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Development of *Heterodera glycines* on Soybean Damaged by Soybean Looper and Stem Canker[1]

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Abstract: Short-term greenhouse studies with soybean (*Glycine max* cv. Bragg) were used to examine interactions between the soybean cyst nematode (*Heterodera glycines*) and two other common pests of soybean, the stem canker fungus (*Diaporthe phaseolorum* var. *caulivora*) and the soybean looper (*Pseudoplusia includens*), a lepidopterous defoliator. Numbers of cyst nematode juveniles in roots and numbers of cysts in soil and roots were reduced on plants with stem cankers. Defoliation by soybean looper larvae had the opposite effect; defoliation levels of 22 and 64% caused stepwise increases in numbers of juveniles and cysts in both roots and soil, whereas numbers of females in roots decreased. In two experiments, stem canker length was reduced 40 and 45% when root systems were colonized by the soybean cyst nematode. The absence of significant interactions among these pests indicates that the effects of soybean cyst nematode, stem canker, and soybean looper on plant growth and each other primarily were additive.

Key words: Diaporthe phaseolorum, Glycine max, Heterodera glycines, insect defoliation, pest complex, *Pseudoplusia includens,* soybean, soybean cyst nematode, soybean looper, stem canker.

The soybean cyst nematode (*Heterodera glycines* Ichinohe), the stem canker fungus (*Diaporthe phaseolorum* (Cke. and Ell.) Sacc. var. *caulivora* Athow and Caldwell), and the soybean looper (*Pseudoplusia includens* (Walker)) are among the most destructive pests of soybean (*Glycine max* (L.) Merrill) in the southeastern United States (5,10). Soybean cyst nematode has a worldwide distribution and is an important nematode pest of soybean in the United States (16,17). Soybean stem canker has emerged as a disease of major economic importance during the past decade (1), and soybean looper ranks among the most important defoliating insect pests of soybean in the region (5).

All three pest species can occur concurrently in soybean fields (11). Although the individual effects of these pests on soybean have been studied, relatively little research has focused on their combined effects on soybean or their effects on each other. Such information is crucial for formulation of pest management strategies. The objectives of this study were 1) to examine development and composition of soybean cyst nematode populations in plants damaged by soybean looper and stem canker and 2) to determine effects of cyst nematode on the severity of stem canker.

Materials and Methods

General procedures: Experiments were conducted in a greenhouse. Seeds of the soybean cultivar Bragg were treated with a commercial, peat-based inoculant of *Bradyrhizobium japonicum* Kirchner (Buchanan) and planted in 7.6-liter plastic pots (five seeds per pot) containing ca. 6 kg autoclaved soil (78% sand, 16% silt, 6% clay, 0.24% organic matter). Soil pH 6.2 was obtained by adding aluminum sulfate (4 g/kg). Seedlings were thinned to two per pot 1 week after emergence. Each pot received 70 μg/kg K as KCl at planting, as dictated by soil nutrient analyses.

The cyst nematode isolate (race 3) was propagated on soybean cultivar Lee 74 in a greenhouse. Cysts were recovered from soil using a modified sugar-flotation technique (6). Cysts were blended in water for ca. 30 seconds to release eggs. Number of eggs per milliliter and egg viability were

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estimated in the laboratory prior to soil infestation. When plants were in the V1 (unifoliate) growth stage (4), 1 ml of egg suspension was pipetted into a single depression (4 cm deep) in the soil between the two plants.

Fungal inoculum was prepared as described by Russin et al. (13). Soybean stems were inoculated by inserting mycelium-infested toothpick sections into vertical incisions (10 mm long) made between the unifoliate and first trifoliate nodes. Mycelium-free toothpick sections were placed similarly in control plants.

Defoliation was accomplished by placing neonate soybean looper larvae on plants and allowing them to feed until pupation (7). The numbers of larvae per plant are listed in methods for individual experiments.

Pots within experiments were completely randomized with factorial arrangement of treatments. Data were analyzed using PROC GLM (15) to test for main treatment effects and interactions. When the number of treatment levels exceeded two, orthogonal contrasts were used to test for differences between levels.

Experiment 1. Nematode–fungus relationships: Treatments consisted of two levels each of nematode and fungus (not inoculated and inoculated), for a total of four treatment combinations each replicated 15 times. Seeds planted in 60 pots on 15 October 1986 were maintained under supplemental fluorescent lighting (16 hours per day) to delay onset of flowering. When plants reached the V1 growth stage (16 days after planting), ca. 1,500 eggs (ca. 60% viability) of soybean cyst nematode were introduced into each of 30 pots. Remaining pots did not receive nematode inoculum. When plants reached V8 (47 days after planting), those in 30 pots (15 with nematodes, 15 without) were inoculated with *D. phaseolorum* var. *caulivora.* Plants in remaining pots did not receive fungal inoculum. Stem canker disease severity was determined by measuring canker lengths 32 days after fungal inoculation (plants in V15 growth stage). At this time, cankers were enlarging rapidly but had not yet caused mortality. Plants were harvested 79 days after planting by severing stems at the cotyledonary node. Leaf areas were determined using an area meter (Li-Cor model LI-3100). Stems and roots were weighed after drying at 60 C for 72 hours. Before drying, root systems of all plants were rated for numbers of visible females and cysts with the following scale: 0 = no cysts, 1 = 1–25 cysts, 2 = 26–50 cysts, 3 = 51–75 cysts, 4 = > 75 cysts. Subsamples of root tissue (ca. 0.5 g fresh weight) were obtained from five replicates of each treatment, cleared, and stained (3); numbers of juveniles and females were counted.

Experiment 2. Nematode–fungus–insect relationships: Treatments consisted of two levels each of nematode and fungus (not inoculated and inoculated) and three levels of soybean looper larvae (zero, low, and moderate), for a total of 12 treatment combinations each replicated 10 times. Seeds planted in 120 pots on 4 December 1986 were maintained under supplemental lighting as described for experiment 1. When plants reached the V1 growth stage (13 days after planting), ca. 500 eggs (ca. 80% viability) of soybean cyst nematode were introduced into each of 60 pots. Twenty days later (plants in V4), plants in 60 pots (30 with nematodes, 30 without) were inoculated with *D. phaseolorum* var. *caulivora* as described for experiment 1. At 43 days after planting (plants in V6), plants in each pot received 0, 6, or 24 neonate soybean looper larvae. Stem canker disease severity was determined 31 days after fungal inoculation (plants in V9). Soybean looper larvae fed for 27 days, at which time defoliation levels of 0, 22, and 64% had been achieved, larvae had reached the pupal stage, and plants were in V11. Plants were harvested 70 days after planting. Leaf areas and dry weights of stems and roots were determined, and percentages of defoliation were calculated on the basis of (mean leaf area of control − mean leaf area of defoliated)/mean leaf area of control × 100. Before drying, root systems were rated for visible cysts as described for ex-

TABLE 1. Effects of soybean cyst nematode and stem canker on selected growth parameters of Bragg soybean in greenhouse experiment 1.

Pest	Level	Leaf area (cm^2)	Stem dry weight (g)	Root dry weight (g)
Soybean cyst nematode	0†	1,880	5.6	2.7
	1	1,703	4.9	2.5
Stem canker fungus	0	2,545	5.9	3.0
	1	1,060	4.6	2.3
Source	df	*F, P*	*F, P*	*F, P*
Nematode	1	1.03, 0.3145	7.59, 0.0079	1.52, 0.2228
Fungus	1	52.34, 0.0001	20.78, 0.0001	22.94, 0.0001
N × F	1	2.88, 0.0953	3.82, 0.0556	0.00, 0.9508
Error mean square	55	624,549.39	1.26	0.35

† 0 = not inoculated; 1 = inoculated.

periment 1. Subsamples of root tissue from four replicates of each treatment were stained, and numbers of soybean cyst nematode juveniles and females were determined as described for experiment 1. Soil samples (150 cm^3) also were taken from these four replicates, and soybean cyst nematode juveniles and cysts were extracted from soil with the sugar-flotation technique and counted.

RESULTS

Plant development: In experiment 1, plants inoculated with soybean cyst nematode had lower ($P < 0.05$) stem dry weights than did control plants (Table 1). All three growth parameters were reduced ($P < 0.05$) in plants with stem cankers. Stem dry weights were affected by a nematode × fungus interaction ($P = 0.0556$). Examination of individual treatment means revealed an antagonistic relationship between nematode and fungus; stem dry weight reductions in plants damaged by both pests were less than the combined reductions caused by each pest individually (Fig. 1).

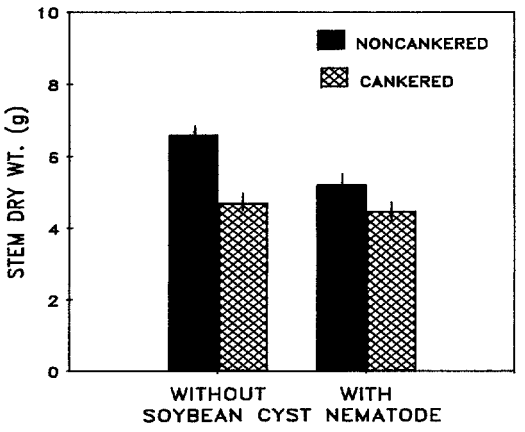

FIG. 1. Individual treatment means for the interaction between soybean cyst nematode and stem canker on stem dry weight in experiment 1. Vertical lines delimit standard errors of means.

Plant growth in experiment 2, terminated 12 February 1987, was ca. one-third that in experiment 1, terminated 2 January 1987 (Tables 1, 2). In experiment 2, soybean cyst nematode did not reduce plant growth (Table 2). Stem canker caused consistent reductions ($P < 0.05$) in leaf areas and stem and root dry weights. Defoliation by soybean looper larvae caused stepwise reductions ($P < 0.05$) in all three plant growth parameters. Leaf area was affected by a nematode × insect interaction ($P = 0.0543$). Individual treatment means revealed an antagonistic relationship between nematode and insect similar to that described for nematode and fungus (Fig. 2).

Nematode development: In experiment 1, stem canker reduced ($P < 0.05$) both cyst rating and the number of juveniles and caused a lesser reduction ($P = 0.0920$) in total nematode number (Table 3).

In experiment 2, the number of cysts in soil was lower ($P < 0.05$) on cankered than on noncankered plants (Table 3). Plants

TABLE 2. Effects of soybean cyst nematode, stem canker, and soybean looper on selected growth parameters of Bragg soybean in greenhouse experiment 2.

Pest	Level	Leaf area (cm^2)	Stem dry weight (g)	Root dry weight (g)
Soybean cyst nematode	0†	656	1.8	1.1
	1	647	1.7	1.0
Stem canker fungus	0	759	1.9	1.1
	1	544	1.7	0.9
Soybean looper	0‡	916	2.1	1.3
	1	710	1.8	1.0
	2	329	1.4	0.7
Contrast	df	*F, P*	*F, P*	*F, P*
0 vs. 1 + 2	1	118.83, 0.0001	46.96, 0.0001	74.56, 0.0001
1 vs. 2	1	82.61, 0.0001	22.13, 0.0001	21.87, 0.0001
Source	df	*F, P*	*F, P*	*F, P*
Nematode	1	0.70, 0.7863	1.74, 0.1905	1.53, 0.2183
Fungus	1	39.40, 0.0001	8.30, 0.0048	13.30, 0.0004
Insect	2	100.72, 0.0001	35.54, 0.0001	48.21, 0.0001
N × F	1	1.06, 0.3062	0.00, 0.9822	0.30, 0.5832
N × I	2	2.99, 0.0543	2.34, 0.1015	2.59, 0.0798
F × I	2	0.91, 0.4070	0.42, 0.6559	1.64, 0.1985
N × F × I	2	0.12, 0.8912	0.11, 0.9002	0.32, 0.7264
Error mean square	108	35,259.90	0.17	0.07

† 0 = not inoculated; 1 = inoculated.
‡ 0 = not infested; 1 = infested with 6 larvae; 2 = infested with 24 larvae.

defoliated by soybean looper larvae had higher ($P < 0.05$) cyst ratings, numbers of juveniles and total nematodes in roots, and numbers of J2 and cysts in soil. Conversely, the number of nematode females was lower ($P < 0.05$) on defoliated than on nondefoliated plants. No significant fungus × insect interactions were detected for any measured parameter.

Canker development: Stem canker severity, as measured by canker length, was 40 and 45% less in experiments 1 and 2, respectively, when roots were colonized by the soybean cyst nematode than when plants had stem cankers alone (Fig. 3). Reisolations from cankered tissue verified presence of the fungus. The nematode × insect interaction did not affect canker length.

DISCUSSION

Plant growth was less in experiment 1 than in experiment 2, probably because of an extended period of low temperatures during the last half of January 1987; however, actual temperatures in greenhouses were not recorded. The cold period probably also increased the time required for soybean looper larvae to develop to pupae from 18 to 21 days in previous studies (unpubl.) to 27 days in our study.

All three pest species primarily had ad-

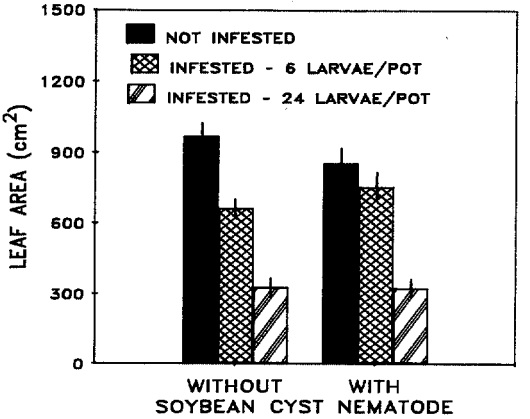

FIG. 2. Individual treatment means for the interaction between soybean cyst nematode and soybean looper on leaf area in experiment 2. Vertical lines delimit standard errors of means.

TABLE 3. Effects of stem canker and soybean looper on soybean cyst nematode populations developing on Bragg soybean in two greenhouse experiments.

			Number of nematodes				
			0.5 g root tissue			150 cm³ soil	
Pest	Level	Cyst rating	Juveniles	Females	Total	J2	Cysts
			Experiment 1				
Stem canker	0†	2.2	23	8	31		
fungus	1	1.4	3	8	11		
Source	df	*F, P*	*F, P*	*F, P*	*F, P*		
Fungus	1	4.81, 0.0374	7.62, 0.0247	0.02, 0.8834	3.66, 0.0920		
			Number of nematodes				
			0.5 g root tissue			150 cm³ soil	
Pest	Level	Cyst rating	Juveniles	Females	Total	J2	Cysts
			Experiment 2				
Stem canker	0	0.8	52	9	61	249	19
fungus	1	0.6	48	8	56	226	10
Soybean	0‡	0.5	27	15	42	165	8
looper	1	0.5	42	9	51	204	13
	2	1.1	80	1	81	342	23
Contrast	df	*F, P*	*F, P*	*F, P*	*F, P*	*F, P*	*F, P*
0 vs. 1 + 2	1	1.92, 0.1719	11.64, 0.0031	15.20, 0.0011	5.65, 0.0288	4.36, 0.0513	5.41, 0.0319
1 vs. 2	1	5.75, 0.0200	10.16, 0.0051	8.03, 0.0110	5.87, 0.0261	5.32, 0.0332	3.58, 0.0746
Source	df	*F, P*	*F, P*	*F, P*	*F, P*	*F, P*	*F, P*
Fungus	1	1.70, 0.1973	0.20, 0.6634	0.07, 0.7885	0.25, 0.6209	0.22, 0.6414	4.41, 0.0501
Insect	2	3.83, 0.0277	10.90, 0.0008	11.62, 0.0006	5.76, 0.0117	4.84, 0.0208	4.50, 0.0261
F × I	2	0.75, 0.4793	1.13, 0.3449	0.40, 0.6779	0.81, 0.4618	0.44, 0.6527	1.33, 0.2892
Error mean square	18	0.63§	553.51	35.97	572.76	14,248.68	106.14

† 0 = not inoculated; 1 = inoculated.
‡ 0 = not infested; 1 = infested with 6 larvae; 2 = infested with 24 larvae.
§ df = 54.

ditive effects on plant growth. The interactions that affected plant growth exhibited a similar antagonistic relationship between pests; i.e., effects of both pests together were less than the combined effects of each pest alone. These results suggest a limit to the amount of growth reduction that was possible under our experimental conditions.

A portion of the nematode population increase on defoliated plants probably resulted from increased densities of nematodes on roots due to smaller root systems; however, the increases in population density were greater than could be attributed to reduced root size alone. For example, plants that sustained a 46% decrease (1.3 vs. 0.7 g) in root dry weight exhibited nearly a threefold increase (27 vs. 80) in density of juveniles (Tables 2, 3). The higher juvenile numbers, higher cyst ratings, and reduced numbers of females suggest an accelerated maturation rate for soybean cyst nematode on roots of defoliated plants. Plants with stem cankers exhibited decreases in overall nematode populations in both roots and soil.

Soybean looper and stem canker could have affected the nematode in several ways. Reduced leaf area was a direct effect of soybean looper on the soybean plants; reductions in root and stem weights were indirect effects of diminished photosynthate in defoliated plants. Therefore, nematode

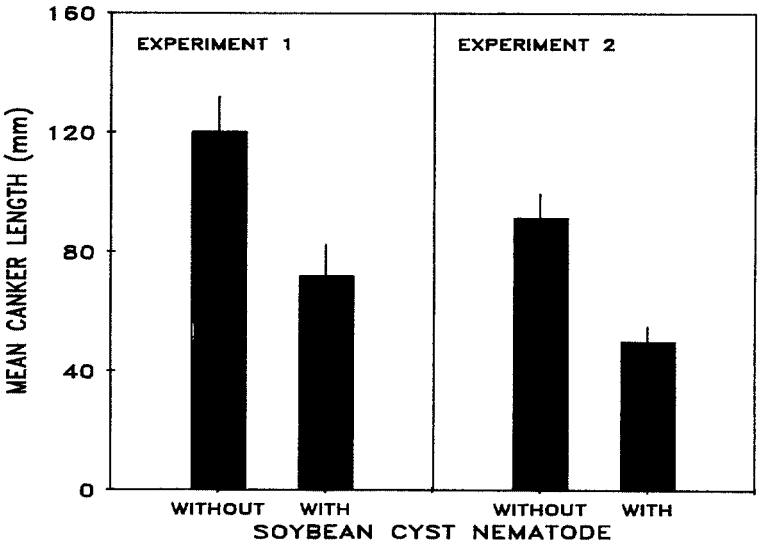

FIG. 3. Lengths of stem cankers on soybean plants noninoculated or inoculated with soybean cyst nematode in two greenhouse experiments. Vertical bars delimit standard errors of means.

population development was enhanced in plants that had lower levels of photosynthate caused by defoliation. Effects of stem canker on plant growth parameters were similar to those of the soybean looper (Tables 1, 2). Despite the similarity, the effects of the fungus on soybean cyst nematode were opposite to those of the insect. Stem canker apparently does not affect cyst nematode indirectly through its effects on the host plants, as does the insect. Rather, the effect may be more direct, such as production of a translocatable substance that can affect nematodes in roots. A toxic metabolite(s) from culture filtrates of *D. phaseolorum* var. *caulivora* induces typical disease symptoms in susceptible plants (2); however, translocation of this toxin into roots and its effects on soybean cyst nematode have not been demonstrated.

Stem canker length consistently was reduced when roots were colonized by the cyst nematode, even though the nematodes had minimal impact on plant growth. This suggests that development of stem cankers may be influenced by subtle physiological changes induced in soybean plants by the cyst nematode. Previous studies on soybean have reported that lengths of stem cankers were reduced on defoliated plants (14) but increased if stems were girdled by the threecornered alfalfa hopper, *Spissistilus festinus* (13). Plant-parasitic nematodes also have been reported to influence stem pathogens on other hosts. *Criconemella xenoplax* has been shown to predispose peach trees to bacterial canker caused by *Pseudomonas syringae* pv. *syringae* (8,9). In addition, *C. xenoplax* feeding in roots was reported to cause biochemical changes in shoots that may have a role in predisposition of peach trees to peach tree short life syndrome (12). *Heterodera glycines* and *D. phaseolorum* var. *caulivora* may have a similar relationship on soybean.

The present results partially describe a complex relationship between soybean cyst nematode, soybean looper, and stem canker on soybean that reflects the extent to which pest species can influence development of one another. These results were obtained from short-term greenhouse studies using only vegetative soybean plants. There is an obvious need for long-term field experiments that can examine these interactions under natural conditions over the full growing season.

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