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Biological interactions among soybean cyst nematode, herbicide injury, and insect defoliation in soybean

Browde, Joseph Arthur, Jr., Ph.D.

Iowa State University, 1993



Biological interactions among soybean cyst nematode, herbicide injury, and insect defoliation in soybean

by

Joseph Arthur Browde, Jr.

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

> Department: Entomology Major: Entomology

Approved:

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In Charge of Major Work ()

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ABSTRACT

Field experiments were conducted during 1988-1991 for quantifying interactions among soybean, <u>Glycine max</u>, stresses from soybean cyst nematode (SCN), Heterodera glycines, acifluorfen plus bentazon herbicides, and green cloverworm SCN and (GCW), <u>Plathypena</u> <u>scabra</u> (Lepidoptera: Noctuidae). soybean responses were evaluated. Treatments were combinations of SCN density, acifluorfen plus bentazon rate, and simulated GCW defoliation level. A method developed for infesting soil with an SCN-root-soil inoculum was used in 1990 and 1991. Herbicide applications and defoliation were imposed at V6 and R2 to R4 stages of soybean development, respectively. Herbicides always stressed soybean, as indicated by visual evaluations of foliar injury. Likewise, defoliation reduced soybean leaf area in 1989, 1990, and 1991. No determinations of leaf area were made in 1988. Herbicides consistently decreased SCN soil densities, although applications never increased soybean seed yield. SCN soil densities generally were unaffected by defoliation. Because of extreme confounding from iron deficiency chlorosis and drought in 1988, data for that year were not used for evaluations of soybean response. Herbicides and defoliation limited soybean growth and yield in 1990 and 1991. Excessive injury from SCN likely precluded similar responses in 1989. Preharvest growth parameters reduced by herbicides included

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leaf area, plant height, pod number, and leaf, pod, and support (stem and petiole) dry weights; while defoliation reduced plant height and leaf dry weight. Moreover, herbicides and defoliation interacted to reduce seed yield over 1990 and 1991. Yield reductions primarily were attributed to decreases in pod number (defoliation) and weight per seed (herbicides). When desired stress from SCN was achieved (1990), interaction with herbicides decreased leaf stomatal conductance, increased visual crop injury, and reduced preharvest growth (leaf area, leaf number, pod number, and pod weight) and seed yield. Additionally, SCN and defoliation interacted in 1990, reducing seed yield. Canopy interception of photosynthetically active radiation was a key mechanism underlying yield reductions from SCN, herbicides, and defoliation. Management recommendations, including adjusted economic decision levels for GCW, were made to deal with SCN x herbicides, SCN x defoliation, and herbicides x defoliation effects.

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GENERAL INTRODUCTION

Numerous biotic (pests) and abiotic stress factors limit crop production. Pest organisms of soybean, for instance, include various arthropods, weeds, nematodes, and pathogens (Hammond et al. 1991). Abiotic factors generally are natural stresses, such as heat and water stress (Raper and Kramer 1987). However, pest-control tactics, particularly herbicides (Owen and Hartzler 1993), also can cause abiotic stress. Many stress factors alone can cause enough injury to reduce crop growth and yield. Yet, single-stress scenarios seldom occur. Rather, multiple stresses that interact to affect crop productivity are common during each growing season .

Because crops usually are stressed by multiple factors during development, crop growth and yield is a result of the physiological integration of all stresses. Nevertheless, most researchers in pest disciplines have assessed impacts of single pests only (Higgins 1985). Consequently, specific host-pest relationships have been characterized, including the development of yield-loss models on which management decisions are based. A potentially invalid assumption of this singlestress approach, however, is that stresses act independently, i.e., no stress interactions (Higgins 1985, Newsom and Boethel 1985, Higley et al. 1993). By considering each stress separate from other concurrent and sequential stresses, models of crop responses, including yield losses, may be inaccurate.

Decision rules for pest suppression (e.g., economic decision levels) based on these models may be erroneous, reducing producer profits and, in some instances, decreasing environmental quality (Pedigo and Higley 1992). Interactions among stresses must be quantified to improve both understanding of crop-stress relationships and pest management.

Because of the great number of stress factors in each agroecosystem, it would be difficult to test all conceivable combinations for potential interactions. A logical approach, therefore, is to evaluate relationships among the most commonly encountered stress factors or those presumed to influence different processes of crop physiology (Poston et al. 1983, Higley et al. 1993).

Research Objectives

Research is proceeding towards more studies on interactive stresses, however, few projects are considering stress interactions across pest disciplines. Consequently, research was conducted during 1988-1991 for quantifying interactions among soybean stresses from soybean cyst nematode (SCN), <u>Heterodera glycines</u> Ichinohe, acifluorfen (5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid) plus bentazon [3-(1-methylethyl)-(1<u>H</u>)-2,1,3-benzothiadiazin-4(3<u>H</u>)-one 2,2dioxide] herbicides, and green cloverworm (GCW), <u>Plathypena</u>

<u>scabra</u> (F.). These stress factors of soybean in the midwestern United States represent frequently encountered stresses associated with nematodes, weeds, and insects.

Specific experimental objectives were:

- To refine and develop methodology for studying effects of multiple stresses in soybean production (Papers I, II, III, and IV).
- To evaluate effects of stresses from acifluorfen plus bentazon and simulated GCW defoliation on SCN populations (Paper II).
- 3) To quantify individual and interactive impacts of stresses from SCN, acifluorfen plus bentazon, and simulated GCW defoliation on soybean growth and yield (Papers III and IV).
- To use obtained yield-loss relationships for developing improved guidelines for nematode, weed, and insect management (Paper IV).

An Explanation of the Dissertation Organization

The contents of this thesis include four manuscripts prepared for publication in scholarly journals. Specifically, papers were formatted for publication in Agronomy Journal (Papers I, III, and IV) and Journal of Nematology (Paper II). The papers are preceded by a literature review and followed by sections for general conclusions, literature cited (for

introduction, literature review, and general conclusions), and acknowledgments.

Literature Review

Soybean Cyst Nematode

Soybean cyst nematode (SCN), <u>Heterodera</u> glycines Ichinohe, is a key pest of soybean in the United States. It is believed that SCN was introduced from Japan into the southern United States (Winstead et al. 1955), where it has been a serious problem for nearly 40 years. Recently, SCN has become an increasingly greater problem in the midwestern United States (Tylka and Sweets 1991, Niblack et al. 1992). Continuous generations of SCN develop in soybean fields each growing season, with a complete life cycle taking approximately 24 days at optimum temperatures. Because of short generational times, low SCN densities at planting can increase dramatically by the end of the growing season (Bonner and Schmitt 1985, Alston and Schmitt 1987). Moreover, SCN is quite persistent in soil, with eggs remaining viable in cysts for as long as 11 years (Inagaki and Tsutsumi 1971). Therefore, SCN problems occur suddenly and persist. Currently advocated control tactics for SCN include crop rotation, resistant cultivars, and nematicides. Nematicides are least recommended because of high costs and inconsistent efficacy (Riggs and Schmitt 1989, Schmitt and Riggs 1989, Tylka and .

Sweets 1991).

Injury by SCN can greatly impact soybean growth, development, and yield. SCN injures soybean by the penetration, cell destruction, and feeding of juvenile nematodes within roots (Dropkin 1989). This feeding and mechanical disruption results in discolored, necrotic root systems that lead to decreases in shoot growth and development (Alston and Schmitt 1987, Wrather and Anand 1988, Alston et al. 1991a, Niblack et al. 1992). Symptoms of injury also may include foliar chlorosis, although chlorosis most commonly occurs in the southern United States (Riggs and Schmitt 1987). Yield losses up to 100% can occur, with yields generally negatively correlated with soil densities of SCN at planting (Francl and Dropkin 1986, Riggs and Schmitt 1987, Schmitt et al. 1987).

Geographical differences in soil texture and SCN race influence symptoms of soybean injury. Typically, yield losses are greatest for coarse-textured soils (Koenning et al. 1988). Races 1 and 2, which are more prevalent in the southern United States, greatly reduce nodulation and nitrogen fixation (Barker et al. 1972). Deficiencies in plant nitrogen may cause the development of foliar chlorosis (Riggs and Schmitt 1987).

Postemergence Herbicides

For soybean, economic losses in yield and control costs associated with weeds likely exceed that for all other pest taxa combined (Jordan et al. 1987). Although crop rotations and cultural practices are included in many weed management programs for soybean, herbicides remain the key tactic for control. Most soybean fields in the United States are treated with herbicides each year. Recently, however, the proportion of herbicide applications made postemergence has increased. In fact, approximately 30 to 40% of the soybean hectarage in the northern soybean producing states is treated annually with a postemergence application. Moreover, an increased reliance on postemergence applications is expected in the future as use of conservation tillage practices increases (McWhorter 1992).

Two herbicides frequently applied postemergence for controlling broadleaf weeds in soybean are acifluorfen and bentazon. Each herbicide alone effectively controls specific weed species. Typically, however, the two compounds are mixed to increase the spectrum of control, and applied during early soybean development (Sorensen et al. 1987, Owen and Hartzler 1993). Both herbicides are considered contact toxins, undergoing little translocation in plant tissues (Ross and Lembi 1985). Additionally, both herbicides ultimately affect contacted cells by disrupting membranes, leading to foliar wilting, chlorosis, and necrosis (Sorensen et al. 1987). However, specific biochemical pathways of activity for

acifluorfen and bentazon differ. Phytotoxic effects of acifluorfen seem unrelated to photosynthesis, despite light being required for activity. Although not completely understood, acifluorfen seems to affect membrane function directly (Ross and Lembi 1985, Sorensen et al. 1987). In contrast, bentazon first interferes with photosynthesis by inhibiting electron transfer in photosystem II (Mine and Matsunaka 1975). Cellular death occurs secondarily, because of the formation of free radicals that then disrupt membranes (Ross and Lembi 1985, Sorensen et al. 1987).

Although less susceptible to mixes of acifluorfen and bentazon than targeted weed species, soybean also may be injured considerably (Owen and Hartzler 1993). Moreover, greater injury may occur when surfactants are added to the spray mix to improve weed control. Because soybean has enzymes capable of degrading acifluorfen (Frear et al. 1983) and bentazon (Connelly et al. 1988), injury to soybean from these herbicides is relatively less than that for susceptible weeds, and yield usually is unaffected (Owen 1986).

Defoliating Insects

Defoliators constitute the most abundant and diverse guild of insects injuring soybean in the United States (Turnipseed and Kogan 1976). Historically, the green cloverworm (GCW), <u>Plathypena scabra</u> (F.), has been the primary defoliator of soybean in the Midwest. In fact, GCW is

considered one of eight major insect pests of soybean in the United States (Hammond et al. 1991). In addition to GCW, other leaf-consuming insects (e.g., bean leaf beetle, <u>Ceratoma</u> <u>trifurcata</u> (Forster), grasshoppers, <u>Femurrubrum</u> spp.) can add to total defoliation of midwestern soybean, increasing economic losses.

Economically damaging populations of GCW occur approximately every two to five years in the Midwest (Hammond et al. 1991). Each year, GCW moths migrate from southerly areas to the Midwest (Wolf et al. 1987), where two generations develop (Hammond et al. 1991). In Iowa, Pedigo et al. (1983) have characterized the seasonal dynamics of GCW larvae according to two types of population configurations, outbreak and endemic. An outbreak configuration is distinguished by a large first generation, followed by a greatly reduced second generation. Defoliation by first-generation larvae, often coinciding with soybean flowering, can cause economic losses. However, epizootics caused by the fungal pathogen Nomuraea rileyi (Farlow) often reduce second-generation densities to noneconomic levels. Alternatively, an endemic configuration is characterized by smaller, noneconomic first and second generations. The number of spring days suitable for migration may be the key factor influencing type of larval configuration (Wolf et al. 1987). Management strategies for GCW include the use of calculated economic decision levels (economic injury levels and economic thresholds) and grower-based sampling

programs (Pedigo and van Schaik 1984).

Relationships between insect defoliation, canopy interception of photosynthetically active radiation (PAR), and yield have been evaluated. Soybean yield relates to the amount of canopy photosynthesis during reproductive phenology (reviewed by Shibles et al. 1987). Canopy photosynthesis, in turn, depends on the amount and utilization of intercepted PAR Because photosynthetic rates of remaining (Monteith 1977). leaf tissue are unaffected by most leaf-mass consuming insects (Poston et al. 1976, Higley 1992), soybean yield reductions from defoliation likely relate to total PAR interception during critical reproductive stages. In fact, Higley (1992) found highly significant linear relationships between PAR interception after simulated insect defoliation and soybean yield. Clearly, soybean canopy size can alter the impact of defoliation on PAR interception. Parameters typically used for predicting soybean yield losses to defoliation (insect density and % defoliation), therefore, may be unreliable across field environments (Higley 1992, Herbert et al. 1993).

<u>Stress</u> Interactions

Most interactions among agricultural stresses are unknown. Previous to this research, no work has considered interactions among soybean stresses from SCN, acifluorfen plus bentazon, and GCW. Separately, however, some interactions involving these factors and other stresses have been

determined, particularly for SCN. Soybean infected by SCN and Fusarium spp. fungi exhibit greater symptoms of Fusarium wilt (Ross 1965, Roy et al. 1989). Lim et al. (1985) reported that symptoms of brown spot, caused by the fungus Septoria glycines Hemmi, increased while symptoms of bacterial pustule, caused by Xanthomonas campestries pv. glycines (Smith) Dye, decreased on SCN-infected soybean. Additionally, Overstreet and McGawley (1988, 1990) found that soybean infection with the fungus Calonectria crotalariae (Loos) Bell increased stress from SCN by making roots more susceptible to penetration by SCN juveniles. In contrast, antagonistic effects of soybean stresses from SCN and stem canker fungus, <u>Diaporthe</u> phaseolorum (Cke. and Ell.) Sacc. var. caulivora Athow and Caldwell, on stem dry weight and canker lesion length have been found (Russin et al. 1989b). Robbins et al. (1990) found only additive effects of SCN, the threecornered alfalfa hopper, Spissistilus festinus Say, and broadleaf weeds on soybean seed yield. Other research indicates that SCN densities increase after defoliation by soybean looper, Pseudoplusia includens Walker (Russin et al. 1989b), and corn earworm, <u>Helicoverpa</u> zea (Alston et al. 1989), and that densities of weeds (Alston et al. 1991a) and corn earworm (Alston et al. 1991b) often increase in SCN-infected soybean.

Some soybean research also has evaluated stress interactions involving insect defoliation (exclusive of SCN) or acifluorfen. Weed biomass has been shown to increase in

insect-defoliated soybean (Helm et al. 1984, Higgins et al. 1984c), although effects of weeds and defoliation on soybean generally are additive (Higgins et al. 1983, 1984a,b). Additionally, Russin et al. (1989a) found that defoliation by soybean looper decreased stem canker length. Huckaba et al. (1988) noted increased phytotoxicity from acifluorfen in soybean previously injured by soybean thrips, <u>Sericothrips</u> <u>variabilis</u> (Beach).

PAPER 1: A METHOD FOR INFESTING SMALL FIELD PLOTS WITH SOYBEAN CYST NEMATODE

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A Method for Infesting Small Field Plots with Soybean Cyst Nematode

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ABSTRACT

Field experimentation with soybean cyst nematode (SCN), Heterodera glycines Ichinohe, is often difficult because of problems in obtaining plots with desired nematode population densities. Therefore, a technique was developed for infesting small field plots with SCN. SCN cysts, eggs, and infective juveniles were mixed with soil and applied with a Scotts Model PF3 drop-fertilizer spreader. Root segments infected with SCN were distributed by hand on top of the infested soil. Both SCN-infected soil and roots then were incorporated with a tractor-drawn Glencoe herbicide incorporator, and plots were planted with SCN-susceptible soybean. SCN egg densities immediately after planting averaged 184 and 100 100-cm⁻³ soil. This represented 78 and 90% of expected densities for 1990 and 1991, respectively. Resultant low soil densities of encysted eggs, which are highly aggregated, likely prevented detection of eggs in some plots. No SCN was detected in uninfested plots at planting, although contamination was noted in some plots at harvest.

INTRODUCTION

Effective determination or establishment of accurate and reliable population densities of plant-parasitic nematodes is important in experimentation evaluating the efficacy of control tactics (4) and for study in quantitative ecology, including nematode-host relationships (2). Difficulty in achieving prescribed densities can lead to inconclusive results because of excessive variation within density levels or indistinct differences among density levels.

Various methods have been used to obtain specific population densities of soybean cyst nematode (SCN), Heterodera glycines Ichinohe, in the field. Soil-applied nematicides have been used (2), but these chemicals often give unreliable control (10) and (or) confound results because of nontarget effects (3). Other researchers (1) have quantified preplanting densities of SCN from a large matrix of plots, enabling plot categorization to ranges of nematode density. However, this method is effective only if an adequate number of plots with desired densities can be found. Soil within microplots often is artificially infested with SCN eggs (6,8,12). However, this technique is not ideally suited for plots larger than microplots because it would require extensive efforts in extracting, counting, and diluting large quantities of eggs. Furthermore, plots are infested with isolated eggs, rather than encysted eggs which are subject to

natural egg-hatch inhibitors (9). Therefore, objectives of this work were to develop and evaluate a technique for infesting small field plots with SCN.

MATERIALS AND METHODS

Greenhouse Culture

SCN was cultured on SCN-susceptible 'Asgrow 2187' soybean in the greenhouse for four months at 26-30°C temperature with a 16:8 (L:D) photoperiod. Two 2-wk-old seedlings were transplanted into 7-liter pots filled with soil (81% sand, 11% silt, and 8% clay) which was sterilized in 1991, but not in 1990. Soil naturally infested with SCN was used in 1990. In 1991, SCN eggs were added to soil within pots just before transplanting. To obtain eggs, cysts were sieved from soil (11) and then eggs were obtained from cysts (5). A single application of a fertilizer solution (N:P:K, 20:20:20) was made immediately after initial transplanting each year. Two increases in numbers of pots were achieved by cutting plant shoots at soil level and dividing the soil medium plus roots between two pots, half filled with sterilized soils. Two 2-wk-old seedlings were transplanted into each pot.

Field Infestation

Fields were infested at the Johnson Farm near Ames, IA, on Webster soils (fine-loamy, mixed, mesic Typic Haplaquolls) in 1990 and 1991. Thirty-two SCN-infested and SCN-uninfested four-row plots (0.76 m wide x 3 m long) were designated each year in alternating infested and uninfested eight-plot strips. Four uninfested buffer rows were included between strips, and

plots within strips were separated by uninfested 2-m alleys.

SCN cultures were harvested 17 May 1990 and 27 May 1991, 14 and 11 d before planting, respectively. Plant shoots were clipped at soil level and discarded. Root systems were removed from the soil, cut in 2- to 3-cm long pieces, and mixed with the infested soil. Soil volumes were approximately 644 and 910 liters in 1990 and 1991, respectively. Mean egg population densities in the soil and roots, determined from 10 (1990) and 8 (1991) 200-cm³ subsamples, were 22 620 (SD = 7974.8) and 3562 (SD = 1302.4) 100 cm⁻³ for 1990 and 1991, respectively. Quantities of 18.9 (1990) and 28.4 (1991) liters were added to each infested plot.

Root segments were extracted from soil by screening through a sieve with 0.3-cm openings. Soil was applied to plots in 51-cm-wide bands using a Scotts Model PF3 dropfertilizer spreader with half of the tines removed (Figs. 1A,1B). Root segments were distributed by hand on top of the infested soil. A single pass with a Glencoe Herbicide Incorporator (Fig. 1C) set to run 13 cm deep was used to mix the inoculum in the soil.

'Asgrow 2187' soybean was planted with a four-row planter (John Deere Model 7000) 31 May 1990 and 7 June 1991, 0 and 9 d after infestation, respectively. A preemergence herbicide application of 2.8 kg a.i. ha^{-1} metolachlor [2-chloro-<u>N</u>-(2ethyl-6-methylphenyl)-<u>N</u>-(2-methoxy-1-methylethyl)acetamide] was made 1 (1990) and 0 (1991) d after planting (DAP). Work

in uninfested areas was completed before that for infested plots.

Nematode Sampling and Data Analysis

SCN egg and second-stage juvenile (J2) populations were sampled near soybean planting and harvest in 1990 (4 and 123 DAP) and 1991 (1 and 117 DAP) for indication of infestation efficacy and population establishment. For each date, 10 2.5cm-diam., 20-cm-deep soil cores were taken systematically from the center 2 m within the 2 middle rows $plot^{-1}$. The 10 soil cores were combined, thoroughly mixed, and cysts and J2s (11) and eggs (5) were extracted from a 100-cm³ aliquant. Final separation of J2s from sediments was with centrifugal flotation (7).

RESULTS AND DISCUSSION

Calculated and sampled population densities of SCN eggs were compared for determining the accuracy of the infestation technique. Approximately 4 280 835 (1990) and 2 022 609 (1991) eggs were added to each infested plot. Based on soil sampling at 20-cm depth, the total soil volume $plot^{-1}$ was 1 828 800 cm³. Consequently, initial postinfestation egg population densities should have averaged 234 (1990) and 111 (1991) 100-cm⁻³ soil. Actual counts among plots at planting ranged from 0 to 600 and 0 to 300 eggs 100 cm^{-3} soil for 1990 and 1991, respectively. Eggs were not detected in one and nine infested plots at planting in 1990 and 1991, respectively, although J2s were recovered from two of the nine plots in 1991. Consequently, SCN was not detected initially in only 8 of the 64 plots over 1990 and 1991. Sampled egg population densities at planting averaged 78% in 1990 and 90% in 1991 of predicted densities (Table 1). Because infestation resulted in low densities of highly aggregated (encysted) eggs, no detection of eggs in some infested plots is more likely indication of imperfect extraction efficiency or sampling error than failure of the infestation technique. Increasing the number of soil cores taken for each plot likely would enhance detection rate and decrease variation.

SCN (egg or J2) was found in all infested plots at harvest both years, supporting 100% infestation incidence.

This result is particularly impressive for 1990 because egg densities were lower at harvest than at planting, although J2 densities increased over the growing season (Table 1). Undetermined environmental factors probably caused the decline in eggs during 1990. Egg and J2 densities were greater at harvest than at planting in 1991.

In addition to establishing prescribed SCN population densities in infested sites, an equally important criterion of an effective infestation method is preventing introduction to uninfested areas. SCN was not detected in any uninfested plots at planting. However, low population densities were detected in 2 and 13 uninfested plots at harvest in 1990 and 1991, respectively. These introductions probably occurred via wind and water movement of infested soil later in the growing season, a likely result of any SCN infestation technique.

There are advantages and disadvantages of all techniques for infesting field plots with SCN. The addition of egg suspensions to soil (6,8,12) may result in more precise egg densities than those obtained with infestation utilizing infested soil and infected roots. Again, efforts required to prepare egg suspensions of specified densities make the egginfestation method unsuitable for many field introductions other than for microplots. Introductions of isolated eggs to soil also subject eggs to an unnatural hatching environment (9), as mentioned previously. In contrast, decreased efforts in inoculum preparation and a more natural inoculum (mostly

encysted eggs) constitute selective advantages of the method described here. Our method, moreover, was successful at establishing accurate soil densities of SCN eggs and preventing contamination of uninfested areas. Consequently, we advocate this method as a means to establish SCN population densities for small-plot research.

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	1990		1991	
Sample date+	Egg (SD)	J2 (SD)	Egg (SD)	J2 (SD)
Planting	184.4 (136.01)	1.6 (2.71)	100.0 (105.30)	0.2 (0.59)
Harvest	130.5 (110.48)	9.9 (8.07)	525.0 (467.96)	25.1 (22.58)

Table 1. Soybean cyst nematode egg and second-stage juvenile (J2) population densities 100-cm⁻³ soil after 1990 and 1991 field infestations.

+ Planting and harvest samples from 32 infested plots at 4 and 123 d after planting in 1990 and 1 and 117 d after planting in 1991.


Figure 1. Application and incorporation of soybean cyst nematode-root-soil inoculum to small field plots. A) Drop-fertilizer spreader applying nematodes mixed with soil. B) Evenly distributed pattern of mix applied by the fertilizer spreader. C) Incorporation of the inoculum into plots with a herbicide incorporator. PAPER 2: RESPONSES OF SOYBEAN CYST NEMATODE POPULATIONS TO A POSTEMERGENCE HERBICIDE MIX AND SIMULATED INSECT DEFOLIATION

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Responses of Soybean Cyst Nematode Populations to a Postemergence Herbicide Mix and Simulated Insect Defoliation¹

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RH: Herbicides, Defoliation, and <u>H. glycines</u>: <u>Browde</u> et al.

ABSTRACT

Field research was conducted during 1988-1991 to determine impacts of the postemergence herbicide mix acifluorfen plus bentazon and simulated green cloverworm, Plathypena scabra, defoliation on population densities of soybean cyst nematode (SCN), <u>Heterodera</u> <u>qlycines</u>, in soil. Responses of natural (1988 and 1989) and artificially infested (1990 and 1991) SCN populations were evaluated. Herbicide applications always caused soybean stress, indicated by foliar chlorosis and necrosis. Likewise, defoliation consistently decreased the soybean canopy. Population densities of SCN eggs were always lower for herbicide-treated than herbicideuntreated plots. These reductions were significant (P < 0.05) in 1988 and over 1990 and 1991. Moreover, herbicide applications caused significant and near significant (P = 0.06) reductions in population densities of SCN second-stage juveniles in 1988 and 1989, respectively. SCN population densities were unaffected by defoliation for all years except 1989, when defoliation significantly increased egg densities. Causal mechanisms for reductions in SCN population densities after application of acifluorfen plus bentazon are unknown.

Key words: acifluorfen, bentazon, defoliation, <u>Glycine</u> <u>max</u>, green cloverworm, <u>Heterodera</u> <u>glycines</u>, <u>Plathypena</u> <u>scabra</u>, postemergence herbicides, soybean, soybean cyst nematode

INTRODUCTION

The soybean cyst nematode (SCN), <u>Heterodera glycines</u> Ichinohe, is a key pest of soybean, <u>Glycine max</u> (L.) Merr., in the United States. Although originally found in the southeastern United States, SCN has spread to the Midwest and is becoming an increasing problem (13,30). Soybean-yield losses to SCN can be extensive, with losses usually correlated to population densities in soil at planting (7,18,25). Additional factors, however, have an impact on yield-loss relationships, particularly SCN race and edaphic factors (25).

Numerous natural abiotic and biotic environmental variables affect SCN population dynamics. Important abiotic factors include soil moisture and temperature. The most researched biotic factors are host characteristics, including root growth, developmental stage, and susceptibility to injury (19,24). Recent studies have begun to investigate interactions among SCN and other pests. Alston et al. (2) and Russin et al. (22) found that SCN population densities increased after insect defoliation of determinate soybean. In contrast, population densities decreased after soybean infection with stem canker fungus, <u>Diaporthe phaseolorum</u> (Cke and Ell.) Sac. var. <u>caulivora</u> Athow and Caldwell (22).

Agricultural chemicals can influence SCN population dynamics. Nitrate fertilizer is toxic to SCN at application rates higher than those used in crop production (20). At

agriculturally acceptable rates of fertilization, however, population densities generally increase more rapidly than densities in unfertilized soil. This stimulative effect on SCN populations is likely indirect, probably related to enhanced root growth following fertilization (11,20).

Pesticides also can impact SCN population densities. Nematicides often reduce early season densities in soybean by delaying root infection or inhibiting nematode development after infection (27,32). Nontarget effects of soil-applied herbicides on SCN have been noted. Preplant applications of alachlor (4) or trifluralin (17) have increased SCN population densities. Moreover, antagonism between alachlor and the nematicide fenamiphos reduces fenamiphos efficacy (26).

Two additional important pest groups that limit soybean production are weeds and defoliating insects. Although weed management programs for soybean may include several control tactics, herbicides remain the key tactic (12). Postemergence applications of acifluorfen plus bentazon herbicides are used commonly for controlling broadleaf weeds in soybean (28). In Iowa (15), applications are recommended during early soybean development, specifically between V3 and V6 growth stages (6). Although soybean is less susceptible to injury from these herbicides than targeted weed species, considerable injury can occur (10). Both acifluorfen and bentazon physically affect leaf cells on contact by disrupting membranes, leading to foliar wilting, chlorosis, and necrosis (28). Additionally,

several defoliating insects of soybean in the Midwest, particularly the green cloverworm (GCW), <u>Plathypena scabra</u> (F.), can cause extensive injury. Economically damaging populations of GCW occur during years of excessive spring immigration of moths from southerly areas (16).

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 pest management. Therefore, research was conducted during 1988-1991 for quantifying impacts of acifluorfen plus bentazon and simulated GCW defoliation on SCN population densities.

MATERIALS AND METHODS

Plot establishment and experimental design: In 1988 and 1989, plots were established at the Iowa State Agronomy Farm on a Harps soil (fine-loamy mesic Typic Calciaquolls) in Boone County, Iowa. A field previously planted to soybean was used both years. In 1990 and 1991, plots were located at the Johnson Farm on Webster soils (fine-loamy, mixed, mesic Typic Haplaquolls) in Story County, Iowa. Separate fields previously cropped in corn-soybean rotations were used these years. SCN-susceptible soybean cultivars were planted all years in a 0.76-m row spacing, at a rate of 85.4 kg seeds/ha. Soybean 'BSR 201' was seeded 26 May 1988, while soybean 'Asgrow 2187' was planted 17 May 1989, 31 May 1990, and 7 June 1991. After seedling emergence, stands were thinned to 25 plants/row-m.

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 for 1988 and 1989, respectively. In 1990 and 1991, a split-plot design (four blocks) was used. Treatments were combinations of two population densities of SCN, three (1988 and 1989) or two (1990 and 1991) rates of acifluorfen plus bentazon, and four levels of simulated GCW defoliation. Treatments were assigned according to a factorial in 1988 and 1989. SCN population

density constituted whole-plot treatments in 1990 and 1991, with four border rows separating adjacent whole plots. Subplots were assigned factorial combinations of herbicide rate and defoliation level. A total of 96, 4-row plots was used in 1988 (4 m long) and in 1989 (5 m long). For both 1990 and 1991, a total of 64 (4 row x 3 m long) subplots was used.

Nematode treatments: Two levels of SCN population density were planned each year. Manipulations of natural populations were done in 1988 and 1989. To establish plots of low density in 1988, aldicarb (3.4 kg AI/ha) was banded over the row and incorporated at planting. No aldicarb was applied to high density plots. Because aldicarb was ineffective, however, alternative methods for establishing SCN population densities were used for subsequent years. In 1989, plots of medium and high at-planting SCN density (Pi) were established by categorizing locations according to density classes (1). Five days after planting (22 May), the field was divided into a matrix of 216, 4-row x 4-m-long plots. Ten, 2.5-cm-d, 20-cm-deep soil cores were taken systematically from the two middle rows of each plot. The ten soil cores were combined, mixed, and SCN eggs were extracted from cysts (3) obtained from sieving (29) a $100-cm^3$ aliquot of soil. Plots then were categorized as medium, medium-high, or high Pi. Forty-eight plots of medium (\overline{x} = 1,006 eggs, SD = 358) and high (\overline{x} = 4,333 eggs, SD = 1,121) Pi were selected for experimentation.

Fields without detectable SCN populations in soil before planting were used for 1990 and 1991 studies. Low Pi plots were achieved in 1990 ($\bar{\mathbf{x}} = 184 \text{ eggs/100-cm}^3$ soil, SD = 136) and 1991 ($\bar{\mathbf{x}} = 100 \text{ eggs/100-cm}^3$ soil, SD = 105) by artificially infesting soil with an SCN-root-soil inoculum before planting (5).

Herbicide treatments: Herbicides were applied at recommended rates (15) each year. In 1988, acifluorfen plus bentazon at 0.14 + 0.84 or 0.56 + 0.84 kg a.i./ha was applied on 3 July (27 days after soybean emergence) when soybean growth stages ranged from V3 to V6. In 1989, acifluorfen plus bentazon applications at 0.28 + 0.56 or 0.56 + 0.84 kg a.i./ha were made on 5 July (38 days after soybean emergence; V6 growth-stage). Acifluorfen (0.56 kg a.i./ha) plus bentazon (0.84 kg a.i./ha) was applied 17 July 1990 (39 days after soybean emergence; V6 growth-stage) and 11 July 1991 (29 days after emergence; V5 to V6 growth-stages). A nonionic surfactant (X-77; Ortho Chemical) was included in all applications (0.5% v/v). Untreated plots also were included each year. To control weeds, trifluralin (1.12 kg a.i./ha) was applied preplant-incorporated in 1988, and metolachlor (2.8 kg a.i./ha) was applied preplant-incorporated in 1989 and preemergence in 1990 and 1991. No interactions between herbicides applied for controlling weeds and acifluorfen plus bentazon treatments were expected. Weed escapes were removed by hand.

Defoliation treatments: Numbers of soybean leaflets removed to simulate GCW feeding rates were determined with a computer program (L. G. Higley, unpublished) based on inputs of daily low and high temperatures. The program incorporated models for GCW consumption (8) and development (9). Defoliation began at the R2 growth-stage (full bloom) and ended at the R4 growth-stage (full pod), coinciding with the characteristic phenology of first-generation GCW larvae in Iowa (16). Specific defoliation intervals were 18 to 29 July 1988, 17 to 28 July 1989, 23 July to 3 August 1990, and 22 July to 2 August 1991. GCW feeding was simulated spatially by removing leaflets by hand from upper canopy strata (14) of the two middle rows. Injury levels corresponding to 0, 6, 12, and 24 GCWs or GCW equivalents/row-m were simulated in 1988 and 0, 9, 18, and 36 in 1989. One GCW equivalent is 54 cm^2 (8), the average amount of leaf area consumed by a GCW larva that completes development. The range of injury was increased to 0, 18, 72, and 144 GCW equivalents/row-m in 1990 and 1991. Non-defoliated plots were walked through and plants were handled daily to minimize confounding. On days 8 and 12 of defoliation, outer rows were sham-defoliated by hand using an upward flailing motion to approximate levels in middle rows. No efforts were made to control natural defoliators because of both low population densities and potential nontarget effects of the control tactic.

Nematode and soybean responses: SCN egg and second-stage juvenile (J2) populations in soil were sampled, as described previously, at intervals of at least 30 days for each growing season (days after planting (DAP) indicated in Fig. 1). Eggs were extracted from cysts as described previously, and J2s were separated from sediments by centrifugal flotation (10).

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. Impact of defoliation on leaf area index (LAI; ratio of leaf to ground area) immediately after defoliation at the R4 growth-stage was determined in 1989, 1990, and 1991. LAIs were calculated from leaf areas measured 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. LAIs were not determined in 1988.

Data analysis: Data for 1988 and 1989 were analyzed by year because of unique levels and arrangements of treatments. Because identical herbicide rates and defoliation levels were imposed on plots with artificially infested SCN populations in 1990 and 1991, data for these years were pooled for analysis. SCN egg and J2 counts were transformed to log₁₀ (x+1) before analysis. Data were subjected to analyses of variance (23)

for partitioning sums of squares among factors and interactions, and for testing effects having single degrees of freedom for significance (P < 0.05). Significant effects of other factors and interactions were isolated by orthogonal comparisons.

RESULTS

Interactive effects of treatments on SCN population densities generally were not significant. The few significant interactions were inconsistent and were believed spurious. Additionally, no interactions with year were detected for analyses of SCN counts pooled over 1990 and 1991. Therefore, main effects of SCN (1988 and 1989 only), herbicide, and defoliation treatments will be presented.

Nematode responses to nematode treatments: Partial (1988 and 1989) and full (1990 and 1991) seasonal dynamics of SCN populations for each SCN treatment in 1988 and 1989 and for artificially infested plots in 1990 and 1991 are presented in Figure 1. Although at-planting populations were not sampled in 1988, the ineffectiveness of aldicarb in reducing egg and J2 population densities was noted on both sampling dates. Differences in SCN densities between plots treated and untreated with aldicarb were not significant for eggs or J2s at 33 DAP or for eggs at 69 DAP. However, J2 densities were significantly greater for aldicarb-treated plots at 69 DAP. Extreme drought likely contributed to the ineffectiveness of aldicarb in 1988.

Both categorization of natural populations (1989) and artificial infestation (1990 and 1991) were successful in establishing plots of different egg and J2 Pi. In 1989, egg and J2 population densities were significantly lower in medium

compared to high Pi plots at 5 and 37 DAP, although differences were not significant at 82 DAP. Patterns of population fluctuation differed between 1990 and 1991, however, SCN was established in 100% of infested plots both years (5).

Soybean and nematode responses to herbicides: Applications of acifluorfen plus bentazon always resulted in detectable soybean injury (Tables 1-3). Because relative evaluations of injury among plots were made, comparison among years is difficult. Nevertheless, marked gradients in crop injury among herbicide treatments in 1988, 1989, and over 1990 and 1991 substantiate that levels of soybean stress were established. High application rates caused significantly greater crop injury than low rates in 1988 and 1989, when three rates were used.

Main effects of herbicide treatments on SCN population densities in soil are presented in Tables 1-3. Egg densities were consistently lower in herbicide-treated plots than in herbicide-untreated plots. These reductions were significant at 69 DAP (35% reduction) in 1988 and at soybean harvest (11% reduction) over 1990 (123 DAP) and 1991 (117 DAP). Moreover, herbicide applications caused significant and near significant (P = 0.06) reductions in J2 densities at 69 DAP in 1988 (33% reduction) and at 82 DAP in 1989 (17% reduction), respectively. No significant effects of application rate on egg or J2 densities were found.

Soybean and nematode responses to defoliation: Defoliation caused significant linear reductions in crop canopies in 1989 (Table 2) and over 1990 and 1991 (Table 3). Average LAIs for plots of highest injury were reduced by 17% in 1989 and by 22% over 1990 and 1991. Although LAIs were not measured in 1988, canopy reductions likely were proportionately similar or greater than those for other years, because soybean growth was limited by drought and irondeficiency chlorosis.

Defoliation impacts on SCN egg and J2 population densities in soil were not significant in 1988 (Table 1) or over 1990 and 1991 (Table 3). However, a significant quadratic relationship between defoliation and egg densities was found in 1989 (Table 2). Egg densities increased with defoliation up to 18 GCW equivalents/row-m, after which, densities decreased.

DISCUSSION

An important result of this study is the indication that acifluorfen plus bentazon reduced SCN egg and J2 population densities. Mechanisms underlying these reductions were not determined, although the effect is probably a consequence of altered soybean physiology. Acifluorfen and bentazon translocate minimally in soybean and bind rapidly to soil colloids (21). 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. Or, herbicide injury may limit root growth, providing fewer sites for nematode feeding. However, herbicides significantly lowered egg densities even in soils of low Pi in 1990 and 1991.

Future research should determine causal mechanisms for lower SCN population densities in soil after applications of acifluorfen plus bentazon. Furthermore, additional efforts should be made in quantifying the impact of these herbicides on population densities at harvest. From a cropping systems perspective, any reduction in the density of an SCN population in soil is economically important to future soybean production (2).

Despite reducing SCN population densities in soil, acifluorfen plus bentazon applications to SCN-infested plots

never significantly increased soybean seed yield (unpublished data). Reductions in soybean yield from SCN generally result from injury during the first 42 DAP (31). Therefore, applications of these herbicides made near V6 soybean development probably are too late to limit important injury. Earlier applications of acifluorfen plus bentazon could be especially cost-effective by limiting yield losses to both weeds and SCN. Additional research should investigate relationships among application timing, SCN population densities, and soybean yield.

Because defoliation significantly reduced SCN egg population densities in soil in 1989 only, no conclusions about relationships between defoliation of indeterminate soybean and SCN populations could be developed. A short interval between defoliation and population assessment in 1988, or low SCN densities in 1990 and 1991 could have precluded detection of significant effects. Increased SCN population densities in soil with defoliated determinate soybean has been attributed to lengthened seed-fill intervals (2) or increased photosynthate partitioning to roots (22). Research involving more timely sampling of defoliated indeterminate soybean in soils of medium or high Pi should be conducted.

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, or economic decision levels for pest control. Researchers in pest disciplines should strengthen their commitments to interdisciplinary studies for improving and sustaining pest management.

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Factor	Crop Response ‡	Egg	J2
HERB§			
none	0%	1,438	83
low	10%	861	52
high	14%	1,006	59
<u>F</u> (df)		2.63 (2,68)	2.64 (2,65)
$\underline{\mathbf{P}} > \underline{\mathbf{F}}$		0.0795	0.0791
Contrast <u>F</u> , <u>P</u> > <u>F</u>			
none vs low+high		4.75, 0.0328	4.78, 0.0324
low vs high		0.55, 0.4609	0.57, 0.4548
DEFOL			
0 GCW/row-m		1,035	70
6 GCW/row-m		1,250	67
12 GCW/row-m		1,038	57
24 GCW/row-m		1,092	65
F (df)		0.54 (3,68)	0.73(3,65)
$\mathbf{P} > \mathbf{F}$		0.6582	0.5379
Contrast <u>F</u> , <u>P</u> > <u>F</u>			
linear		0.04, 0.8421	0.15, 0.6998
quadratic		0.06, 0.8072	1.74, 0.1918

§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. Table 2. Main effects of acifluorfen plus bentazon (HERB) and simulated green cloverworm (GCW) defoliation (DEFOL) on soybean cyst nematode egg and second-stage juvenile (J2) population densities/100-cm³ soil at 82 days after planting in 1989.[†]

Factor	Crop response [‡]	Egg	J 2
HERB [§]			
none	0%	5,188	256
low	98	4,472	196
high	19%	4,841	227
<u>F</u> (df)		0.38 (2,72)	2.49 (2,72)
$\underline{P} > \underline{F}$		0.6869	0.0897
Contrast <u>F</u> , <u>P</u> > <u>F</u>			
none vs low+high		0.74, 0.3920	3.54, 0.0638
low vs high		0.01, 0.9077	1.44, 0.2334
DEFOL			
0 GCW/row-m	3.57	3,933	224
9 GCW/row-m	3.08	4,817	234
18 GCW/row-m	2.97	5,625	216
36 GCW/row-m	2.95	4,958	231
<u>F</u> (df)		1.72 (3,72)	0.19 (3,72)
<u>P > F</u>		0.1707	0.9026
Contrast \underline{F} , $\underline{P} > \underline{F}$			
linear		0.64, 0.4263	0.00, 0.9438
quadratic		4.22, 0.0436	0.00, 0.9498

+Log₁₀(x+1) used for analyses; untransformed means reported. Date of sampling corresponded to 33 and 10 days after herbicide and defoliation treatments, respectively. +Crop injury and leaf area index for herbicide and defoliation treatments, respectively.

§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.

Crop Factor response ‡	Pre-harvest		Harv	Harvest	
	Crop response †	Egg	J2	Egg	J2
HERBS					
none	0%	138	9	346	15
high	24%	105	8	309	20
$\underline{\mathbf{F}}$ (df)		0.91(1,49)	1.06 (1,49)	3.95 (1,49)	0.90 (1,49)
$\underline{P} > \underline{F}$		0.3460	0.3079	0.0526	0.3466
DEFOL					
0 GCW/row-m	4.30	128	10	269	19
18 GCW/row-m	4.03	95	9	344	14
72 GCW/row-m	3.56	98	7	381	21
144 GCW/row-m	3.34	162	8	317	16
<u>F</u> (df)		0.18 (3,49)	0.36 (3,49)	0.09 (3,49)	0.74 (3,49)
$\underline{P} > \underline{F}$		0.9126	0.7815	0.9634	0.5331
Contrast <u>F</u> , <u>P</u>	> <u>F</u>				
linear		0.02, 0.8881	0.75, 0.3907	0.05, 0.8240	0.22, 0.6411
quadratic		0.00, 0.9862	0.28, 0.5991	0.22, 0.6411	1.74, 0.1933

Table 3. Main effects of acifluorfen plus bentazon (HERB) and simulated green cloverworm (GCW) defoliation (DEFOL) on soybean cyst nematode egg and second-stage juvenile (J2) population densities/100-cm³ soil before and at soybean harvest over 1990 and 1991.

 \pm Log₁₀(x+1) used for analyses; untransformed means reported. Pre-harvest = 90 days after planting, 43 days after herbicides, and 26 days after defoliation in 1990; 76 days after planting, 42 days after herbicides, and 20 days after defoliation in 1991. Harvest = 123 days after planting, 76 days after herbicides, and 59 days after defoliation in 1990; 117 days after planting, 83 days after herbicides, and 61 days after defoliation in 1991.

+Crop injury and leaf area index for herbicide and defoliation treatments, respectively, averaged over SCN-infested and SCN-noninfested plots. §High = 0.56 kg a.i./ha acifluorfen + 0.84 kg a.i./ha bentazon.

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Figure 1. Changes in SCN egg and second-stage juvenile (J2) population densities as affected by aldicarb in 1988 and at-planting SCN density (Pi) in 1989-1991. Data for artificially infested plots only are presented for 1990 and 1991.



Days after planting

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AND DEFOLIATING INSECTS

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PAPER 3: GROWTH OF SOYBEAN STRESSED BY NEMATODES, HERBICIDES

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Growth of Soybean Stressed by Nematodes, Herbicides, and Defoliating Insects

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RH: BROWDE ET AL.: MULTIPLE STRESSES AND SOYBEAN GROWTH

ABSTRACT

Generally, soybean [Glycine max (L.) Merr.] responses to combinations of stress factors are unknown. Therefore, research was conducted in 1989, 1990, and 1991 for quantifying growth responses of soybean to combined stresses from soybean cyst nematode (SCN) (Heterodera glycines Ichinohe), acifluorfen {5-[2-chloro-4-(trifluoromethyl)phenoxy]-2nitrobenzoic acid} plus bentazon [3-(1-methylethyl)-(1H)-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide herbicides, and green cloverworm (GCW) [Plathypena scabra (F.)]. Treatments were combinations of at-planting SCN density, acifluorfen plus bentazon rate, and simulated GCW defoliation level. Plant growth was quantified at V4, R2, and R4 stages of development. Herbicide stress was assessed by leaf stomatal conductance and visual estimates of foliar injury. Despite always causing visual injury, herbicides only limited growth [(leaf area, plant height, pod number, and dry weights (leaf, pod, and stem and petiole)] in 1990 and 1991. Likewise, defoliation reduced leaf area immediately after defoliation for each year, but growth reductions (plant height and leaf dry weight) were noted for 1990 and 1991 only. Although herbicides and defoliation affected growth additively, defoliation of herbicide-injured plants in 1990 and 1991 caused proportionately greater reductions in quantity and quality of remaining foliage. Excessive injury from SCN likely

desensitized plants to herbicides and defoliation in 1989. When controlled stress from SCN was achieved (1990), interaction with herbicides caused a significant reduction in pod number and near significant reductions in leaf area, leaf number, and pod weight. Consequently, applications of acifluorfen plus bentazon to SCN-infected soybean may cause more-than-additive reductions in soybean growth.

INTRODUCTION

Common stress factors of soybean [Glycine max (L.) Merr.] in the midwestern United States include soybean cyst nematode (SCN) (Heterodera glycines Ichinohe), acifluorfen {5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid} and bentazon [3-(1-methylethyl)-(1H)-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide] herbicides, and green cloverworm (GCW) [Plathypena scabra (F.)]. Juvenile SCN infect and feed on soybean roots (Dropkin, 1989), often reducing growth (Alston and Schmitt, 1987; Wrather and Anand, 1988; Alston et al., 1991; Niblack et al., 1992) and yield (Francl and Dropkin, 1986; Riggs and Schmitt, 1987; Schmitt et al., 1987). Acifluorfen and bentazon typically are mixed and applied postemergence during early soybean development to control broadleaf weeds (Sorensen et al., 1987; Owen and Hartzler, 1993). Both herbicides physically affect leaf cells on contact by disrupting membranes, leading to foliar wilting, chlorosis, and necrosis (Sorensen et al., 1987). Although soybean is less susceptible to these herbicides than targeted weeds, considerable injury can occur (Owen and Hartzler, However, subsequent soybean growth and yield usually 1993). are unaffected (Owen, 1986). Economically damaging densities of GCW develop every two to five years in the Midwest (Hammond et al., 1991). Defoliation by first-generation GCW larvae often coincides with soybean flowering (Pedigo et al., 1983)

and can limit growth and yield (Hammond and Pedigo, 1982; Higgins et al., 1983; Ostlie and Pedigo, 1985).

Numerous factors stress crops each growing season. Most pest research, however, has only assessed impacts of single pests (Higgins, 1985). Interactive stresses must be characterized to improve understanding of crop-stress relationships and, thus, pest management. Characterizations should include preharvest responses, as well as yield, so that mechanisms underlying yield responses may be determined. Therefore, research was conducted in 1989, 1990, and 1991 to quantify soybean responses to combined stresses from SCN, acifluorfen plus bentazon, and GCW. Preharvest growth responses are presented; yield responses and management recommendations are reported elsewhere (Browde et al., 1993c).
MATERIALS AND METHODS

Plot Establishment and Experimental Design

Plots in 1989 were located at the Iowa State Agronomy Farm near Boone, IA, on a Harps soil (fine-loamy, mesic Typic Calciaquolls) planted previously to soybean. In 1990 and 1991, plots were established at the Johnson Farm near Ames, IA, on Webster soils (fine-loamy, mixed, mesic Typic Haplaquolls) planted previously to corn. SCN-susceptible 'Asgrow 2187' soybean was planted at a seeding rate of 85.4 kg ha⁻¹ in 0.76-m rows on 17 May 1989, 31 May 1990, and 7 June 1991. Stands were thinned to 25 plants row-m⁻¹ after emergence.

A completely random design with four replications was used in 1989, while a split-plot design with four blocks was used in 1990 and 1991. Treatments were combinations of two at-planting SCN soil densities (SCN Pi), three (1989) or two (1990 and 1991) rates of acifluorfen plus bentazon, and four levels of simulated GCW defoliation. A factorial arrangement of treatments was made in 1989. In 1990 and 1991, SCN Pi constituted whole plots, with four border rows between plots. Subplots were assigned factorial combinations of herbicide rate and defoliation level. Consequently, 96 (4 m long; 1989) and 64 (3 m long; 1990 and 1991) 4-row plots were used. Herbicide applications and defoliation were imposed sequentially after establishing SCN Pi.

Treatment Applications

In 1989, 48 plots of medium ($\bar{x} = 1006 \text{ eggs } 100 \text{ cm}^{-3} \text{ soil}$, SD = 358) and high ($\bar{x} = 4333 \text{ eggs } 100 \text{ cm}^{-3} \text{ soil}$, SD = 1121) SCN Pi were established after sampling and categorizing locations in a naturally infested field according to SCN density (Alston and Schmitt, 1987). Fields without detectable SCN populations in soil before planting were used for 1990 and 1991 studies. Thirty-two plots of low SCN Pi were achieved in 1990 ($\bar{x} = 184 \text{ eggs } 100 \text{ cm}^{-3} \text{ soil}$, SD = 136) and 1991 ($\bar{x} = 100$ eggs 100 cm⁻³ soil, SD = 105) by infesting soil with an SCNroot-soil inoculum before planting (Browde et al., 1993a).

Herbicides were applied at recommended rates (Owen and Hartzler, 1993) at the V6 stage of soybean development (Fehr et al., 1977). In 1989, acifluorfen plus bentazon at 0.28 + 0.56 or 0.56 + 0.84 kg a.i. ha⁻¹ was applied on 5 July [38 d after emergence (DAE)]. Acifluorfen (0.56 kg a.i. ha⁻¹) plus bentazon (0.84 kg a.i. ha⁻¹) was applied 17 July 1990 (39 DAE) and 11 July 1991 (29 DAE). A nonionic surfactant (X-77; Ortho Chemical) was included in all applications (0.5% v/v). Untreated plots were included each year. A preplantincorporated (1989) or preemergence (1990 and 1991) application of 2.8 kg a.i ha⁻¹ metolachlor [2-chloro-<u>N</u>-(2ethyl-6-methylphenyl)-<u>N</u>-(2-methoxy-1-methylethyl)acetamide] was made to control weeds. No interactions between herbicides applied for weed control and acifluorfen plus bentazon were expected. Weed escapes were removed by hand.

A computer program (L. G. Higley, unpublished) was used for calculating defoliation rates (leaflets $plot^{-1} day^{-1}$) from inputs of daily low and high temperatures. The program incorporated models for GCW consumption (Hammond et al., 1979a) and development (Hammond et al., 1979b). Leaf area removed was quantified daily with a leaf area meter (LI-COR 3100, LI-COR, Lincoln, NE) so that deviations in rates could be adjusted in subsequent defoliation. Defoliation began at R2 (full bloom) and ended at R4 (full pod) stages of soybean development, thereby coinciding with the characteristic phenology of first-generation GCW larvae in Iowa (Pedigo et al., 1983).

Leaflets were removed by hand from upper canopy strata (Ostlie, 1984) of the two middle rows during 17 to 28 July (50-61 DAE) 1989, 23 July to 3 August (48-59 DAE) 1990, and 22 July to 2 August (40-51 DAE) 1991. Injury levels corresponding to 0, 9, 18, and 36 GCWs or GCW equivalents rowm⁻¹ were simulated in 1989. One GCW equivalent is 54 cm² (Hammond et al., 1979a), the average amount of leaf area consumed by a GCW larva that completes development. Injury levels of 0, 18, 72, and 144 GCW equivalents row-m⁻¹ were used in 1990 and 1991. Non-defoliated plots were walked through and plants were handled (Higgins et al., 1983) to minimize confounding from compaction and thigmomorphogenesis, respectively. On days 8 and 12 of defoliation, outer rows of defoliated plots were sham-defoliated by hand using an upward

flailing motion to approximate levels in middle rows. No efforts were made to control natural defoliators because of both low population densities and potential nontarget effects of the control tactic.

Soybean Responses

Measurements of leaf stomatal conductance and visual evaluations of crop injury were made to quantify stress from herbicides. Conductance was measured on three plants plot⁻¹. Measurements were taken from the middle leaflet of the uppermost fully expanded trifoliate with a porometer (LI-COR 1600, LI-COR, Lincoln, NE) at 2 (7 July 1989 and 19 July 1990) or 3 (14 July 1991) and 7 (24 July 1990 and 18 July 1991) or 8 (13 July 1989) d after applications (DAA). At least a 7-d interval between applications and final measurements ensured that leaves used at last sampling dates had unfolded and expanded following applications. Visual assessments of foliar chlorosis and necrosis (0 to 100% scale; 0 = no symptoms, 100 = plant death) were made at 7 (12 July 1989 and 18 July 1991) or 8 (25 July 1990) and 20 (25 July 1989), 21 (1 August 1991), or 22 (8 August 1990) DAA.

Plants were destructively sampled at V4, R2, and R4 stages of development (average for untreated plots) for characterizing plant growth after applications of each stress factor. V4 samples were taken before herbicides and defoliation on 27 June 1989 (30 DAE), 6 July 1990 (31 DAE),

and 5 July 1991 (23 DAE). R2 samples were taken after herbicides and immediately before defoliation on 11 July 1989 (44 DAE), 19 July 1990 (45 DAE), and 21 July 1991 (39 DAE). R4 samples were taken immediately after defoliation on 31 July 1989 (64 DAE), 6 August 1990 (62 DAE), and 6 August 1991 (55 DAE). For each stage, four (1989) or three (1990 and 1991) plants were harvested from the two middle rows plot⁻¹. Parameters recorded included plant height, vegetative (Vstage) and reproductive (R-stage) developmental stages, branch number, node number, pod number, leaf number, lowest leafbearing node, and leaf area. Additionally, dry weights (96 hr at 60° C) were obtained for leaves, pods, and supports (stem and petiole).

Data, by year, were subjected to analyses of variance (SAS Institute, 1988) for partitioning sums of squares among factors and interactions and for testing effects having single degrees of freedom for significance (P < 0.05). For factors and interactions with more than a single degree of freedom, significant relationships among means were determined with orthogonal comparisons.

RESULTS AND DISCUSSION

Stomatal Conductance and Visual Crop Injury

Herbicides consistently stressed soybean (Table 1). Conductance at 2 (1989 and 1990) or 3 (1991) DAA was significantly (1989 and 1990) or nearly significantly (P = 0.07; 1991) lower for leaves contacted by herbicides than for untreated leaves. Moreover, conductance was lowered significantly by the increased herbicide rate in 1989. A similar response was observed for visual crop injury at approximately 1 and 3 wk after applications. Applications always resulted in visually injured canopies, including significantly more injury from the higher rate in 1989. Reduced turgor of foliage contacted by herbicides likely caused leaf stomatal closure and eventual chlorosis and necrosis of some leaf tissues.

No physical effects of herbicides were found on foliage developing after applications. In fact, leaf conductance at 7 DAA was significantly greater for herbicide-treated (374.1 mmol $H_20 \ m^{-2} \ s^{-1}$) than untreated (287.7 mmol $H_20 \ m^{-2} \ s^{-1}$) plants in 1990 and for herbicide-treated (769.2 mmol $H_20 \ m^{-2} \ s^{-1}$) than untreated (599.8 mmol $H_20 \ m^{-2} \ s^{-1}$) plants in 1991. These increases may have resulted from altered water relations, i.e., more water available per noninjured, actively transpiring leaf. No impact of herbicides on the conductance of newly developed leaves was found at 8 DAA in 1989, possibly

caused by relatively drier soils. Nevertheless, canopy growth subsequent to applications always made foliar injury less apparent by approximately 3 wk after applications (Table 1).

SCN Pi also affected stomatal conductance and visual injury from herbicides (Table 1). In 1989, conductance measured at 2 DAA was significantly lower for high than medium SCN Pi. However, neither conductance at 7 DAA nor visual injury were affected. Although no main effects of SCN Pi were found in 1990, SCN and herbicide stresses interacted significantly to reduce conductance (Fig. 1) and to increase visual injury at 8 DAA (Table 1). Root injury by SCN likely limited water uptake, thereby contributing to stomatal closure in 1989 and 1990. Subsequent visual injury, however, was increased only in 1990. No impact of SCN Pi on conductance nor visual injury was found in 1991, when SCN densities were lowest.

Defoliation significantly affected evaluations of crop injury from herbicides at approximately 3 wk after applications in 1990 and 1991, but not in 1989 (Table 1). Because defoliation of herbicide-treated soybean exposed injured, lower leaves in 1990 and 1991, defoliation and visual injury were related linearly. Evaluations of injury made relatively earlier during defoliation and (or) lower levels of defoliation probably kept defoliation from influencing injury assessments in 1989.

V4 Growth Responses

Higher SCN Pi never decreased plant growth at V4. Alternatively, plants injured by low SCN Pi had significantly more nodes (5.5) and leaves (6.5) than noninjured plants (5.2 nodes and 6.1 leaves) in 1991. Injury from low densities of other parasitic nematodes has been shown to stimulate plant growth (Nickle, 1984).

R2 Growth Responses

As at V4, plant growth at R2 never was reduced by higher SCN Pi (Table 2). In contrast, leaf areas and leaf dry weights were significantly lower for medium compared to high SCN Pi in 1989. These effects are confusing because conductance measured just 4 d before R2 samples (Table 1) indicated more stress for plants injured by high SCN Pi. No effects of SCN Pi on growth at R2 were found in 1990. As for V4 samples, injury from SCN generally increased plant growth in 1991. Only branching, however, was increased significantly.

Herbicides significantly limited plant growth at R2 in 1991, but not in 1989 or 1990 (Table 2). At 10 DAA in 1991, herbicide-injured plants had significantly lower plant heights, leaf areas, and dry weights compared to untreated plants. Although herbicide-injured plants also had significantly more leaves, this resulted from a significant increase in lower-leaf retention rather than greater leaf

production. An interval of only 2 d between applications and R2 plant samples likely precluded the detection of similar growth reductions in 1990. No impact of herbicides on growth was found at 6 DAA in 1989. Herbicide rate, however, did affect growth significantly. Unexpectedly, plants injured by the high rate had greater vegetative development and more branches, nodes, and leaves than plants injured by the low rate. Causal mechanisms for these rate effects are unknown, although growth responses may relate to herbicide effects on SCN population dynamics (Browde et al. 1993b). No SCN Pi x herbicides effects on R2 growth were significant.

<u>R4</u> Growth Responses

Fewer main effects of SCN Pi on growth were found at R4 (Table 3) than at earlier developmental stages. In 1989 and 1991, trends of greater growth for plants injured by higher SCN Pi persisted, although increases were significant only for height in 1989 and pods in 1991. Alternatively, injury from SCN limited many growth parameters in 1990, but none significantly.

Intervals between herbicide applications and R4 samples were similar each year (26, 20, and 26 d for 1989, 1990, and 1991, respectively), however, herbicides significantly limited plant growth in 1990 and 1991 only (Table 3). Plant height, leaf area, pod number, and dry weights were reduced in 1990 and 1991. Furthermore, as noted for R2 samples in 1991,

herbicide-injured plants retained more lower leaves than untreated plants. Delayed abscission of lower leaves may have been caused by physical injury to these leaves. Or, because herbicides also reduced leaf area, leaf retention may have resulted from delayed leaf senescence (Higley, 1992). In addition, plants injured by herbicides in 1990 had delayed vegetative development and fewer nodes and branches. More growth parameters may have been reduced by herbicides in 1990 than in 1991 because of greater injury (Table 1) and (or) applications made slightly later in soybean development. Despite considerable visual injury from herbicides in 1989, no impact of herbicides on plant growth was found. Excessive stress from SCN probably desensitized soybean responses to other stresses that year.

SCN Pi x herbicides effects on plant growth at R4 were found in 1990 and 1991. Although no main effects of SCN Pi were noted in 1990, SCN and herbicide stresses significantly interacted to reduce plant height (Fig. 1). Similar interactions were nearly significant for leaf area (P = 0.10), pods (P = 0.07), and pod weight (P = 0.09). Therefore, greater foliar injury from herbicide applications to SCNinfected soybean in 1990 (Table 1) significantly limited subsequent plant growth. In contrast, impact of SCN Pi and herbicides on lower leaf retention was significantly less than additive in 1991 (Fig. 2). This response may be attributed to subtle differences in senescence rates of lower leaves.

Defoliation significantly impacted plant growth each year (Table 3). Leaf removal caused linear reductions in leaf area for 1989, 1990, and 1991 and in leaf dry weight for 1990 and 1991. A quadratic relationship also was significant for leaf area in 1991, implying compensatory leaf growth at the highest level of injury (144 GCW equivalents $row-m^{-1}$). Because support and pod dry weights were unaffected in 1990 and 1991, decreases in total shoot dry weight for those years resulted primarily from reductions in leaf dry weight. Despite no impact on leaf dry weight in 1989, support dry weights were reduced linearly.

Defoliation also significantly affected plant morphology (Table 3). Only 3- and 4-d intervals existed between defoliation and R4 plant samples for 1990 and 1991, respectively. For both years, however, plant height was reduced linearly. Because stage of vegetative development was unaffected, height reductions resulted from shortened internodes. Quadratic relationships existed between defoliation and both leaf and node number for 1991. Because respective linear coefficients were not significant, these effects are believed spurious. A 4-d interval also existed between defoliation and plant samples in 1989, but no main effects of defoliation were found. Excessive stress from SCN and (or) an insufficient range of defoliation may have precluded the detection of defoliation effects in 1989. Α significant SCN Pi x herbicides x defoliation effect on

branching was found in 1989 but also was believed spurious.

Longer intervals between defoliation and R4 plant samples may have enabled the detection of additional defoliation effects, including interactions with other stresses. Still, leaf area, leaf weight, and plant height were reduced in 1990 These soybean responses to simulated GCW and 1991. defoliation have been reported elsewhere (Hammond and Pedigo, 1982; Higgins et al., 1983; Ostlie and Pedigo, 1985). Because common defoliation rates were applied across treatments, no herbicides x defoliation effect on leaf area was expected nor occurred. Defoliation of plants injured vs. uninjured by herbicides, however, resulted in markedly different plant canopies in 1990 and 1991. Because of less leaf area for herbicide-injured plants, defoliation was proportionately greater for these plants than for those noninjured by herbicides. In fact, removal of 144 GCW equivalents of leaf area row-m⁻¹ caused 31 and 16% defoliation for plants injured and noninjured by herbicides, respectively, averaged over 1990 and 1991. Defoliation also caused greater decreases in canopy quality for herbicide-injured plants because of injured lower leaves (Table 1). These herbicides x defoliation reductions in canopy quantity and quality likely would cause similar decreases in canopy photosynthesis, leading to more-thanadditive reductions in soybean growth.

Important interactive stresses were determined with this research. In 1990, the only year in which desired stress from

SCN was achieved, SCN Pi and herbicides interacted to reduce leaf stomatal conductance, increase visual injury from herbicides, and reduce plant height. Similar reductions were nearly significant for leaf area, pod number, and pod dry weight. Because impacts of defoliation were greater for herbicide-injured plants, more-than-additive reductions in soybean growth after acifluorfen plus bentazon applications, at or near the V6 stage of development, and GCW defoliation are likely. Relationships between earlier applications of these herbicides and stresses from SCN and GCW are unknown and should be investigated.

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Table 1. Main effects of at-planting soybean cyst nematode density (SCN Pi) and acifluorfen plus bentazon rate (HERB) on stomatal conductances of leaves contacted by herbicides and SCN Pi, HERB, and simulated green cloverworm (GCW) defoliation (DEFOL) on % visual crop injury from herbicides.+

Factor	19	989		19	990		1991				
	Conductance (mmol H ₂ 0 m ⁻² s ⁻¹)	Crop injury 1 wk	Crop injury 3 wk	Conductance (mmol H ₂ 0 m ⁻² s ⁻¹)	Crop injury 1 wk	Crop injury 3 wk	Conductance (mmol H ₂ 0 m ⁻² s ⁻¹)	Crop injury 1 wk	Crop injury 3 wk		
SCN Pi=											
None				366.6	27.5	8.6	297.1	19.7	9.9		
Low				335.9	30.3	10.3	315.2	18.8	11.6		
Medium	388.5	15.0	5.0		•-						
High	366.2	13.0	5.3								
$P > \tilde{F}$	*	NS	NS	NS	*	NS	NS	NS	NS		
HERB§											
None	415.7	0.0	0.0	427.0	0.0	0.0	325.0	0.0	0.0		
Low	378.4	8.9	3.2								
High	338.7	19.1	7.2	277.5	28.9	9.5	287.1	19.2	10.8		
P > F											
None vs. Any	**			**			NS				
Low vs. High	**	**	**								
DEFOL											
0 GCW row-m ⁻¹			5.1			5.6			6.4		
9 GCW row-m ⁻¹			5.2				·				
18 GCW row-m ⁻¹			5.1			6.9			7.2		
$36 \text{ GCW row-m}^{-1}$			5.2								
72 GCW row-m ⁻¹						11.9			13.1		
144 GCW row-m ⁻¹						13.5			16.2		

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P > F						
Linear	 	NS	 	**	 	**
Quadratic	 	NS	 	NS	 	NS

*, **, and NS = significant at the 0.05 and 0.01 probability levels, and not significant, respectively.

- + 1989: 1-wk crop injury assessed at 7 d after HERB, 3-wk crop injury assessed at 20 d after HERB and 8 d after DEFOL onset; 1990: 1-wk crop injury assessed at 8 d after HERB and 2 d after DEFOL onset, 3-wk crop injury assessed at 22 d after HERB and 5 d after DEFOL completion; 1991: 1-wk crop injury assessed at 7 d after HERB, 3-wk crop injury assessed at 21 d after HERB and 10 d after DEFOL onset.
- at 7 d after HERB, 3-wk crop injury assessed at 21 d after HERB and 10 d after DEFOL onset. = Low = means of 100 (1990) and 184 (1991) eggs 100-cm⁻³ soil; medium = mean of 1006 eggs 100-cm⁻³ soil; high = mean of 4333 eggs 100-cm⁻³ soil.
- § Low = 0.28 kg a.i. ha⁻¹ acifluorfen + 0.56 kg a.i. ha⁻¹ bentazon; high = 0.56 kg a.i. ha⁻¹ acifluorfen + 0.84 kg a.i. ha⁻¹ bentazon.

Factor		Ht (cm)	Vst	Rst	Brches	Nodes	Leaves	Lowest leaf- bearing node	Leaf area (cm ²)	Leaf wt (g)	Support wt (g)	Total shoot wt (g)
							1989 (A)					
SCN Pi												
Medium	(1)	30.5	7.92	1.99	1.28	11.6	10.7	1.80	444.3	1.95	1.59	3.55
High HERB	(2)	32.0	8.10	1.98	1.30	12.1	11.1	1.90	510.4	2.19	1.80	3.98
None	(1)	31.1	7.99	2.00	1.40	11.8	11.0	1.87	501.0	2.15	1.76	3.91
Low	(2)	30.9	7.87	1.99	1.01	11.2	10.2	1.88	454.3	1.93	1.59	3.52
High	(3)	31.7	8.17	1.97	1.47	12.4	11.4	1.80	476.8	2.14	1.73	3.86
							1990 (B)	,				
SCN Pi												
None	(1)	34.1	7.68	2.04	0.97	10.2	9.4	1.98	497.4	1.97	1.64	3.61
Low HERB	(2)	35.6	7.74	2.07	1.01	10.5	9.5	2.03	479.8	1.96	1.68	3.64
None	(1)	35.0	7.62	2.07	0.99	10.2	9.3	1.99	503.8	1.96	1.71	3.67
High	(3)	34.8	7.80	2.04	0.99	10.5	9.6	2.02	473.3	1.96	1.61	3.58
							1991 (C))				
SCN Pi												
None	(1)	37.5	8.90	2.15	1.46	13.0	12.8	1.49	703.5	2.58	2.14	4.72
Low HERB	(2)	39.0	8.96	2.14	1.61	13.7	13.3	1.39	751.1	2.75	2.32	5.08
None	(1)	41.7	8.82	2.18	1.40	12.9	12.3	1.74	854.2	2.96	2.66	5.61
High	(3)	34.8	9.04	2.12	1.68	13.8	13.8	1.14	600.4	2.38	1.81	4.19

Table 2. Effects of at-planting soybean cyst nematode density (SCN Pi)+ and acifluorfen plus bentazon rate (HERB)+ on soybean growth plant⁻¹§ at R2 (full flower) development.

Years (A,B,C) with significant (P < 0.05) comparisons:

SCN Pi											
1 vs. 2			 С				Α	Α	Α	Α	
HERB											
1 vs. 2+3			 								
2 vs 3		Α	 Α	Α	А						
1 vs. 3	С		 		С	С	C	С	C	С	
SCN Pi x HERB			 								

Plants harvested 44 d after emergence and 6 d after HERB in 1989; 45 d after emergence and 2 d after HERB in 1990; and 39 d after emergence and 10 d after HERB in 1991.

- \pm Low = means of 100 (1990) and 184 (1991) eggs 100-cm⁻³ soil; medium = mean of 1006 eggs 100-cm⁻³ soil; high = mean of 4333 eggs 100 cm^{-3} soil. $\pm \text{ Low} = 0.28 \text{ kg a.i.} \text{ ha}^{-1}$ acifluorfen + 0.56 kg a.i. ha⁻¹ bentazon; high = 0.56 kg a.i. ha⁻¹ acifluorfen
- + 0.84 kg a.i. ha^{-1} bentazon.

§ Ht = plant height; Vst and Rst = vegetative and reproductive development, respectively; brches = branches.

Factor		Ht (cm)	Vst	Rst	Brches	Nodes	Pods	Leaves	Lowest leaf- bearin node	Leaf area g (cm ²)	Leaf wt (g)	Pod wt (g)	Support wt (g)	Total shoot wt (g)
	<u> </u>						1989 (Ά)						
SCN Pi														
Medium	(1)	58.8	13.4	3.87	2.41	23.2	24.7	19.3	4.88	956.9	3.64	0.47	4.87	8.96
High	(2)	62.2	13.6	3.96	2.23	22.9	26.1	18.6	5.00	958.6	3.88	0.51	5.19	9.58
HERB														
None	(1)	61.3	13.4	3.95	2.37	22.6	25.9	18.6	4.87	1008.4	3.82	0.51	5.25	9.57
Low	(2)	61.0	13.5	3.90	2.34	23.2	25.2	19.3	4.97	964.9	3.73	0.49	4.98	9.20
High	(3)	59.2	13.5	3.89	2.25	23.2	25.1	18.9	4.98	899.9	3.74	0.48	4.86	9.07
DEFOL						•								
0 GCW	row-m ⁻¹	62.4	13.5	3.92	2.50	24.1	27.5	19.9	5.13	1087.9	4.25	0.53	5.76	10.54
9 GCW	row-m ⁻¹	60.1	13.4	3.87	2.22	22.5	25.2	18.4	4.82	938.3	3.64	0.48	4.86	8.97
18 GCW	row-m ⁻¹	59.8	13.4	3.95	2.33	22.4	23.0	18.4	4.93	906.5	3.54	0.49	4.74	8.77
36 GCW	row-m ⁻¹	59.8	13.5	3.92	2.23	23.1	25.9	19.0	4.88	898.2	3.64	0.47	4.77	8.88
							1990	(B)						
SCN Pi														
None	(1)	63.3	12.4	4.02	2.70	20.5	35.4	17.4	3.78	898.6	3.33	1.04	4.17	8.55
Low	(2)	60.2	12.3	4.02	2.81	20.6	33.4	17.0	3.72	866.4	3.26	0.94	4.05	8.28
HERB														
None	(1)	66.0	12.6	4.09	3.10	21.7	38.8	18.6	3.87	1040.2	3.78	1.21	4.90	9.95
High	(3)	57.6	12.2	3.95	2.42	19.4	30.0	15.8	3.64	724.7	2.83	0.78	3.32	6.93
DEFOL														
0 GCW	row-m ⁻¹	62.0	12.2	4.02	2.80	19.8	34.9	17.3	3.80	957.3	3.44	0.98	4.00	8.46
18 GCW	row-m ⁻¹	63.6	12.3	4.04	2.35	20.6	35.4	17.4	3.79	1000.8	3.89	1.06	4.60	9.55
72 GCW	row-m ⁻¹	63.0	12.4	4.08	2.47	20.2	31.9	17.0	3.67	860.2	3.30	0.99	4.18	8.47
144 GCW	row-m-1	58 5	12 6	3 94	3 41	21 5	31 9	17 2	3 75	711 6	2.58	0.95	3.66	7.19

Table 3. Effects of at-planting soybean cyst nematode density (SCN Pi)+, acifluorfen plus bentazon rate (HERB)+, and simulated green cloverworm (GCW) defoliation (DEFOL) on soybean growth plant⁻¹§ at R4 (full pod) development.

SCN Pi	•													
None	e (1)	73.3	13.6	4.00	3.01	24.2	33.8	21.2	2.83	1373.3	5.16	1.40	6.30	12.9
Low	(2)	74.8	13.9	3.98	3.42	26.1	36.9	22.9	2.85	1508.0	5.57	1.56	6.79	13.9
HERB	•													
None	e (1)	81.1	13.8	4.00	3.32	24.9	38.9	22.1	3.05	1637.4	6.00	1.79	7.68	15.5
High	ı (3)	67.0	13.8	3.98	3.11	25.4	31.8	22.0	2.63	1243.8	4.72	1.16	5.41	11.3
DEFOL		-												
0	GCW row-m	77.1	14.1	4.00	3.77	26.4	36.3	23.5	2.93	1662.4	6.10	1.48	7.25	14.8
18	GCW row-m	75.5	13.7	3.98	2.51	23.5	35.7	20.9	2.92	1457.5	5.51	1.66	6.54	13.7
72	GCW row-m	74.8	13.6	3.98	3.19	23.5	33.8	20.4	2.80	1314.2	4.94	1.45	6.18	12.6
144	GCW row-m	⁻¹ 68.8	13.8	4.00	3.40	27.3	35.6	23.4	2.72	1328.4	4.90	1.33	6.22	12.4

1991 (C)

Years (A,B,C) with significant (P < 0.05) comparisons:

SCN Pi													
1 vs. 2	А	-	-	-	-	-	С	-	-	-	-	-	-
HERB													
1 vs. 2+3	-	-	-	-	-	-	-	-	-	-	-	-	-
2 vs 3	-	-	-	-	-	-	-	-	-	-	-	-	-
1 vs. 3	BC	В	В	В	В	BC	В	BC	BC	BC	BC	BC	BC
DEFOL													
Linear	BC	-	-	-	-	-	-	-	ABC	BC	-	Α	BC
Quadratic	-	-	-	-	С	-	С	-	С	-	-	-	-
SCN Pi X HERB	В	-	-	-	-	-	-	С	-	-	-	-	-
SCN Pi x DEFOL	-	-	-	-	-	-	-	-	-	-	-	-	-
HERB x DEFOL	-	-	-	-	-	-	-	-	-	-	-	-	-
SCN Pi x HERB	-	-	-	Α	-	-	-	-	-	-	-	-	-
x DEFOL													

Plants harvested 64 d after emergence, 26 d after HERB, and 3 d after DEFOL in 1989; 62 d after emergence, 20 d after HERB, and 3 d after DEFOL in 1990; 55 d after emergence, 26 d after HERB, and 4 d after DEFOL in 1991.

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Table 3 (continued).

- + Low = means of 100 (1990) and 184 (1991) eggs 100-cm⁻³ soil; medium = mean of 1006 eggs 100-cm⁻³ soil; high = mean of 4333 eggs 100-cm⁻³ soil. = Low = 0.28 kg a.i. ha⁻¹ acifluorfen + 0.56 kg a.i. ha⁻¹ bentazon; high = 0.56 kg a.i. ha⁻¹ acifluorfen + 0.84 kg a.i. ha⁻¹ bentazon.
- § Ht = plant height; Vst and Rst = vegetative and reproductive development, respectively; brches = branches.

Figure 1. Interactive effects of at-planting soybean cyst nematode density (SCN Pi) and acifluorfen plus bentazon rate (Herbicides) on stomatal conductance of leaves contacted by herbicides and plant height at R4 soybean development in 1990.

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Figure 2. Interactive effect of at-planting soybean cyst nematode density (SCN Pi) and acifluorfen plus bentazon rate (Herbicides) on lowest leaf-bearing node at R4 soybean development in 1991.

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PAPER 4: SOYBEAN YIELD AND PEST MANAGEMENT AS INFLUENCED BY NEMATODES, HERBICIDES, AND DEFOLIATING INSECTS

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Soybean Yield and Pest Management as Influenced by Nematodes, Herbicides, and Defoliating Insects

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RH: BROWDE ET AL.: MANAGEMENT OF SOYBEAN INTERACTIVE STRESSES

ABSTRACT

Pest management strategies based on crop losses from single stress factors may be inadequate for multiple-stress scenarios. Consequently, experiments were conducted in 1989, 1990, and 1991 for quantifying interactive effects of stresses from soybean cyst nematode (SCN) (Heterodera glycines Ichinohe), acifluorfen (5-[2-chloro-4-(trifluoromethyl) phenoxy]-2-nitrobenzoic acid) plus bentazon [3-(1-methylethyl) -(1H)-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide] herbicides, and green cloverworm (GCW) [Plathypena scabra (F.)] on seed yield of soybean. Treatments were combinations of at-planting SCN density, acifluorfen plus bentazon rate, and simulated GCW defoliation level. Plot yield and yield components (pods plant⁻¹, seeds pod⁻¹, weight seed⁻¹) were determined. Herbicides and defoliation, independently, reduced yield in 1990 and 1991. Additionally, herbicide and defoliation stresses interacted over 1990 and 1991 to decrease yield. Excessive injury from SCN likely desensitized responses to herbicides and defoliation in 1989. No main effects of atplanting SCN density on yield were found. However, stresses from SCN and herbicides and from SCN and defoliation interacted in 1990, lowering yield. Yield reductions from defoliation were attributed to fewer pods plant⁻¹, while herbicides decreased pods $plant^{-1}$ (1990) and weight seed⁻¹ (1990 and 1991). Interactive effects on seed yield were not

explained by analysis of yield components. Low-risk management recommendations were made to deal with interactions. Because of the consistent herbicides x defoliation effect on yield, lower GCW economic injury levels were developed for soybean treated with acifluorfen plus bentazon. To account for potential SCN x herbicides and SCN x defoliation effects on yield, soybean growers will be advised to consider adjusting weed and insect management strategies for SCN-infested fields.

INTRODUCTION

Numerous biotic and abiotic stress factors limit crop production. For biotic factors on soybean [Glycine max (L.) Merr.], nematodes, weeds, and defoliating insects account for over 85% of economic losses (Hammond et al., 1991). Most research on abiotic factors has concerned natural stresses, such as heat and water stress (Raper and Kramer, 1987). However, pest-control tactics, particularly herbicides (Owen and Hartzler, 1993), can add to abiotic stress. Many stress factors alone can cause sufficient injury to reduce crop growth and yield. Yet, single-stress scenarios seldom exist. Rather, multiple stresses are common during each growing season that interact to affect crop productivity. Economic decision rules currently used for pest management decisions generally are based on models of yield losses to single pests (Higgins, 1985; Newsom and Boethel, 1985). If stress interactions exist, use of these rules limits producer profits and, in some instances, reduces environmental quality (Pedigo and Higley, 1992). Interactions among important stresses must be quantified to improve understandings of crop-stress relationships and pest management.

Soybean is a crop stressed by multiple factors most growing seasons. Important stress factors of soybean in the midwestern United States are soybean cyst nematode (SCN) (<u>Heterodera glycines</u> Ichinohe), acifluorfen (5-[2-chloro-4-

(trifluoromethyl)phenoxy]-2-nitrobenzoic acid) and bentazon [3-(1-methylethyl)-(1<u>H</u>)-2,1,3-benzothiadiazin-4(3<u>H</u>)-one 2,2dioxide] herbicides, and green cloverworm (GCW) [<u>Plathypena</u> <u>scabra</u> (F.)]. SCN injures soybean by the penetration, cell destruction, and feeding of juvenile nematodes within roots (Dropkin, 1989). Although preharvest responses vary, SCNinfected plants often are stunted and have poorly developed canopies (Alston and Schmitt, 1987; Wrather and Anand, 1988; Alston et al., 1991a,b; Niblack et al., 1992). Yield losses usually are correlated with SCN densities in soil at planting (Francl and Dropkin, 1986; Riggs and Schmitt, 1987; Schmitt et al., 1987).

Postemergence applications of acifluorfen plus bentazon frequently are made during early soybean development to control broadleaf weeds (Sorensen et al., 1987; Owen and Hartzler, 1993). Both herbicides physically affect leaf cells on contact by disrupting membranes, leading to foliar wilting, chlorosis, and necrosis (Sorensen et al., 1987). Soybean also may be injured (Owen and Hartzler, 1993), although injury is relatively less than that for weeds, and yield usually is unaffected (Owen, 1986).

Historically, the GCW has been the primary defoliator of soybean in the Midwest (Hammond et al., 1991). Economic losses generally are associated with feeding by larvae of the first generation, typically coinciding with flowering soybean (Pedigo et al., 1983). Management strategies for GCW on

soybean include the use of calculated economic decision levels and grower-based sampling programs (Pedigo and van Schaik, 1984).

Some work on interactive stresses involving SCN (Lim et al., 1985; Russin et al., 1989; Robbins et al., 1990; Alston et al., 1991a,b), acifluorfen (Huckaba et al., 1988), or GCW (Higgins et al., 1983; Higgins et al., 1984a,b,c) has been conducted. However, no reports have considered stress interactions among this combination of factors. Consequently, research was conducted in 1989, 1990, and 1991 for evaluating effects of combined stresses from SCN, acifluorfen plus bentazon, and GCW on seed yield of soybean. Results would be used for adjusting guidelines for managing the nematode-weedinsect complex in soybean.

MATERIALS AND METHODS

Plot Establishment and Experimental Design

Plots were established at the Iowa State Agronomy Farm near Boone, IA, on a Harps soil (fine-loamy, mesic Typic Calciaquolls) in 1989, and at the Johnson Farm near Ames, IA, on Webster soils (fine-loamy, mixed, mesic Typic Haplaquolls) in 1990 and 1991. Soils were planted previously to soybean in 1989 and to corn in 1990 and 1991. SCN-susceptible 'Asgrow 2187' soybean was seeded at 85.4 kg ha⁻¹ in 0.76-m rows on 17 May 1989, 31 May 1990, and 7 June 1991. Stands were thinned to 25 plants m⁻¹ after emergence.

A completely random design with four replications was used in 1989, while a split-plot design with four blocks was used in 1990 and 1991. Treatments were combinations of two at-planting SCN soil densities (SCN Pi), three (1989) or two (1990 and 1991) rates of acifluorfen plus bentazon, and four levels of simulated GCW defoliation. Treatments were assigned, according to a factorial, to 96 4-m-long plots in 1989. In 1990 and 1991, whole plots were assigned SCN Pi, while subplots were assigned factorial combinations of herbicide rate and defoliation level. Whole plots were separated by four border rows. Totals of 64 3-m-long subplots were used for 1990 and 1991 studies. Herbicides and defoliation were imposed sequentially after establishing SCN Pi.
Treatment Applications

A field naturally infested with SCN was used in 1989. Forty-eight plots of medium ($\bar{x} = 1006 \text{ eggs } 100 \text{ cm}^{-3} \text{ soil}$, SD = 358) and high ($\bar{x} = 4333 \text{ eggs } 100 \text{ cm}^{-3} \text{ soil}$, SD = 1121) SCN Pi were selected after sampling and categorizing locations according to SCN Pi classes (Alston and Schmitt, 1987). Fields without detectable SCN populations in soil were infested with an SCN-root-soil inoculum before planting (Browde et al., 1993) to achieve 32 plots of low SCN Pi in 1990 ($\bar{x} = 184 \text{ eggs } 100 \text{ cm}^{-3} \text{ soil}$, SD = 136) and in 1991 ($\bar{x} = 100 \text{ eggs } 100 \text{ cm}^{-3} \text{ soil}$, SD = 105).

Herbicides were applied at recommended rates (Owen and Hartzler, 1993) at the V6 stage of soybean development (Fehr et al., 1977). Acifluorfen plus bentazon was applied at 0.28 + 0.56 or 0.56 + 0.84 kg a.i. ha⁻¹ on 5 July 1989 [38 d after emergence (DAE)]. On 17 July 1990 (39 DAE) and 11 July 1991 (29 DAE), applications of 0.56 kg a.i. ha⁻¹ acifluorfen plus 0.84 kg a.i. ha⁻¹ bentazon were made. All applications (0.5% v/v) included a nonionic surfactant (X-77; Ortho Chemical). Additionally, untreated plots were included each year. Weeds were controlled by a preplant-incorporated (1989) or preemergence (1990 and 1991) application of 2.8 kg a.i. ha⁻¹ metolachlor [2-chloro-<u>N</u>-(2-ethyl-6-methylphenyl)-<u>N</u>-(2-methoxy-1-methylethyl)acetamide] or by hand removal. No interactions between herbicides applied for controlling weeds and acifluorfen plus bentazon were expected.

Defoliation rates (leaflets plot⁻¹ day⁻¹) were calculated from inputs of daily low and high temperatures into a computer program (L. G. Higley, unpublished). Models for GCW consumption (Hammond et al., 1979a) and development (Hammond et al., 1979b) were incorporated into the program. To simulate feeding by first-generation larvae, leaflets were removed by hand from upper canopy strata (Ostlie, 1984) during R2 (full bloom) to R4 (full pod) stages of soybean development. Specific intervals were 17 to 28 July (50-61 DAE) 1989, 23 July to 3 August (48-59 DAE) 1990, and 22 July to 2 August (40-51 DAE) 1991. Leaf area removed was quantified daily with a leaf area meter (LI-COR 3100, LI-COR, Lincoln, NE) so that deviations in rates could be adjusted in subsequent defoliation.

Injury levels corresponding to 0, 9, 18, and 36 (1989) or 0, 18, 72, and 144 (1990 and 1991) GCWs or GCW equivalents row-m⁻¹ were simulated in the two middle rows. One GCW equivalent is 54 cm² (Hammond et al., 1979a), the average amount of leaf area consumed by a GCW larva that completes development. Outer rows were sham-defoliated by hand using an upward flailing motion to approximate levels in middle rows. Non-defoliated plots were walked through and plants were handled (Higgins et al., 1983) to minimize confounding. No efforts were made to control natural defoliators because of both low population densities and potential nontarget effects of the control tactic.

Soybean Responses

Efficacy of factors in causing crop stress was assessed by visual evaluations of injury from herbicides, leaf area index (LAI; ratio of leaf to ground area), and canopy interception of photosynthetically active radiation (PAR). Visual ratings of foliar chlorosis and necrosis (0 to 100% scale; 0 = no symptoms, 100 = plant death) were made at 7 (12) July 1989 and 18 July 1991) or 8 (25 July 1990) and 20 (25 July 1989), 21 (1 August 1991), or 22 (8 August 1990) d after herbicide applications (DAA). LAIs were calculated from measurements of plant leaf area (LI-COR 3100, LI-COR, Lincoln, NE) taken immediately after defoliation at R4 soybean development. Leaf areas were measured from four (1989) or three (1990 and 1991) plants from the two middle rows plot⁻¹ on 31 July 1989 (64 DAE), 6 August 1990 (62 DAE), and 6 August 1991 (55 DAE). Interception of PAR by the soybean canopy, particularly during seed fill, is a critical parameter relating to canopy photosynthesis and yield (Shibles et al., 1987; Higley, 1992). Therefore, determinations of % interception of PAR were made at R4 and R5 (beginning seed) stages of soybean development by using a 0.76-m-long line quantum sensor (LI-COR 191, LI-COR, Lincoln, NE). Assessments at R4 were made on 28 July 1989 (61 DAE), 4 August 1990 (60 DAE), and 9 August 1991 (58 DAE). Assessments at R5 were made on 16 August 1989 (80 DAE), 13 August 1990 (69 DAE), and 19 August 1991 (68 DAE). For each plot, % interception was

calculated from the ratio of incident radiation below (two measurements) to above (one measurement) the canopy. Measurements were made in full sunlight within approximately 1 hr of solar noon. Calculations of % interception for R4 and R5 were averaged to provide representative values of interception during early to middle stages of seed fill.

Plants were harvested at soybean maturity to quantify treatment effects on seed yield. Yield components (pod number plant⁻¹, seed number pod^{-1} , and weight seed⁻¹) were determined from four (1989) or five (1990 and 1991) plants sampled from rows two and three on 26 September 1989 (121 DAE), 26 September 1990 (113 DAE), and 30 September 1991 (110 DAE). Remaining plants from these rows (8 row-m plot⁻¹) were harvested and threshed with a stationary small-plot thresher for plot yields on 28 September 1989 (123 DAE). Only the center 2 m of these rows (4 row-m plot⁻¹) was harvested for plot yields on 27 September 1990 (114 DAE) and 1 October 1991 (111 DAE).

<u>Data Analysis</u>

Data, by year, were subjected to analyses of variance (SAS Institute, 1988) for partitioning sums of squares among factors and interactions and for testing effects having single degrees of freedom for significance (P < 0.05). Significant effects of other factors and interactions were isolated by orthogonal comparisons. Regression analyses were used for

calculating coefficients for models of yield losses to defoliation.

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RESULTS AND DISCUSSION

Visual Crop Injury and Leaf Area Index

Herbicide applications always stressed soybean, as indicated by visually injured canopies at approximately 1 wk after applications (Tables 1-3). Moreover, injury was significantly greater for the high than low rate in 1989. Although canopy growth made injury less apparent at approximately 3 wk after applications (data not presented), herbicide-treated soybean had significantly lower LAIs at R4 development (Tables 2 and 3). No significant effect of herbicides on LAI was found in 1989, despite similar intervals between applications and LAI assessments among years. Excessive stress from both medium and high SCN Pi likely desensitized soybean responses to herbicides in 1989.

SCN Pi affected crop injury from herbicides and LAI (SCN Pi x herbicides) in 1990 (Table 2), but not in 1989 (Table 1) or 1991 (Table 3). At 8 DAA in 1990, crop injury was significantly greater for soybean infected than not infected with SCN. Mechanistic bases for this interaction probably relate to impacts of SCN and herbicide stresses on leaf turgor (unpublished data). Furthermore, this acute SCN Pi x herbicides effect on canopy quality almost similarly reduced LAI at R4 development (P = 0.10). No effect of SCN Pi on crop injury was found at 22 DAA in 1990 (data not presented). Similar stress for SCN Pi levels in 1989 and extremely low SCN

densities in 1991 likely precluded impacts of SCN Pi on crop injury and LAI for these years.

Defoliation also affected LAI and crop injury from herbicides. Defoliation consistently caused linear reductions in LAI (Tables 1-3). Including a quadratic component improved the relationship in 1991. Additionally, defoliation affected evaluations of crop injury at 22 and 21 DAA in 1990 and 1991, respectively (data not presented). Because defoliation was completed in 1990 (5 d after defoliation completion) and nearly completed in 1991 (10 d after defoliation onset), defoliation and crop injury were related linearly (P < 0.01) both years. Earlier evaluations of crop injury (8 d after defoliation onset) and (or) lower defoliation levels may have precluded a similar relationship in 1989.

Plot Yield and Light Interception

Stress factors impacted plot yield in 1990 and 1991, but not in 1989 (Tables 1-3). Despite lower yields corresponding to increased levels of herbicides and defoliation in 1989, differences were not significant. Again, excessive stress from SCN likely was confounding in 1989. Furthermore, stress from SCN Pi alone never influenced yield. Main effects of herbicides and defoliation on yield were found in 1990 and 1991. Herbicides reduced yield by 21% in 1990 and 8% in 1991. The greater reduction in 1990 may be attributed to increased injury from herbicides for that year (Table 2). Yield

responses to defoliation in 1990 and 1991 were explained best by quadratic and linear models, respectively.

Three important interactive effects on yield also were found (Fig. 1). When stress from SCN was achieved (1990), SCN Pi and herbicides significantly interacted (P < 0.05) to reduce yield. This response at soybean maturity confirmed the importance of similar interactions before harvest on visual crop injury and LAI. Moreover, SCN Pi and defoliation significantly interacted (P < 0.05) to reduce yield in 1990. Herbicides x defoliation effects on yield were significant (P < 0.05) in 1990 and near significant (P = 0.09) in 1991. Because herbicide rates and defoliation levels were identical for 1990 and 1991, yields for these years also were pooled for analysis to test the consistency of herbicide and defoliation effects so that applicable yield-loss models for GCW could be developed. In this analysis, the herbicides x defoliation effect was highly significant (P < 0.01) with no interaction with year. These data support the conclusion that soybean infected with SCN or treated with these herbicides (near V6 development) is more susceptible to yield reductions from GCW.

Canopy interception of PAR during seed fill (Tables 1-3) was a key physiological mechanism underlying yield reductions to SCN, herbicides, and defoliation. Confirming results for LAI, SCN Pi and herbicides interacted to decrease PAR interception in 1990 (P < 0.05; Fig. 2). Because identical defoliation rates were applied across treatments, only

additive impacts of defoliation on LAI at R4 development were expected and occurred (Tables 2 and 3). Defoliation of various-sized canopies, however, markedly affected PAR interception. Independently, stresses from SCN (Table 2) and herbicides (Tables 2 and 3) limited canopy growth. Consequently, reductions in LAI from defoliation were proportionately greater for SCN- and herbicide-stressed This was confirmed by SCN Pi x defoliation (P = soybean. 0.07; 1990) and herbicides x defoliation (P < 0.01; pooled over 1990 and 1991) effects on PAR interception (Fig. 2). Because these effects resembled those for yield (Fig. 1), yield losses caused by stress factors in this research can be attributed mostly to PAR interception. In fact, PAR interception explained a majority of the variation in yield each year (Fig. 3). These relationships involving PAR interception are particularly noteworthy because of possible confounding from herbicide-injured lower leaves, especially for defoliated canopies in 1990 and 1991.

Yield Components

Impacts of treatments on soybean yield components are presented in Tables 1-3. Generally, only main effects were detected. One SCN x herbicides x defoliation effect on pods $plant^{-1}$ was detected in 1989 but was believed spurious. Small sampling units (n = 5 plants) probably precluded the detection of interactions in 1990 and 1991 that would explain

interactive effects on plot yield.

As for plot yield, yield components were affected significantly in 1990 and 1991 only. SCN-infected plants had lower individual seed weights in 1990, although no main effect of SCN Pi on plot yield was found for that year. Herbicides reduced plot yield by decreasing pod number plant⁻¹ (1990) and weight seed⁻¹ (1990 and 1991). Greater injury from herbicides in 1990 (Table 2) seemingly reduced pod set as well as seed fill for that year. Defoliation caused linear reductions in pod number plant⁻¹ in 1990 and 1991. No impacts of defoliation on other yield components were found. Ostlie and Pedigo (1985) also attributed yield losses from GCW defoliation during R2 soybean development primarily to decreases in pod number.

Nematode, Weed, and Insect Management

Interactive stresses determined here have important ramifications on SCN, weed, and GCW management. Stresses from SCN and herbicides and from SCN and defoliation interacted to reduce yield in 1990. Furthermore, defoliation of herbicidestressed soybean in 1990 and 1991 resulted in more-thanadditive yield losses. These results support the increased susceptibility of SCN-infected soybean to both acifluorfen plus bentazon and GCW defoliation and of soybean treated with acifluorfen plus bentazon to GCW defoliation. In contrast, 1989 data imply that soybean greatly stressed by SCN is less

susceptible to herbicide and defoliation stresses. Practically, however, it is unlikely that soils infested with 1000 or more SCN eggs 100 cm⁻³ would be planted to SCNsusceptible soybean. Yield responses for 1989, therefore, provide little useful information for management.

Based on these results, soybean producers will be advised that current recommendations for weed and insect management may be inadequate for fields infested with SCN. Guidelines for weed management (Owen and Hartzler, 1993) do not incorporate yield losses potentially caused by postemergence applications of acifluorfen plus bentazon to these fields. Likewise, economic decision levels for GCW (Ostlie and Pedigo, 1985) do not reflect interactions between SCN and defoliation stresses. Consequently, management strategies, e.g., economic decision rules, presently used for weed and insect management for soybean fields infested with SCN may be restricting producer profits. Additional research with other SCN Pi levels and herbicide rates should be conducted for improved understanding of these interactions. Nevertheless, soybean growers should consider using alternative herbicides and (or) weed-control tactics for broadleaf weed management and lower economic decision levels for GCW management.

Because yield reductions from herbicides and defoliation over 1990 and 1991 were more than additive, economic decision levels for GCW should reflect herbicide use. Regressions of yield on GCW density pooled over 1990 and 1991 resulted in

yield-loss estimates of 0.17 and 0.34 g insect⁻¹ for soybean untreated and treated with acifluorfen plus bentazon, respectively. Discrepancies between our yield-loss estimate of 0.17 g insect⁻¹ for simulated GCW defoliation and previously published estimates of 0.46 (Hammond and Pedigo, 1982), 0.22 (Higgins et al., 1984a) and 0.21 (Ostlie and Pedigo, 1985) g insect⁻¹ likely relate to differences in canopy development as affected by soil moisture. The previous studies were conducted during conditions of moderate to severe drought (Hammond and Pedigo, 1982) and mild drought to normal moisture (Higgins et al., 1984a; Ostlie and Pedigo, 1985). In contrast, our research was conducted during conditions of normal to above normal moisture. Using procedures developed by Hammond and Pedigo (1982), separate economic injury levels were calculated for soybean untreated and treated with acifluorfen plus bentazon (Table 4) using our yield-loss estimates and combinations of pest management costs (\$17.30- $(0.19-0.38 \text{ kg}^{-1})$ and soybean market values $(0.19-0.38 \text{ kg}^{-1})$. Τn fact, relationships between GCW defoliation and yield may differ for other herbicide rates and (or) application times. For low-risk management, however, soybean producers should use these economic injury levels or those calculated from updated control costs and market values.

By invalidating the assumption that soybean stresses from SCN, acifluorfen plus bentazon, and GCW act independently, these results substantiate the importance of characterizing

interactive stresses for pest management. Applications of acifluorfen plus bentazon to soybean fields infested with SCN may have undesirable economic consequences. Additionally, use of economic decision levels for GCW not accounting for SCN and herbicide stresses could be limiting producer profits. Furthermore, evaluations of canopy interception of PAR indicate the importance of characterizing key aspects of crop physiology for providing mechanistic understandings of yield Indication of PAR interception as the unifying losses. explanation for yield losses in this work improves understanding of these interactions and enables prediction of other interactions. For instance, similar interactions are likely between many canopy-limiting factors, in addition to SCN and acifluorfen plus bentazon, and defoliating pests. In the future, assessments of canopy should be an integral component of decision making for management of defoliators. Interactive stresses must be understood and appropriately managed if pest management is to remain a viable component of sustainable agriculture.

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Table 1. Effects of at-planting soybean cyst nematode soil density (SCN Pi), acifluorfen plus bentazon rate (HERB), and simulated green cloverworm (GCW) defoliation (DEFOL) on % visual crop injury from herbicides, leaf area index (LAI) at R4 development, % canopy interception of photosynthetically active radiation (PAR) averaged over R4 and R5 development, and seed yield of soybean in 1989.+

	Crop injury							
Factor		LAI	PAR intercept	Yield (g row-m ⁻¹)	Pods plant ⁻¹	Seeds pod ⁻¹	Weight (g) seed ⁻¹	
SCN Pi+								
Medium	15.0	3.14	69.2	. 125.8	24.9	2.22	0.122	
High	13.0	3.14	71.1	135.6	24.4	2.20	0.124	
$P > \overline{F}$	NS	NS	NS	NS	NS	NS	NS	
HERB§								
None	0.0	3.31	72.2	132.6	25.0	2.25	0.122	
Low	8.9	3.16	70.5	131.2	24.8	2.17	0.126	
High	19.1	2.95	67.6	128.2	24.2	2.22	0.122	
$P > \bar{F}$								
None vs. Any		NS	NS	NS	NS	NS	NS	
Low vs. High	**	NS	NS	NS	NS	NS	NS	
DEFOL								
0 GCW row-m ⁻	1	3.57	72.7	135.0	26.2	2.27	0.124	
9 GCW row-m ⁻	1	3.08	69.4	129.7	24.4	2.16	0.127	
18 GCW row-m ⁻	1	2.97	70.3	134.0	24.2	2.21	0.121	
36 GCW row-m ⁻	1	2.95	68.0	124.1	23.9	2.21	0.121	
P > F								
Linear		*	NS	NS	NS	NS	NS	
Quadratic		NS	NS	NS	NS	NS	NS	
				•				

Vield components

Significant (P < interactions (see text)	(0.05)							
SCN x HERB		No	No	No	No	No	No	
SCN x DEFOL		No	No	No	No	No	No	
HERB x DEFOL		No	No	No	No	No	No	
SCN x HERB		No	No	No	Yes	No	No	
x DEFOL				•				
						•		

*, **, and NS = significant at 0.05 and 0.01 probability levels, and not significant, respectively.

+ Crop injury assessed at 7 d after HERB; LAI assessed at 64 d after emergence, 26 d after HERB, and 3 d after DEFOL; PAR interception assessed at 61 and 80 d after emergence; yield and yield components assessed at 123 and 121 d after emergence, respectively. # Medium = mean of 1006 eggs 100-cm⁻³ soil; high = mean of 4333 eggs 100-cm⁻³ soil. § Low = 0.28 kg a.i. ha⁻¹ acifluorfen + 0.56 kg a.i. ha⁻¹ bentazon; high = 0.56 kg a.i. ha⁻¹ acifluorfen + 0.84 kg a.i. ha⁻¹ bentazon.

Table 2. Effects of at-planting soybean cyst nematode soil density (SCN Pi), acifluorfen plus bentazon rate (HERB), and simulated green cloverworm (GCW) defoliation (DEFOL) on % visual crop injury from herbicides, leaf area index (LAI) at R4 development, % canopy interception of photosynthetically active radiation (PAR) averaged over R4 and R5 development, and seed yield of soybean in 1990.+

					Yiel	Yield components			
Factor	Crop injury	LAI	PAR intercept	Yield (g row-m ⁻¹)	Pods plant ⁻¹	Seeds pod ⁻¹	Weight (g) seed ⁻¹		
SCN Pi+				-					
None	27.5	2.95	76.8	218.8	25.7	2.53	0.132		
Low	30.3	2.84	75.1	209.6	24.0	2.49	0.126		
P > F	* NS NS			NS	NS	**			
HERB§									
None	0.0	3.41	82.6	239.2	27.9	2.54	0.133		
High	28.9	2.38	69.3	189.3	22.0	2.49	0.126		
P > F		**	**	**	**	NS	*		
DEFOL	_								
0 GCW row	v-m ⁻¹	3.14	79.5	229.1	26.2	2.47	0.129		
18 GCW row	w-m ⁻¹	3.28	80.7	231.9	28.4	2.51	0.136		
72 GCW row	w-m ⁻¹	2.82	76.5	215.8	24.2	2.53	0.127		
144 GCW row	w-m ⁻¹	2.33	67.1	180.0	21.1	2.53	0.126		
P > F									
Linear		**	**	**	**	NS	NS		
Quadratic		NS	*	*	NS	NS	NS		

Significant (P -	< 0.05)						
interactions							
(see text)							
SCN x HERB		NS	Yes	Yes	No	No	No
SCN x DEFOL		NS	No	Yes	No	No	No
HERB x DEFOL		NS	Yes	Yes	No	No	No
SCN x HERB		NS	No	No	No	No	No
x DEFOL							

*, **, and NS = significant at 0.05 and 0.01 probability levels, and not significant, respectively.

+ Crop injury assessed at 8 d after HERB; LAI assessed at 62 d after emergence, 20 d after HERB, and 3 d after DEFOL; PAR interception assessed at 60 and 69 d after emergence; yield and yield components assessed at 114 and 113 d after emergence, respectively. \pm Low = mean of 184 eggs 100-cm⁻³ soil. § High = 0.56 kg a.i. ha⁻¹ acifluorfen + 0.84 kg a.i. ha⁻¹ bentazon.

Table 3. Effects of at-planting soybean cyst nematode soil density (SCN Pi), acifluorfen plus bentazon rate (HERB), and simulated green cloverworm (GCW) defoliation (DEFOL) on % visual crop injury from herbicides, leaf area index (LAI) at R4 development, % canopy interception of photosynthetically active radiation (PAR) averaged over R4 and R5 development, and seed yield of soybean in 1991.+

					Yield components			
Factor	Crop injury	LAI	PAR intercept	Yield (g row-m ⁻¹)	Pods plant	Weight (g) seed ⁻¹		
SCN Pi+								
None	19.7	4.50	90.1	251.9	26.8	2.61	0.162	
Low	18.8	4.95	91.1	255.3	26.6	2.63	0.157	
P > F	NS	' NS	NS	NS	NS	NS	NS	
HERB§								
None	0.0	5.37	93.3	264.7	26.3	2.62	0.165	
High	19.2	4.08	87.8	242.5	27.1	2.61	0.154	
P > F		**	**	**	NS	NS	**	
DEFOL								
0 GCW ro	w-m ⁻¹	5.45	95.2	262.5	28.8	2.59	0.162	
18 GCW ro	$w-m^{-1}$	4.78	91.8	259.6	27.1	2.62	0.160	
72 GCW ro	$w - m^{-1}$	4.31	90.7	253.3	26.6	2.63	0.159	
144 GCW ro	w-m ⁻¹	4.36	84.5	239.1	24.3	2.62	0.157	
P > F								
Linear		*	**	**	**	NS	NS	
Quadratic		*	NS	NS	NS	NS	NS	

Significant (P < interactions (see text)	< 0.05)						
SCN x HERB		No	No	No	No	No	No
SCN x DEFOL		No	No	No	No	No	No
HERB x DEFOL		No	No	No	No	No	No
SCN x HERB		No	No	No	No	No	No
x DEFOL							

*, **, and NS = significant at 0.05 and 0.01 probability levels, and not significant, respectively.

+ Crop injury assessed at 7 d after HERB; LAI assessed at 55 d after emergence, 26 d after HERB, and 4 d after DEFOL; PAR interception assessed at 58 and 68 d after emergence; yield and yield components assessed at 111 and 110 d after emergence, respectively. \neq Low = mean of 100 eggs 100-cm⁻³ soil. § High = 0.56 kg a.i. ha⁻¹ acifluorfen + 0.84 kg a.i. ha⁻¹ bentazon.

			Pest mana	agement cos	sts, \$ ha ⁻¹	^l (\$ ac ⁻¹)		-
	17.3	(7.00)	19.8	(8.00)	22.2	(9.00)	24.7	(10.00)
Soybean market value, \$ kg ⁻¹ (\$ bu ⁻¹)	Herb	None	Herb	None	Herb	None	Herb	None
0.19 (5.00)	. 20.4	40.7	23.3	46.6	26.1	52.2	29.0	58.1
0.23 (6.00)	16.8	33.6	19.2	38.5	21.6	43.2	24.0	48.0
0.27 (7.00)	14.3	28.6	16.4	32.8	18.4	36.8	20.4	40.9
0.30 (8.00)	12.9	25.8	14.8	29.5	16.5	33.1	18.4	36.8
. 0.34 (9.00)	11.4	22.7	13.0	26.0	14.6	29.2	16.2	32.5
0.38 (10.00)	10.2	20.4	11.6	23.3	13.0	16.1	14.5	29.0

Table 4. Economic injury levels for green cloverworm (insects row-m⁻¹) on flowering soybean after (Herb) and without (None) postemergence applications of acifluorfen plus bentazon herbicides.

Presented values based on 0.76-m row spacing and 25 plants m^{-1} .

.

Figure 1. Effects of at-planting soybean cyst nematode density (SCN Pi) x acifluorfen plus bentazon rate (Herbicides) on soybean yield in 1990 (A; P < 0.05), SCN Pi x simulated green cloverworm (GCW) defoliation on yield in 1990 (B; P <0.05), and Herbicides x GCW defoliation on yield pooled over 1990 and 1991 (C; P < 0.01).</p>



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Figure 2. Effects of at-planting soybean cyst nematode density (SCN Pi) x acifluorfen plus bentazon rate (Herbicides) on % canopy interception of photosynthetically active radiation (PAR) during soybean seed fill in 1990 (A; P < 0.05), SCN Pi x simulated green cloverworm (GCW) defoliation on PAR interception during seed fill in 1990 (B; P = 0.07), and Herbicides x GCW defoliation on PAR interception during seed fill pooled over 1990 and 1991 (C; P < 0.01).</p>





Defoliation (QCWs row-m⁻¹)



Figure 3. Regressions of soybean seed yield on % canopy interception of photosynthetically active radiation (PAR) during seed fill for 1989, 1990, and 1991.



GENERAL CONCLUSIONS

All objectives of this research (pp. 3-4) were accomplished. Because of the success of this work, methods used here are recommended for similar studies on stress interactions (objective 1). Procedures used for simulating defoliation (Ostlie 1984) and characterizing soybean stress (e.g., plant sampling, canopy interception of photosynthetically active radiation (PAR)) were developed elsewhere (Higgins et al. 1983, Higgins et al. 1984a,b, unpubl. protocols CSRS/USDA Regional Research Project S-219). Other methods were developed specifically for this work, including a technique for infesting soil with soybean cyst nematode (SCN). Use of this technique resulted in SCN densities at 78 and 90% of expected densities for 1990 and 1991, respectively. This method is advocated for establishing soil densities of SCN for various research, including that for stress interactions.

In retrospect, the success of this work can be attributed to techniques used for establishing precise levels of all stresses. Effective experiments, particularly those involving interactions, are dependent on the precision of treatments. With lower levels of precision in this research, for instance, large experimental errors may have caused the erroneous conclusion of no interactions among SCN, acifluorfen plus bentazon, and defoliation stresses. In this regard, suitable

methods for simulating stresses (e.g., simulated defoliation) should be considered.

Impacts of acifluorfen plus bentazon herbicides (applied at V6 soybean development) and green cloverworm (GCW) defoliation (simulated during R2 to R4 soybean development) on SCN populations in soil were evaluated (objective 2). Herbicide applications caused significant reductions in SCN egg densities in 1988, and pooled over 1990 and 1991. Similar reductions were significant for densities of SCN infective juveniles in 1988 and near significant in 1989. Because these herbicides translocate minimally in soybean and bind rapidly to soil colloids, direct contact of parent compounds with SCN in roots or soil is unlikely. Causal mechanisms for density decreases, therefore, probably relate to stress-induced alterations in soybean physiology. Future work should be directed towards determining precise mechanisms. Despite decreasing SCN densities, herbicide applications to plots infested with SCN never resulted in significant yield increases. Additional research relating earlier herbicide. applications, SCN densities, and yield should be conducted. SCN densities generally were unaffected by simulated GCW defoliation, although egg densities were increased significantly in 1989.

Individual and interactive effects of at-planting SCN density (Pi), acifluorfen plus bentazon rate, and simulated GCW defoliation level on soybean growth (objective 3) and

yield (objective 4) were found. Because of extreme environmental confounding from iron deficiency chlorosis and drought in 1988, only 1989, 1990, and 1991 data were used for evaluations of soybean response. Herbicides always caused soybean stress, as indicated by lower stomatal conductance and by visual crop injury (foliar chlorosis and necrosis). Likewise, defoliation reduced plant leaf area and leaf area index for each year. However, herbicide and defoliation stresses limited soybean growth and yield in 1990 and 1991 only. Excessive field-wide stress from SCN likely precluded SCN Pi effects and desensitized responses to herbicides and defoliation in 1989. At R4 soybean development for both years, leaf area, plant height, pod number, and leaf, pod, and support (stem and petiole) dry weights were lower for plants treated than untreated with herbicides. Defoliation limited plant height and leaf dry weight at R4 soybean development for both years. Herbicides and defoliation, individually, reduced plot yield in 1990 and 1991. Yield reductions from herbicides were attributed to decreased numbers of pods per plant (1990) and weight per seed (1990 and 1991), while defoliation decreased numbers of pods per plant (1990 and 1991). Herbicide and defoliation stresses also interacted to reduce plot yield pooled over 1990 and 1991, although similar effects on yield components were not detected. Because these stresses similarly affected yield and canopy interception of PAR during seed fill, PAR interception provided a physiological

explanation for yield reductions from herbicides and defoliation.

Although different densities of SCN were established each year, stress from SCN Pi alone generally did not affect soybean growth and yield. Similar stress for SCN Pi levels in 1989 and extremely low SCN densities in 1991 likely precluded responses to SCN Pi. In 1990, however, SCN Pi and herbicides interacted to lower stomatal conductance and increase visual crop injury. Greater injury for SCN-infected soybean treated with herbicides resulted in significant (pod number) and near significant (leaf area, leaf number, and pod weight) SCN Pi x herbicides effects on reducing plant growth at R4 soybean development. Moreover, SCN Pi interacted with herbicides and with defoliation in 1990 to reduce seed yield, although no interactive effects on yield components were found. As for herbicide and defoliation effects, SCN Pi x herbicides and SCN Pi x defoliation effects on yield and canopy interception of PAR were similar. PAR interception during seed fill, therefore, was a key physiological mechanism underlying all yield reductions to SCN, herbicides, and defoliation in this research.

Low-risk management recommendations were made to deal with the interactive stresses determined here (objective 4). Because of the increased susceptibility of soybean treated with acifluorfen plus bentazon to yield losses from defoliation, separate economic injury levels for GCW were
calculated for soybean treated and untreated with these herbicides. Calculations were based on yield-loss estimates of 0.17 (no herbicides) and 0.34 (herbicides) g per insect and combinations of pest management costs and soybean market values. Soybean growers will be encouraged to use these levels for GCW management. Because of potential SCN x herbicides and SCN x defoliation effects on yield, growers should consider using alternative herbicides and (or) weedcontrol tactics for broadleaf weed management and lower economic decision levels for GCW in SCN-infested fields. Use of these recommendations should sustain grower profits. Results of this work substantiate the importance of characterizing and appropriately managing interactive stresses to ensure that pest management remains a viable component of agriculture in the future.

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