Severity of Soybean Stem Canker Disease Affected by Insect-Induced Defoliation

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ABSTRACT

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Greenhouse studies using the soybean (*Glycine max*) cultivar Bragg showed a significant ($P < 0.0001$) negative relationship ($Y = 102.636 - 1.066X + 0.006X^2$) between lengths of stem cankers incited by *Diaporthe phaseolorum* var. *caulivora* and defoliation caused by larvae of the soybean looper, *Pseudoplusia includens*. Reductions in length were greatest when cankers developed at times when defoliation was just completed. Plants allowed 22 or 43 days to recover from 30% defoliation grew sufficient new foliage so that leaf areas did not differ significantly from those of nondefoliated plants. Consequently, lengths of stem cankers on defoliated and nondefoliated plants also did not differ significantly at these times. Nitrogen provided to plants by fertilizing with NH_4NO_3 or by treating seeds with commercial *Bradyrhizobium japonicum* inoculant before planting increased plant growth and decreased stem canker lengths.

Additional keywords: insect-disease interactions, integrated pest management

More than 25 species of insects, pathogens, and weeds have been listed as pests of soybean (*Glycine max* (L.) Merr.) in the southeastern United States, and all of these can have individual and combined effects on yield (11). The need for thresholds for pest complexes in addition to those for individual species has been stressed (11). Historically, however, research has concentrated on injury caused by individual species and relatively little attention has been given to the almost innumerable interactions that probably occur among these species. Research on pest interactions is necessary if comprehensive integrated pest management programs for soybean are to be developed.

Recent efforts in Louisiana have focused on interactions among important soybean pests. Lengths of stem cankers, caused by *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. var. *caulivora* Athow & Caldwell, were greater on stems with basal girdles caused by the threecornered alfalfa hopper (*Spissistilus festinus* Say) than on nongirdled stems (12). Girdles also predisposed soybean stems to infection by *Sclerotium rolfsii* Sacc. (6). However, these girdles had little effect on incidence and severity of pod and stem blight and stem anthracnose, caused by *Phomopsis sojae* Leh. and *Colletotrichum truncatum* (Schw.) Andrus & Moore, respectively (13). Other studies have demonstrated changes in incidence of certain seedborne microorganisms in soybean seeds damaged by stink bug feeding (14). Results from recent greenhouse experiments indicated that populations of soybean cyst nematode (*Heterodera glycines* Ichinohe) were increased in plants defoliated by the soybean looper (*Pseudoplusia includens* (Walker)) but decreased in plants damaged by the stem canker fungus (15). Also, stem canker lengths were reduced in plants damaged by soybean cyst nematode (15).

The stem canker fungus and the soybean looper are major pests of soybean in the Southeast. Stem canker was first reported from the region in 1973 and since that time has become a serious problem, causing losses in 1983 that exceeded $59 million (1,5,10). Soybean looper is a member of a complex of lepidopterous defoliators that also includes velvetbean caterpillar (*Anticarsia gemmatalis* Hübner) and green cloverworm (*Plathypena scabra* (F.)) (17). Losses and costs of control for these pests in the southeastern region totaled more than $37 million in 1984 (4).

Although infection occurs in early vegetative stages, symptoms of stem canker disease generally are not visible until soybean plants are in reproductive stages (1). Soybean looper and velvetbean caterpillar are migratory pests and also are not usually present in soybean fields until late in the season. However, defoliation of soybean by nonmigrating species such as green cloverworm and bean leaf beetle (*Cerotoma trifurcata* (Forster)) can occur in Louisiana throughout the growing season. Therefore, stem cankers can develop in soybean plants at different times relative to the occurrence of defoliation, i.e., before damage occurs, when defoliation is ongoing or recently completed, or after defoliation has occurred and plants have had an opportunity to recover by producing new foliage. The objectives of this study were: 1) to determine the effect of defoliation by soybean looper larvae on severity of stem canker disease and 2) to examine canker severity in soybean plants that had recovered from defoliation by producing new foliage. Studies to meet these objectives further revealed that added nitrogen also had a pronounced effect on stem canker severity, and preliminary results from these studies are presented.

MATERIALS AND METHODS

General procedures. All experiments were conducted in a greenhouse. Susceptible soybean cultivar Bragg was planted at a rate of five seeds per pot in 7.6-L plastic pots that contained about 6 kg of sandy loam soil. Seed in experiment 1 were treated as described in procedures for that experiment; seed in experiments 2 and 3 were treated before planting with commercial inoculant of the N_2-fixing bacterium *Bradyrhizobium japonicum* (Kirchner) Buchanan (The Nitragin Company, Milwaukee, WI). After emergence, plants were thinned to two uniform seedlings per pot, which constituted an experimental unit. In experiment 1, soil was amended with a total of 22 mg/kg of phosphorus

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($Ca(H_2PO_4)_2$) and 40 mg/kg of potassium (KCl), added in two equal applications at 28 and 58 days after planting. A separate lot of sandy loam soil from the same source was used in experiments 2 and 3 and was amended at planting with 70 mg/kg of KCl and with 4 g/kg of $Al_2(SO_4)_3$ to obtain soil pH 6.2. Soil from both lots was amended according to recommendations based on soil test results.

Inoculum of the stem canker fungus was prepared as described by Russin et al (12). Soybean plants were inoculated by inserting mycelium-infested toothpick sections into vertical incisions (1 cm long) made in the stem between the unifoliate and first trifoliate nodes. In previous studies, this technique resulted in a 3- to 4-wk delay between inoculation and initial development of visible canker symptoms. Control plants were wounded similarly but received sterile toothpick sections. Canker length was measured 6–8 days after initial symptom development and served as an estimate of stem canker disease severity. Lengths of cankers on both stems in a pot were averaged to obtain a mean canker length per experimental unit.

Defoliation was accomplished by infesting soybean plants with neonate soybean looper larvae, which were allowed to feed until acceptable levels of defoliation were achieved (8,9). Plants were examined daily and larvae found on noninfested plants were removed. These larvae, in addition to those that wandered off plants, were replaced on the basis of the degree of defoliation sustained by an experimental unit relative to others in the same treatment.

All experiments were established in a completely random design. Data were analyzed as described for each experiment.

Experiment 1. This was an initial study to test the effect of insect defoliation on severity of stem canker disease. It was conducted in conjunction with another preliminary study that examined canker disease severity in response to added nitrogen. Treatments consisted of a single level of stem canker fungus (inoculated), two levels of insect infestation (0 and 25 larvae per pot), and three levels of nitrogen: 1) nonamended sandy loam soil, 2) seeds coated with commercial bacterial inoculant before being planted in nonamended soil, or 3) seeds not treated and soil amended at 20 and 36 days after planting with a total of 66 mg/kg of nitrogen (NH_4NO_3). There were six treatment combinations and each was replicated eight times. Seed were planted in 48 pots on 30 January 1986, and pots were assigned at random to receive one of the nitrogen treatments. At the V4 growth stage (3) (34 days after planting), the stem canker fungus was inoculated into plants in all pots. Nine days later (plants in V7), plants in 24 pots were infested with soybean looper larvae; plants in the remaining pots were not infested. Larvae fed for 18 days, by which time defoliation was completed and most larvae had pupated. Stem canker lengths were measured 35 days after fungal inoculation (plants in V12). Plants then were harvested by cutting stems at cotyledonary nodes. Leaf areas per two plants were determined using an area meter (Li-Cor LI-3100), and stem weights per two plants were measured after drying at 60 C for 72 hr. Data were analyzed using the PROC GLM procedure of SAS (16). When the number of treatment levels exceeded two, orthogonal contrasts were used to compare treatments.

Experiment 2. This experiment estimated severity of stem canker disease over a range of defoliation levels. Treatments consisted of a single level of stem canker fungus (inoculated) and four levels of insect infestation (0, 10, 20, and 40 larvae per pot), for a total of four treatment combinations, each replicated 10 times. Seed were planted in 40 pots on 13 August 1987. Thirty-four days after planting (plants in V7), plants in all pots were infested with neonate soybean looper larvae at one of the four described densities. Five days after infestation (plants in R1), the stem canker fungus was inoculated into plants in all pots. Larvae fed for 21 days, by which time defoliation was completed and larvae had developed to prepupal or pupal stages. Lengths of stem cankers were measured 5 days later (plants in R3). Plants then were harvested, leaf areas and stem dry weights were determined, and percentages for defoliation and resultant stem weight reduction were calculated. Data were analyzed using the PROC REG procedure of SAS (16). Regression equations were calculated from individual data points, with percent defoliation and percent reduction in stem weight as independent variables.

Experiment 3. This experiment examined severity of stem canker disease in soybean plants recovering from insect-induced defoliation. Treatments consisted of a single level of stem canker fungus (inoculated), two levels of insect infestation (0 and 10 larvae per pot), and three times for stem canker development (0, 22, and 43 days postdefoliation), for a total of six treatment combinations, each replicated 10 times. Seed were planted in 60 pots on 29 June 1987, and pots were assigned at random to one of three groups. All plants in the first group of 20 pots were inoculated with the stem canker fungus 30 days after planting (plants in V6). Two days later, plants in 30 pots (10 from each group) were infested with soybean looper larvae, while plants in remaining pots were not infested. Larvae fed for 20 days, by which time defoliation was completed and larvae had pupated (plants in V11). Lengths of stem cankers were measured at this time. Plants were harvested and leaf area, stem weight, and number of nodes were determined. Reproductive stage of plants also was determined, based on a modification of the system of Fehr et al (3) in which a value of zero was assigned to plants not yet in reproductive stages.

Plants in the second group of 20 pots were inoculated similarly, 1 day after harvest of plants in the first group (plants in V11). Lengths of stem cankers in these plants were measured 21 days after inoculation (plants in R2). Thus, these cankers developed in stems of soybean plants that had recovered from defoliation for 22 days. Plants were harvested and growth parameters were measured as described.

Plants in the third group of 20 pots were inoculated similarly 3 days after harvest of plants in the second group. Lengths of resultant stem cankers were measured 18 days after inoculation (plants in R5). Thus, these cankers developed in stems of soybean plants that

Table 1. Selected growth parameters for and lengths of stem cankers in greenhouse-grown soybean cv. Bragg plants as influenced by insect defoliation and added nitrogen

Variable	Treatment	Leaf area (cm^2)	Stem dry weight (g)	Stem canker length (mm)
Defoliation	0[a]	3,705	13.3	72.1
	1	1,744	7.5	44.7
		$P = 0.0001$	0.0001	0.0001
Nitrogen	0[b]	2,256	8.8	80.9
	1	2,711	10.0	47.8
	2	3,208	12.6	43.5
		$P = 0.0001$	0.0001	0.0001
Contrast				
0 vs. 1 and 2		$P = 0.0003$	0.0001	0.0001
1 vs. 2		$P = 0.0225$	0.0001	0.6301

[a]0 = Nondefoliated, 1 = defoliated 53%.
[b]0 = Nonamended sandy loam soil, 1 = seeds coated with commercial *Bradyrhizobium japonicum* inoculant before being planted in nonamended soil, 2 = seeds not treated and soil amended with 66 mg/kg of NH_4NO_3.

had recovered from defoliation for 43 days. Plants were harvested and growth parameters were measured as described. Data were analyzed using the PROC GLM procedure of SAS.

RESULTS

Experiment 1. Defoliation by soybean looper larvae reached 53% (Table 1). This decreased plant growth significantly, as indicated by a 44% reduction in stem dry weight. In addition, lengths of stem cankers were reduced 38% below those in nondefoliated controls.

Both NH_4NO_3 and commercial *B. japonicum* inoculant increased plant growth significantly, as reflected by greater leaf areas and stem dry weights (Table 1). Increases in these parameters observed in response to NH_4NO_3 were greater than those in response to commercial inoculant. Both treatments reduced canker lengths relative to the nonamended control, but differences between treatments were not significant.

A significant ($P = 0.0011$) interaction was noted between the effects of defoliation and nitrogen on canker length (Fig. 1). An examination of individual treatment means showed that the reduction in stem canker length in response to defoliation was diminished when plants received supplemental nitrogen from either NH_4NO_3 or commercial *B. japonicum* inoculant.

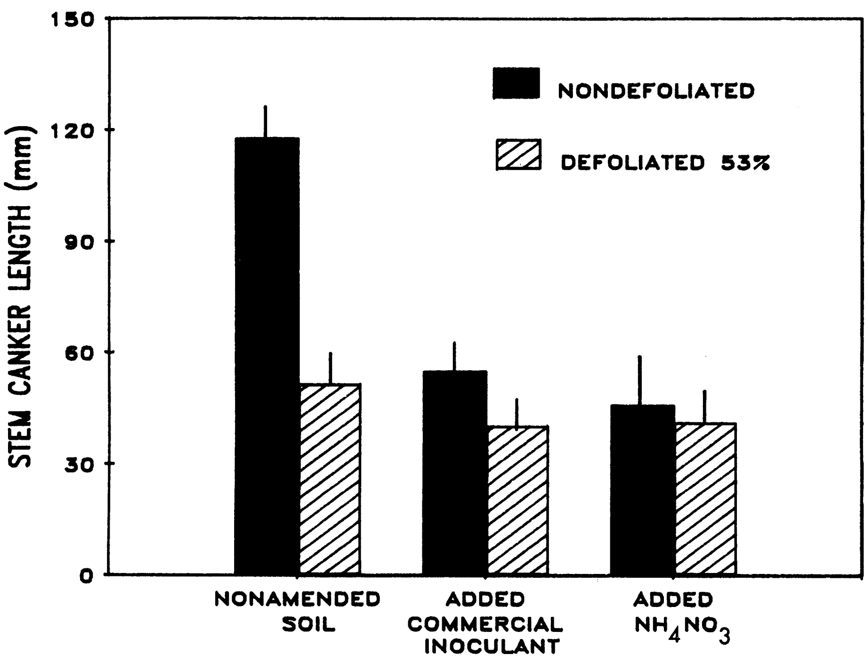

Fig. 1. Lengths of soybean stem cankers in response to insect-induced defoliation and additional nitrogen supplied by commercial bacterial inoculant or NH_4NO_3. Vertical lines delimit standard errors of means for eight replicates.

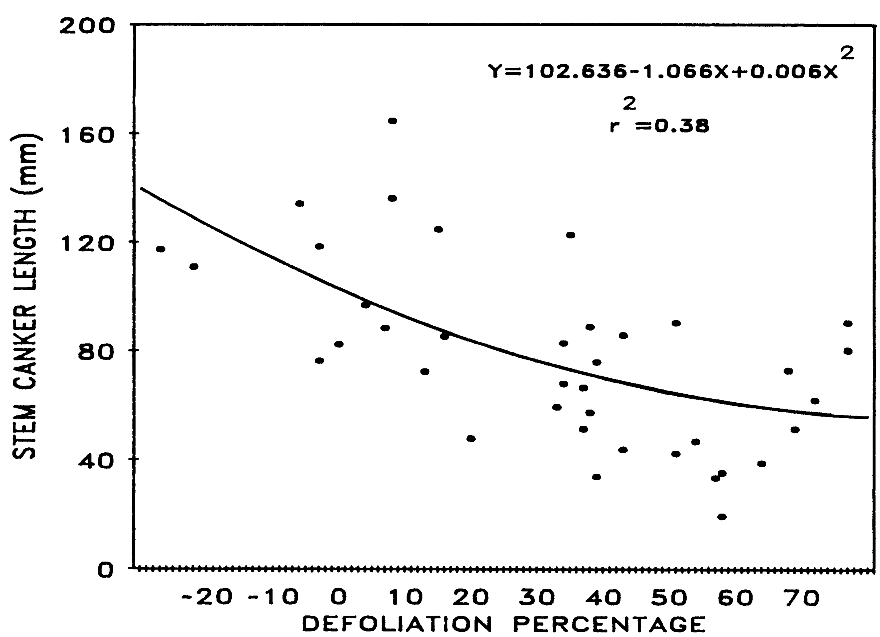

Fig. 2. Relationship between lengths of soybean stem cankers and insect-induced defoliation. Data points reflect leaf areas of plants in individual pots relative to the mean for leaf area of plants in the nondefoliated treatment.

Experiment 2. Mean defoliation levels of 0, 27, 47, and 59% resulted from infestation levels of 0, 10, 20, and 40 larvae per pot, respectively. Lengths of stem cankers in these plants ranged from 17 to 170 mm across all defoliation levels. A significant ($P < 0.0001$) reduction in stem canker length occurred as levels of insect defoliation increased (Fig. 2). Lengths of stem cankers did not decrease significantly ($P = 0.1354$) in response to reductions in stem dry weight that resulted from defoliation.

Experiment 3. Defoliation by soybean looper larvae reached 30%, which did not reduce stem weight or number of nodes significantly (Table 2). Nevertheless, this level of defoliation reduced canker lengths 46% when inoculations were timed so that cankers developed at 0 days postdefoliation. By 22 days postdefoliation, growth of new foliage had reduced the difference in leaf area between nondefoliated and defoliated treatments to 5%. Values for stem weight, number of nodes, and reproductive stage did not differ significantly between treatments. Lengths of cankers that developed in plants at this stage of recovery also did not differ significantly (Table 2). By 43 days postdefoliation, values for leaf area of treatments remained within 5% of each other and lengths of stem cankers did not differ significantly. However, a significant difference in plant reproductive stage between treatments was apparent at this time, which resulted in delayed development of pods on defoliated plants. Pods and stems inadvertently were weighed together, which likely resulted in the significantly lower stem weights for defoliated plants (Table 2).

DISCUSSION

Severity of soybean stem canker disease has been reported in the literature to be increased by drought stress (1) and insect girdling injury (12) but decreased by potash fertilization (2) and soybean cyst nematode infection (15). Results from the present studies showed that disease severity was reduced in plants defoliated by soybean looper. This effect was consistent across a wide range of defoliation levels. However, the relatively low coefficient of determination ($r^2 = 0.38$) between canker length and percent defoliation under controlled greenhouse conditions suggested that other, as yet unidentified factor(s) also had a marked influence on disease severity.

Soybean plants subjected to 30% defoliation showed nearly complete recovery of leaf area by 22 days postdefoliation, and leaf areas of both defoliated and nondefoliated plants remained similar at 43 days postdefoliation. Canker lengths also did not differ significantly between treatments at these times. Thus, 30% defoliation had only a temporary impact on disease severity, which diminished as defoliation stress was relieved by growth of new foliage. However, patterns of recovery in response to more severe defoliation can be quite different from that seen in our study. Layton and Boethel (9) showed that soybean plants defoliated 73% by soybean looper had not recovered leaf area by 5 wk postdefoliation. In fact, the absolute difference in leaf area between treatments at this time was more than double that at 0 days postdefoliation. This difference occurred because the reduction in leaf area due to decreased leaf growth following defoliation was greater than the initial leaf area lost due to insect feeding (9). As a result, the adverse effect of such severe defoliation on leaf area probably would be evident for periods of time much longer than the 22 days observed in our study in response to 30% defoliation. The impact of such prolonged defoliation stress on severity of stem canker disease is unknown.

The significant negative relationship between canker length and defoliation percentage but not stem dry weight indicated that disease severity was related more to current leaf area than to cumulative plant growth. This observation is supported by data in Table 2, which show that significant differences in canker length corresponded more closely to leaf area than to stem dry weight, number of nodes, or reproductive stage. Preliminary results have shown that stems from defoliated plants contained levels of glucose, fructose, and sucrose that differed significantly from those in nondefoliated plants (D. H. Picha, J. S. Russin, and M. B. Layton, *unpublished*). It is possible that stem sugar levels altered by defoliation might have affected stem canker severity in our tests.

Our results also demonstrated that lengths of stem cankers were reduced on plants that received added nitrogen either directly through fertilization or indirectly through symbiotic nitrogen fixation. Although levels of nitrogen in plants were not determined, the resultant stepwise increases in plant growth in response to added inoculant and fertilizer (Table 1) suggest that these treatments provided different levels of nitrogen to plants. Huber (7) reported that specific nutrients, including nitrogen, can decrease disease severity in several ways, including increasing tolerance to disease, facilitating escape from disease, enhancing physiological resistance to disease, or reducing pathogen virulence. It is not known which of these was present in our system.

Table 2. Selected growth parameters for and lengths of stem cankers in greenhouse-grown soybean cv. Bragg plants at three times after insect-induced defoliation

Days after defoliation	Treatment[a]	Leaf area (cm^2)	Number of nodes	Reproductive stage[b]	Stem dry weight (g)	Stem canker length (mm)
0	0	2,126	11.0	0	6.8	81.9
	1	1,486	10.8	0	5.7	44.1
		P = 0.0035	0.6340	···	0.0913	0.0050
22	0	5,141	19.8	1.9	16.9	69.8
	1	4,882	19.4	1.2	14.4	52.4
		P = 0.6642	0.7519	0.2986	0.3777	0.0765
43	0	6,942	22.2	4.9	48.4[c]	53.8
	1	6,607	21.2	3.5	32.1[c]	46.7
		P = 0.7377	0.2994	0.0051	0.0028	0.4890

[a]0 = Nondefoliated, 1 = defoliated 30%.
[b]Modified from Fehr et al (3) by assigning a value of zero to plants not yet in reproductive stages.
[c]Includes weights of developing pods (pods had not yet developed at earlier dates).

The necessity for controlling the timing of canker symptom development in conjunction with defoliation required the use of the toothpick inoculation technique. However, use of this technique precluded determination of defoliation effects on the natural infection process and resultant disease incidence. Primary inoculum of the stem canker fungus initiates infection during early vegetative stages of soybean development (1). At that time, soybean in Louisiana can be damaged by early-season defoliation caused by insects such as green cloverworm and bean leaf beetle. Future research will address whether insect-induced defoliation can affect incidence of the fungus early in the season as well as disease severity later in the season.

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LITERATURE CITED

1. Backman, P. A., Weaver, D. B., and Morgan-Jones, G. 1985. Soybean stem canker: An emerging disease problem. Plant Dis. 69:641-647.
2. Chambers, A. Y. 1987. Effects of potash fertilization on severity of soybean stem canker. (Abstr.) Proc. South. Soybean Dis. Workers 14:90.
3. Fehr, W. R., Caviness, C. E., Burmood, D. T., and Pennington, J. S. 1971. Stage of development descriptions for soybeans, *Glycine max* (L.) Merrill. Crop Sci. 11:929-931.
4. Hamer, J. L. 1985. Report of Insect Detection, Evaluation, and Prediction Committee. Southeastern Branch, Entomological Society of America. 9:12.
5. Harville, B. G., Berggren, G. T., Snow, J. P., and Whitam, H. K. 1986. Yield reductions caused by stem canker in soybean. Crop Sci. 26:614-616.
6. Herzog, D. C., Thomas, J. W., Jensen, R. L., and Newsom, L. D. 1975. Association of sclerotial blight with *Spissistilus festinus* girdling injury on soybean. Environ. Entomol. 4:986-988.
7. Huber, D. M. 1980. The role of mineral nutrition in defense. Pages 381-406 in: Plant Disease. An Advanced Treatise. Vol. 5. How Plants Defend Themselves. J. G. Horsfall and E. B. Cowling, eds. Academic Press, New York.
8. Layton, M. B., and Boethel, D. J. 1987. Reduction in N_2 fixation by soybean in response to insect-induced defoliation. J. Econ. Entomol. 80:1319-1324.
9. Layton, M. B., and Boethel, D. J. 1988. Recovery of N_2 fixation in soybean following insect-induced defoliation. J. Econ. Entomol. 81:1319-1324.
10. Mulrooney, R. P. 1985. Soybean disease loss estimate for southern United States in 1983. Plant Dis. 69:92.
11. Newsom, L. D., and Boethel, D. J. 1986. Interpreting multiple pest interactions in soybean. Pages 232-255 in: Integrated Pest Management in Major Agricultural Systems. R. E. Frisbee and P. L. Adkisson, eds. Texas Agricultural Experiment Station MP-1616.
12. Russin, J. S., Boethel, D. J., Berggren, G. T., and Snow, J. P. 1986. Effects of girdling by the threecornered alfalfa hopper on symptom expression of soybean stem canker and associated soybean yields. Plant Dis. 70:759-761.
13. Russin, J. S., Newsom, L. D., Boethel, D. J., and Sparks, A. N., Jr. 1987. Multiple pest complexes on soybean: Influences of threecornered alfalfa hopper injury on pod and stem blight and stem anthracnose diseases and seed vigour. Crop Prot. 6:320-325.
14. Russin, J. S., Orr, D. B., Layton, M. B., and Boethel, D. J. 1988. Incidence of microorganisms in soybean seeds damaged by stinkbug feeding. Phytopathology 78:306-310.
15. Russin, J. S., Layton, M. B., Boethel, D. J., McGawley, E. C., Snow, J. P., and Berggren, G. T. 1989. Development of *Heterodera glycines* on soybean damaged by soybean looper and stem canker. J. Nematol. 21:108-114.
16. SAS Institute. 1985. SAS User's Guide: Statistics. Version 5 ed. SAS Institute, Cary, NC. 956 pp.
17. Turnipseed, S. G. 1973. Insects. Pages 545-572 in: Soybeans: Improvement, Production, and Uses. B. E. Caldwell, ed. American Society of Agronomy, Madison, WI.