliation in 1988; and 82 days after planting, 33 days after herbicide application, and 10 days after defoliation in 1989.

‡ Visual rating of percent foliar injury and leaf area index for herbicide and defoliation treatments, respectively. Herbicide-untreated plots were not included in analyses of visual herbicide injury.

§ 1988: low = 0.14 kg a.i./ha acifluorfen + 0.84 kg a.i./ha bentazon, high = 0.56 kg a.i./ha acifluorfen + 0.84 kg a.i./ha bentazon; 1989: low = 0.28 kg a.i./ha acifluorfen + 0.56 kg a.i./ha bentazon, high = 0.56 kg a.i./ha acifluorfen + 0.84 kg a.i./ha bentazon.

‖ None, low, med, and high levels simulated based on 0, 6, 12, and 24 green cloverworms/m row in 1988; and 0, 9, 18, and 36 green cloverworms/m row in 1989.

TABLE 2. Effects of acifluorfen plus bentazon (Herb) and simulated green cloverworm defoliation (Defol) on *Heterodera glycines* egg and second-stage juvenile (J2) population densities/100 cm^3 soil in artificially infested plots before and at soybean harvest in 1990–91.†

Treatment	Crop response‡	Midseason Egg	Midseason J2	Harvest Egg	Harvest J2
Herb§					
None	0%	138	9	346	15
High	24%	105	8	309	20
F (df)		0.91 (1,49)	1.06 (1,49)	3.95 (1,49)	0.90 (1,49)
$P > F$		0.35	0.31	0.05	0.35
Defol‖					
None	4.30	128	10	269	19
Low	4.03	95	9	344	14
Med	3.56	98	7	381	21
High	3.34	162	8	317	16
F (df)	7.55 (3,98)	0.18 (3,49)	0.36 (3,49)	0.09 (3,49)	0.74 (3,49)
$P > F$	0.00	0.91	0.78	0.96	0.53
Contrast *F*, $P > F$					
Linear	20.82, 0.00	0.02, 0.89	0.75, 0.39	0.05, 0.82	0.22, 0.64
Quadratic	1.81, 0.18	0.00, 0.99	0.28, 0.60	0.22, 0.64	1.74, 0.19

† Data were $\log_{10}(x + 1)$ transformed for analyses but untransformed means are reported. Midseason soils were sampled at 90 days after planting, 43 days after herbicide application, and 26 days after defoliation in 1990; and 76 days after planting, 42 days after herbicide application, and 20 days after defoliation in 1991. Harvest soil samples for nematode assay were collected at 123 days after planting, 76 days after herbicide application, and 59 days after defoliation in 1990; and 117 days after planting, 83 days after herbicide application, and 61 days after defoliation in 1991.

‡ Visual rating of percent foliar injury and leaf area index for herbicide and defoliation treatments, respectively; average of data from SCN-infested and noninfested plots. Herbicide-untreated plots were not included in analyses of visual herbicide injury.

§ High = 0.56 kg a.i./ha acifluorfen + 0.84 kg a.i./ha bentazon.

‖ None, low, med, and high levels simulated based on 0, 18, 72, and 144 green cloverworms/m row.

from plots with the highest level of defoliation were reduced by 17% in 1989 and by 22% in 1990–91.

Defoliation impacts on SCN egg and J2 population densities in soil were not significant in 1988 (Table 1) or 1990–91 (Table 2). There was a quadratic relationship between defoliation and egg population densities in 1989 (Table 1). Egg population densities increased with defoliation up to 18 GCW equivalents/m of row then decreased.

DISCUSSION

The indication that the herbicides and defoliation studied impact SCN populations is important. Many biotic and abiotic factors, including agricultural chemicals, affect the population dynamics of SCN (25). Other researchers have noted nontarget effects of soil applications of fertilizers (12,21) and herbicides (4,18,26,28,29). As indicated previously, population densities of SCN increase in response to fertilizers (12,21) and the herbicides alachlor (4) and trifluralin (18) applied at field rates. Further, alachlor antagonizes the nematicidal activity of fenamiphos (28), while metribuzin increases nematode control by aldicarb (26).

Postemergence applications of herbicides can also impact the population dynamics of SCN. This study indicates that mixtures of acifluorfen plus bentazon, applied postemergence, reduce SCN egg and J2 population densities. Mechanisms underlying the reductions in SCN population densities by acifluorfen plus bentazon were not determined. The soil applied herbicides alachlor (4) and trifluralin (18) affect SCN directly by increasing egg hatch (alachlor and trifluralin) and root penetration by infective J2 (trifluralin). In contrast, the effect of this postemergence herbicide mix is probably indirect, i.e., a consequence of altered soybean physiology. Acifluorfen and bentazon translocate minimally in soybean and bind rapidly to soil colloids (22). Direct contact of parent chemicals with nematodes in soil or roots, therefore, is unlikely. Herbicide applications may alter the type or release of root exudates, affecting the host-finding behavior of SCN, or cause the production of toxic metabolites. Most likely, however, herbicide injury may limit root growth, providing fewer sites for nematode feeding. Decreases in SCN population densities from other soybean stress factors, including drought and weeds (2) and some preemergence herbicides (26), may be attributed to reductions in general host fitness. The mechanism needs to be determined, especially because of economic impacts (2).

Although simulated insect defoliation increased SCN egg population densities in 1989 only, this result agrees with the research of others (2,23). Studying defoliation by corn earworm, *Helicoverpa zea* Boddie, Alston et al. (2) attributed greater numbers of SCN eggs in soil at harvest for defoliated soybean to delayed plant maturity. The resultant extended period of vegetative growth enabled SCN to continue reproduction. Russin et al. (23) attributed increased egg production on soybean defoliated by soybean looper, *Pseudoplusia includens* Walker, to a greater partitioning of photosynthate to roots. The reason that defoliation increased egg densities in this study is unknown. Since the increase in egg numbers in soil occurred within 10 days after terminating defoliation, an altered partitioning of photosynthate to roots is a plausible explanation.

Understanding and quantifying interactions between SCN and frequently encountered stress factors is important for pest management. Alterations in SCN population densities by herbicides or defoliating insects could influence the selection and (or) timing of pest-control tactics. Major commitments to interdisciplinary research will be needed for improving and sustaining pest management.

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