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

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ABSTRACT

Greenhouse studies using the soybean (Glycine max (L.) Merr.) cultivar Bragg showed a significant ($P < 0.001$) 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$_4$NO$_3$ 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 Athes & Caldwell, were greater on stems with basal girdles caused by the threecornered alfalfa hopper (Spissistilus festivus 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 seed borne 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.
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 larval 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 larval 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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>Leaf area (cm²)</th>
<th>Stem dry weight (g)</th>
<th>Stem canker length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defoliation</td>
<td>0</td>
<td>3.705</td>
<td>13.3</td>
<td>72.1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1.744</td>
<td>7.5</td>
<td>44.7</td>
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<td></td>
<td></td>
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<td>P = 0.0001</td>
<td>0.0001</td>
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<tr>
<td>Nitrogen</td>
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<td>2.256</td>
<td>8.8</td>
<td>80.9</td>
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<tr>
<td></td>
<td>1</td>
<td>2.711</td>
<td>10.0</td>
<td>47.8</td>
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<tr>
<td></td>
<td>2</td>
<td>3.208</td>
<td>12.6</td>
<td>43.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P = 0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>Contrast</td>
<td>0 vs. 1 and 2</td>
<td>P = 0.003</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>1 vs. 2</td>
<td>P = 0.0225</td>
<td>0.0001</td>
<td>0.6301</td>
</tr>
</tbody>
</table>

0 = Nondefoliated, 1 = defoliated 53%; 0 = Nonamended sandy loam soil, 1 = seeds coated with commercial Brachyzygum japonicum inoculant before being planted in nonamended soil, 2 = seeds not treated and soil amended with 66 mg/kg of NH₄NO₃.

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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$_4$NO$_3$ 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$_4$NO$_3$ 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$_4$NO$_3$ or commercial *B. japonicum* inoculant.

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

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**Fig. 1.** Lengths of soybean stem cankers in response to insect-induced defoliation and additional nitrogen supplied by commercial bacterial inoculant or NH$_4$NO$_3$. 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.
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

<table>
<thead>
<tr>
<th>Days after defoliation</th>
<th>Treatment</th>
<th>Leaf area (cm²)</th>
<th>Number of nodes</th>
<th>Reproductive stage</th>
<th>Stem dry weight (g)</th>
<th>Stem canker length (mm)</th>
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<tr>
<td>0</td>
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<td>1</td>
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<td>21.2</td>
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<td>0.4890</td>
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</tbody>
</table>

0 = Nondefoliated, 1 = defoliated 30%.

1 Modified from Fehr et al. (3) by assigning a value of zero to plants not yet in reproductive stages.

2 Includes weights of developing pods (pods had not yet developed at earlier dates).

physiological resistance to disease, or reducing pathogen virulence. It is not known which of these was present in our system.

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.

ACKNOWLEDGMENTS
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LITERATURE CITED