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Induced Resistance to Bean Leaf Beetle (Coleoptera: Chrysomelidae) in Soybean

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Abstract  Induced resistance in soybean, Glycine max (L.) Merrill, to the bean leaf beetle, Cerotoma trifurcata (Forster), was investigated in greenhouse experiments using the treatments of mechanical injury, a chemical inducer (ActigardTM, Novartis Crop Protection Inc., Greensboro, NC) and defoliation by the bean leaf beetle and the soybean looper, Pseudoplusia includens (Walker). Experiments were conducted on soybean PI 227687, two soybean cultivars (Colfax and Williams 82) and a soybean germplasm line (HC95-24MB). Dual-choice feeding-preference tests with bean leaf beetle adults were used to assess induced resistance. Adult beetles were collected from soybean fields 2 to 5 d prior to the feeding preference tests. Pairwise comparisons of leaflets from treated and untreated (control) plants indicated that soybean looper herbivory was a better inducer than other treatments. Herbivory by bean leaf beetle feeding and Actigard following artificial defoliation also were found to induce resistance to the bean leaf beetle. Mechanical injury alone elicited a significantly lower induced response in plants than the other induction treatments.

Key Words  Soybean, induced resistance, bean leaf beetle, chemical inducer, Actigard

Soybean, Glycine max (L.) Merrill, is one of the most important grain and oil seed legumes produced in the United States. Previous research has demonstrated that soybean plant phytochemicals are active against herbivory as well as other biotic and abiotic stresses (Lin and Kogan 1990). Variability in the level of resistance to herbivores has been found among soybean cultivars and lines (Kogan 1972). This variability is the result of differences in the constitutive phytochemical resistance, based on low concentrations of reducing sugars and isoflavonoids which are both involved in some aspects of resistance.

The soybean plant responds to physical and biotic stresses through the enhanced synthesis of compounds previously present or through the de novo synthesis of "defensive chemicals", such as phytoalexins. This quantitative and/or qualitative enhancement of a plant's defense mechanisms against pests in response to extrinsic physical or chemical stimuli was termed "induced resistance" by Kogan and Paxton (1983). These resulting wound-induced plant responses may be part of a general defensive reaction, because some wound-evolved phytochemicals are similar to those developed during pathogen infection.

Induction responses in plants can be elicited by various sources, such as infection...
by pathogens (Karban et al. 1987), herbivory by insects, mechanical damage (e.g., artificial defoliation) and foliar application of chemicals. This response also was found to increase the activity of lipoxygenases (Bi et al. 1994) and peroxidases (Bi and Felton 1995). Bi et al. (1994) reported that fourth-instar corn earworm, Helicoverpa zea (Boddie), larval growth rates were reduced by 39% after 24 h of feeding and by 27% after 48 h when larvae fed on previously wounded V3 foliage compared with undamaged foliage. Smith (1985), Lin et al. (1990b), Fischer et al. (1990), and Lin and Kogan (1990) reported induced soybean responses against the soybean looper, Pseudoplusia includens (Walker), and the Mexican bean beetle, Epilachna varivestis Mulsant. The levels of induced resistance differed with the type of injury (i.e., soybean looper feeding injury and artificial injury); herbivory-feeding injury was considered to be a better inducer than mechanical injury (Lin et al. 1990b). The involvement of chemicals in eliciting induced responses was reported by Friedrich et al. (1996) against plant pathogens, while Liu et al. (1993) and Lin et al. (1990a) reported this phenomenon involving insects.

The bean leaf beetle, Cerotoma trifurcata (Forster), is considered a sporadic pest of soybean in Nebraska, but in recent years has increased in importance, primarily because of the rapid increase in soybean acreage. The bean leaf beetle has previously been reported to be unaffected by high doses of a certain class of phytoalexins, the glyceollins (Fischer et al. 1990). Bean leaf beetle herbivory caused decreased foliage suitability to corn earworm, as reported by Felton et al. (1994). Thus far, no studies have been published to determine if bean leaf beetle injury causes induced resistance to its subsequent feeding in soybean. Therefore, experiments were conducted to determine if: (1) induction of resistance in soybean by and to the bean leaf beetle occurs, (2) different factors are involved in inducing the resistance; and (3) the induction of resistance occurs in soybean germplasm and cultivars adapted to the north central region of the United States.

Materials and Methods

We studied the soybean PI 227687 in these greenhouse experiments as this soybean plant introduction exhibited induced resistance to the soybean looper and Mexican bean beetle in previous studies (Smith 1985, Chiang et al. 1987, Lin et al. 1990 a,b). We also included the soybean cultivar ‘Colfax’ (Reg. no. CV-319, PI 573008) (Graef et al. 1994) and a germplasm line HC95-24MB (Reg. no. GP-271, PI 604463) (Cooper and Hammond 1999) which were compared with the susceptible cultivar ‘Williams 82’ (Reg. no. 222, PI 518671) as the control (Bernard and Cremeens 1988).

Seeds were planted in 15-cm plastic pots, with a sterile (soil) mixture of (3:2:1, top soil/sand/vermiculite) and were grown in a greenhouse maintained at 30 ± 5°C, 70 ± 10% RH, and a 14:10 (L:D) photoperiod with supplementary metal halide illumination. The definition of developmental stages of soybean plants followed Fehr et al. (1971). Adult bean leaf beetles were collected from fields at University of Nebraska Lincoln Agricultural Research Development Center in Saunders Co., NE, and from fields at the University East Campus and Havelock Farms in Lancaster Co. Fourth-instar soybean looper larvae for the initial herbivory were obtained from a colony maintained on artificial diet at the USDA-ARS, Southern Field Crops Insect Laboratory at Stoneville, MS. The chemical inducer Actigard™ was obtained from Novartis Crop Protection Inc., Greensboro, NC 27419. Actigard is a synthetic benzothiadiazole compound
formulated as 50% a.i. in wettable granular form and was used to induce resistance to diseases (Friedrich et al. 1996, Dann et al. 1998) and insects (Inbar et al. 1998).

Plants at the V6 growth stage received the following treatments: (1) bean leaf beetle feeding, (2) soybean looper feeding, (3) artificial defoliation with a paper punch or cork borer to remove about 25% of leaf tissue, (4) 25% artificial defoliation by tearing leaflets with no. 1 insect pins followed by 25 ppm Actigard spray, and (5) no treatment (control). An additional treatment of 25 ppm Actigard spray alone was included in experiments with the three soybean entries adapted to the North Central region used in this study: HC95-24MB, Colfax and Williams 82. This treatment was not applied to PI 227687 plants due to insufficient number of plants and adult beetles for feeding preference tests. All the potted plants (including the control plants) were covered with cages (radius-7.62 cm and height-60.96 cm) constructed of clear Lexan® FR-60 film (GE Plastics-Polymers, Pittsfield, MA) and fitted with vents for aeration and handling of the insects. Each potted plant was considered an experimental unit. Treatments were assigned randomly to plants in a completely randomized fashion. Each treatment was replicated 20 times. Defoliation by soybean looper and bean leaf beetle was achieved by placing three to four fourth-instar larvae and six to eight adults, respectively, on the trifoliates for 24 to 48 h. This resulted in 25 to 30% defoliation of the first five trifoliates. The 25 ppm Actigard solution was prepared in the laboratory and sprayed as a mist on plants covering the first five trifoliates. The sixth trifoliates was used for bean leaf beetle feeding preference tests 2 wks after plants were injured.

**Dual choice feeding preference test.** Dual-choice tests were conducted 2 wks after application of treatments to assess bean leaf beetle feeding preferences. Six leaflet disks from each pair of experimental plants, assigned as control and treatment, were obtained and arranged in an alternating pattern around the bottom of a Petri dish (100 × 15 mm). Similar dual choice feeding preference tests were used by Lin et al. (1990b) in induced resistance studies in soybean. Adult bean leaf beetles were starved for 24 h prior to the tests and were supplied with water. Only beetles collected within the previous 2 wks were used for these tests. Four starved adult bean leaf beetles were released into each Petri dish and allowed to feed for 4 to 6 h. The remaining leaflet disk area was measured using a LICOR-3000 area meter (LICOR, Lincoln, NE). Differences in consumed leaflet disk area of treatment (T) and control (C) were used to compute the statistical significance of differences in feeding preferences in paired comparisons between T and C and to calculate the feeding-preference index (Pl), where \( Pl = 2T/(T + C) \) (Kogan and Goeden 1970, Kogan 1972). The Pl values range from 0 to 2 with Pl = 1 indicating no feeding preference for either control or treatment disks, Pl > 1 indicating preference for treatment disks, and Pl < 1 indicating preference for control leaflet disks. Data were analyzed using the PROC GLM procedure (SAS Institute 1997), and means were compared using the LSD computed at \( \alpha = 0.05 \).

**Results and Discussion**

Results from the experiments with PI 227687 demonstrated that resistance was induced by bean leaf beetle herbivory, soybean looper herbivory, and artificial defoliation (Table 1). The mean preference indices of bean leaf beetle adult feeding and soybean looper larval feeding were less than 1.0 indicating that these treatments induced resistance against subsequent bean leaf beetle adult feeding. Plants me-
Table 1. Mean (±SE) bean leaf beetle feeding preference index (PI) for a plant introduction, two soybean cultivars and one germplasm line

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PI 227687†</th>
<th>Colfax</th>
<th>HC 95-24MB</th>
<th>Williams 82</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean Looper feeding</td>
<td>0.361 ± 0.044 b</td>
<td>0.420 ± 0.026 c</td>
<td>0.424 ± 0.024 c</td>
<td>0.435 ± 0.021 c</td>
</tr>
<tr>
<td>Artificial defoliation +</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actigard spray</td>
<td>0.435 ± 0.060 b</td>
<td>0.494 ± 0.035 bc</td>
<td>0.483 ± 0.050 bc</td>
<td>0.488 ± 0.024 bc</td>
</tr>
<tr>
<td>Bean Leaf Beetle feeding</td>
<td>0.500 ± 0.051 b</td>
<td>0.531 ± 0.040 b</td>
<td>0.537 ± 0.048 b</td>
<td>0.564 ± 0.030 b</td>
</tr>
<tr>
<td>Artificial Defoliation</td>
<td>0.887 ± 0.058 a</td>
<td>0.740 ± 0.044 a</td>
<td>0.744 ± 0.034 a</td>
<td>0.753 ± 0.028 a</td>
</tr>
</tbody>
</table>
| *Within the same column, means followed by the same letter are not significantly different (P < 0.05), by protected LSD.** Probability values for the F-test of treatments in each test soybean.† Actigard spray was not used as a treatment for PI 227687.
Mechanically injured showed little induction response when compared to other treatments, as their mean PI values were nearly 1.0. Actigard also was found to be effective in inducing resistance by enhancing the effect of artificial defoliation. The differences in acceptability of leaf tissues from treated plants compared to untreated plants were taken as the effect of treatments on bean leaf beetle. These differences are considered as variations in resistance levels in comparison with constitutive resistance.

Bean leaf beetle injury induced resistance in the cultivars Colfax and Williams 82, as well as the soybean germplasm line HC95-24MB. There were significant differences among the treatments for all soybean entries (Table 1). Artificial defoliation induced resistance, but induction was significantly lower than all other treatments. Inducing the plants with soybean looper feeding was numerically at least among the highest inducers for all three soybean entries. The mean PI for bean leaf beetle feeding in Colfax was similar to that caused by Actigard spray and artificial defoliation followed by Actigard spray (Table 1). All treatments induced resistance that resulted in significantly less feeding by bean leaf beetle than on the untreated controls as indicated by PI values less than 1.0.

On HC95-24MB foliage, induction by soybean looper feeding, artificial defoliation followed by Actigard spray, Actigard spray and bean leaf beetle feeding were significantly different from artificial defoliation. On Williams 82 foliage, results were similar to those observed in Colfax. Artificial defoliation again caused significantly less induction than other treatments. The other three treatments, bean leaf beetle feeding, Actigard spray and artificial defoliation followed by Actigard spray, were not significantly different from one another but elicited greater induction than artificial defoliation.

Earlier studies have documented that the soybean exhibits induced response from various inducers to soybean looper and Mexican bean beetle (Lin et al. 1990b, Fischer et al. 1990, Lin and Kogan 1990). The soybean plant also has exhibited induced resistance to certain fungal pathogens in response to initial infection by pathogens (Karban et al. 1987) and treatment with chemical inducers (Dann et al. 1998).

Results of our experiments suggest that bean leaf beetle and soybean looper herbivory as well as Actigard spray induced resistance in soybean. Soybean looper was used for inducing resistance because we found soybean looper feeding easier to regulate and a continuous, reliable source of insects was available. Bean leaf beetle was chosen as a test insect because it is a major economic pest of soybeans in the Midwest, and the effects of induced resistance on this pest have not previously been documented. The induction of insect resistance in soybean by soybean looper herbivory has both antibiotic and antixenotic effects on both Mexican bean beetle and soybean looper with superior effects on Mexican bean beetle (Lin and Kogan 1990).

This is the first report that bean leaf beetle induces resistance to its subsequent feeding in soybean. Moreover, this is the first experimental report of Actigard inducing resistance to bean leaf beetle in soybean. A dosage of 25 ppm Actigard induced resistance against subsequent bean leaf beetle feeding. Actigard demonstrated induced resistance against leafminer, Liriomyza trifolii (Burgess), and white flies, Bemisia argentifolii Bellows and Perring, in tomato (Inbar et al. 1998). Actigard also induced resistance (systemic acquired resistance) in soybean to the pathogen Sclerotinia sclerotiorum (Lib.) de Bary in both field and greenhouse environments (Dann et al. 1998). In all our experiments, we found that soybean looper feeding was among
the treatments that induced the highest level of resistance to the bean leaf beetle, and induction was lowest after mechanical damage (punching holes into the leaflets/trifoliates). Our results also indicated that there were no significant differences in induced resistance to the bean leaf beetle between the three soybean entries adapted to the north central region of the U.S. Induced resistance response in soybean appears to be independent of constitutive varietal resistance and dependent on the type of inducing injury or stress to the plant.

Soybean researchers will be better able to take advantage of induced resistance if they have a more thorough understanding of the extent to which it occurs. Future research should focus on assessing induced resistance under field conditions in additional soybean cultivars and germplasm adapted to the north central region. At the basic level, additional research is necessary to understand the mechanisms responsible for regulating the phytochemical production, which apparently mediates induced resistance.

Acknowledgments

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