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## Cross-Resistance and Resistance Longevity as Induced by Bean Leaf Beetle, *Cerotoma trifurcata* and Soybean Looper, *Pseudoplusia includens* herbivory on Soybean

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### Abstract

Cross-resistance, and longevity of resistance, induced by the bean leaf beetle, *Cerotoma trifurcata*, was studied IN the soybean PI 227687 that exhibited induced response in earlier studies. Bean leaf beetle adults and soybean looper, *Pseudoplusia includens*, larvae were used to induce resistance and to determine beetle feeding preference. Beetles were collected from soybean fields 2 to 5 days prior to the feeding preference test. The level of cross-resistance induced by soybean looper herbivory to subsequent bean leaf beetle feeding was higher when compared to cross-resistance induced by bean leaf beetle herbivory against subsequent feeding by soybean looper. Further, herbivory by the bean leaf beetle also induced resistance against soybean looper feeding. In the longevity study, leaflets from treated plants were collected 5, 10, 12, 14, 16, 20 and 25 days after initiation of feeding. Pairwise comparisons of leaflets from plants treated by bean leaf beetle herbivory with untreated plants revealed that induced responses were highest 14 and lowest 25 days after initiation of feeding. On other sampling days, levels of induced response varied with the sampling day.

**Keywords:** soybean, induced resistance, longevity, cross-resistance

### Abbreviation:

DAH days after herbivory

### Introduction

Kogan and Paxton (1983) defined induced resistance as the qualitative and/or quantitative enhancement of a plant's defense mechanisms against pests in response to extrinsic physical or chemical stimuli. From the perspective of herbivore population dynamics, induced resistance is any change in a plant resulting from damage that imparts a negative effect on herbivores (Karban and Baldwin 1997). Insect herbivory has been shown to induce resistance in soybean plants (Lin *et al.*, 1990b, Fischer *et al.*, 1990, Lin and Kogan 1990) against the soybean looper, *Pseudoplusia includens* and the Mexican bean beetle, *Epilachna varivestis*.

The bean leaf beetle, *Cerotoma trifurcata*, is a sporadic pest of soybean in Nebraska, but has increased in importance primarily because of the rapid increase in soybean acreage. In regions where both *P. includens* and *C. trifurcata* are present, *P. includens* attacks plants after *C. trifurcata* (Hammond *et al.*, 1991). For our purposes we define cross-resistance as resistance to one species of insect induced by another species of insect and *vice versa*. Therefore, cross-resistance to *P. includens* due to previous *C. trifurcata* injury in the form of induced resistance would be beneficial to the plant.

In an earlier study by Felton *et al.* (1994), bean leaf beetle herbivory affected larval growth rates and reduced the suitability of foliage to the corn earworm, *Helicoverpa zea*, in soybean. Inbar *et al.*, (1998) demonstrated the effectiveness of the chemical injury inducer, Actigard™ (Novartis Crop Protection Inc., Greensboro, NC), in providing cross-resistance to various insect pests and pathogens in the tomato, *Lycopersicon esculentum*. However, no studies have reported cross-resistance in soybean to one insect species after induction by another insect's herbivory and *vice versa*.

Earlier studies (Srinivas *et al.*, in press) indicated that soybean induced resistance to *C. trifurcata* can be caused either by *C. trifurcata* feeding, or *P. includens* feeding. Foliar application of Actigard™ was also effective. However, the literature on the longevity or duration of protection offered by induced resistance is very limited. Earlier research in host-pathogen systems documented that protection could be effective for a week or more (McIntyre and Dodds 1979, Chaudary *et al.*, 1983). Recent reports on the duration of induced resistance in host-pathogen systems have been documented in barley (Pelcz and Wolfgang 1986), cotton (Liu *et al.*, 1990) and cucumber (Dalisay and Kuc 1995). Underwood (1998) demonstrated that induced resistance against *E. varivestis* lasted

three days after damage in soybean. Stout *et al.*, (1996b) reported persistence of proteinase inhibitors and oxidative enzymes like polyphenol oxidase, peroxidase and lipoxygenase for at least 21 days after induction in damaged tomato leaflets. Thus far, no studies have been conducted to demonstrate the longevity of bean leaf beetle induced resistance in soybean.

The objectives of this study were to investigate: 1) cross-resistance to *P. includens* feeding after *C. trifurcata* herbivory, 2) cross-resistance to *C. trifurcata* feeding after *P. includens* herbivory and 3) the longevity of induced resistance due to *C. trifurcata* feeding in soybean against subsequent *C. trifurcata* feeding.

## Materials and Methods

Seeds of soybean, *Glycine max* (L.) Merrill, PI 227687 were planted in 15 cm plastic pots, in a sterile soil mixture (consisting of a 3:2:1 mixture of top soil:sand:vermiculite) and 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 developmental stages of soybean plants were defined as described by Fehr *et al.*, (1971).

Adult bean leaf beetle *C. trifurcata* (Forster) were collected from fields at University of Nebraska-ARDC in Saunders County, NE and from fields at the University of Nebraska East Campus and Havelock farms in Lancaster County, NE. Soybean looper, *Pseudoplusia includens* (Walker), larvae were obtained from a colony maintained on artificial diet at the USDA-ARS, Southern Field Crops Insect Laboratory at Stoneville, MS.

### Cross-Resistance

Treatments of *C. trifurcata* feeding, *P. includens* feeding, and untreated (control) plants were applied (one plant per pot) at the V6 (vegetative stage with six nodes) stage of growth in a completely randomized fashion. All the potted plants (including the controls) 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), fitted with vents for aeration and handling of the insects. There were a total of 18 replications in this study and each potted plant was considered an experimental unit. 25-30% defoliation was produced by placing three or four fourth and fifth instar *P. includens* larvae or six-to-eight *C. trifurcata* adults, on first

five trifoliates for 24-48 h. The apical trifoliates were used for *C. trifurcata* feeding preference tests 2 weeks after plants were injured.

### Longevity of Induced Resistance

In the longevity study, treatments of *C. trifurcata* feeding and untreated (control) were applied randomly to soybean plants at V6 stage (one plant per pot). All the potted plants (including the control plants) were covered with cages as described above and each caged plant was considered an experimental unit. Five plants each were sampled on 5, 10, 12, 14, 16, 20 and 25 days after herbivory (DAH) and five more from uninjured control plants. There were ten replications in this study. Defoliation (25-30%) by *C. trifurcata* was produced by placing six-to-eight adults on the first top five trifoliates for 24 h. The sixth trifoliolate or the top trifoliates were used for *C. trifurcata* feeding-preference tests. Only beetles collected within the previous 2 weeks were used for these tests.

Dual-choice tests were conducted to assess *C. trifurcata* and *P. includens* feeding preferences on induced plants. Soybean looper and *C. trifurcata* feeding were treatments in the cross-resistance study and *C. trifurcata* feeding was the treatment in the longevity study. Six leaflet disks from each pair of experimental plants, assigned as control (C) and treatment (T), were obtained and arranged in an alternating pattern around the bottom of a Petri dish (100 x 15 mm). In these tests, adult *C. trifurcata* were starved and supplied with only water for 24 h prior to the tests. Four starved adult *C. trifurcata* were released into each Petri dish, allowed to feed for 4 to 6h, and the remaining leaflet disk area was measured using a LICOR-3000 area meter (LI-COR, Lincoln, NE). In the cross-resistance study, feeding preferences of 5<sup>th</sup> instar *P. includens* larvae were determined by allowing larvae that had been pre-starved for 2h before the test to feed for 4 to 6h. Differences in consumed treatment and control leaflet disk area were used to compute the statistical significance of differences in feeding preferences and to calculate the feeding-preference index (PI), where  $PI = 2T/(T + C)$  (Kogan and Goeden 1970, Kogan 1972). The PI values ranged from 0 to 2, with PI=1 indicating no feeding preference for either control or treatment disks, PI > 1 indicating preference for treatment disks, and PI < 1 indicating preference for control leaflet disks. Data were analyzed using PROC GLM procedure (SAS Institute 1997) and means were compared using the LSD (Least Significance Difference) computed at  $\alpha = 0.05$ .

## Results

The acceptability of leaflet disks between treated and untreated plants during feeding was taken as the effect of treatments on *C. trifurcata* and *P. includens* feeding. These differences are taken as variations in resistance levels in comparison with constitutive resistance (Lin *et al.*, 1990b).

### Cross-Resistance

Results from the experiments to demonstrate induced cross-resistance in PI 227687 showed that different levels of cross-resistance were induced by *C. trifurcata* and *P. includens* herbivory (Table 1). The initial herbivory of *C. trifurcata* induced cross-resistance to subsequent *P. includens* feeding. However, leaflets from plants treated with *P. includens* feeding induced higher cross-

**Table 1.** Mean (± SE) feeding preference index (PI) values for soybean looper and bean leaf beetle fed soybean PI 227687 leaflets expressing cross resistance induced by bean leaf beetle and soybean looper feeding, respectively.

Treatment	Mean PI index <sup>1</sup>
Bean Leaf Beetle feeding	0.595 ± 0.020 a
Soybean Looper feeding	0.443 ± 0.015 b
<i>P</i> -value <sup>2</sup>	0.0120

<sup>1</sup> Means followed by the same letter are not significantly different ( $P < 0.05$ ), by protected LSD

<sup>2</sup> Probability values for the *F*-test of treatments in each test soybean (df = 1)

resistance to subsequent *C. trifurcata* feeding where the mean PI was 0.443. Cross-resistance induced by *P. includens* feeding against subsequent *C. trifurcata* feeding, was statistically significant (F Value = 7.05 and Pr > F = 0.0120).

#### Longevity of Induced Resistance

*C. trifurcata* injury-induced resistance in soybean PI 227687 to subsequent feeding by *C. trifurcata* varied throughout the duration of experiment (Fig. 1 and Table 2). The induced response was numerically highest at 14 DAH (PI = 0.573) compared to all other sampling days and was not significantly different from 12 and 16 DAH. The feeding preference was lowest at 25 DAH (0.812) and was not significantly different from 5 and 20 DAH. The PI at 10 DAH was not significantly different from all the other sampling days except 14 DAH. The longevity of induced resistance varied between sampling days after treatment (F Value = 4.19 and Pr > F = 0.0040) indicating significant difference in the longevity of the induction response. Since the data have a quantitative variable, regression analysis was done using PROC REG. The data did not fit the linear model (F value = 1.39 and Pr > F = 0.2469), but did fit the quadratic model (F value = 9.469 and Pr > F = 0.0006). This indicates that the induced resistance increased for a certain period of time and then decreased.

#### Discussion

Induced resistance to herbivores, particularly arthropods, has been observed in soybean, tomato, potato, wheat and cotton (Kogan and Paxton 1983, Stout *et al.*, 1996a, Karban and Baldwin 1997). The response of soybean to various biotic and abiotic stresses that induce resistance may involve the production of natural products, phytoalexins or plant pathogenesis-related proteins (Kogan and Paxton 1983, Benhamou and Theriault 1992, Ebel and Cosio 1994). This phenomenon is not well understood, and its persistence in deterring herbivory after elicitation has been documented in only a few insect-plant systems.

Previous studies have documented cross-resistance in other host-pest systems including tomato and potato (Inbar *et al.*, 1998, Singh and Singh 1995). In our experiments, *P. includens* feeding elicited a higher level of cross-resistance against *C. trifurcata* feeding than *C. trifurcata* induction of cross-resistance against *P. includens* feeding. This is the first report of *C. trifurcata* -induced cross-resistance to *P. includens* in soybean. However, Felton *et al.* (1994)

**Table 2.** Mean ( $\pm$  SE) feeding preference index (PI) values for different sampling days of bean leaf beetle fed soybean PI 227687 leaflets expressing induced response by bean leaf beetle feeding.

Days after Herbivory (DAH)	Mean PI index <sup>1</sup>
5	0.761 $\pm$ 0.074 ab
10	0.707 $\pm$ 0.040 abc
12	0.601 $\pm$ 0.048 cd
14	0.573 $\pm$ 0.026 d
16	0.643 $\pm$ 0.042 bcd
20	0.732 $\pm$ 0.059 ab
25	0.812 $\pm$ 0.035 a
P-value <sup>2</sup>	0.0040

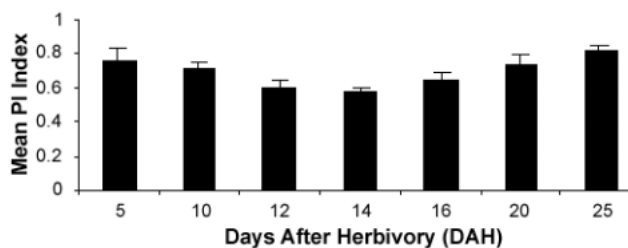
<sup>1</sup> Means followed by the same letter are not significantly different ( $P < 0.05$ ), by protected LSD

<sup>2</sup> Probability values for the *F*-test of treatments in each test soybean (df = 6)

reported the effect of *C. trifurcata* feeding on corn earworm. Similarly, inoculum containing the pathogen, *Phytophthora megasperma* induced phytochemical defenses and deterred *E. varivestis* feeding on soybean (Kogan and Fischer 1991). Inbar *et al.*, (1998) reported cross-resistance in tomato plants where there was a significant reduction of leafminer, *Liriomyza* spp., larval densities after treatment with Actigard™.

This is the first report of the longevity of *C. trifurcata*-induced resistance in soybean. Bentur and Kalode (1996) demonstrated induced resistance in rice to the Asian rice gall midge, *Orseolia oryzae*, to be effective for about 4 weeks after infestation. This research establishes that soybean induced resistance from *C. trifurcata* feeding is effective for approximately the same duration, although the induction effect began to subside at about 14 days after the first initial feeding by *C. trifurcata*. In an earlier study by Underwood (1998), the induced response was effective for three days after damage against *E. varivestis* in soybean. Resistance diminished by 15 days after damage and by 20 days all four genotypes used in the studies exhibited significant induced susceptibility. No such susceptibility was observed in our experiments although the induced response decreased to 0.81 (PI) by 25 DAH. The longevity of induced resistance produced by *C. trifurcata* was two weeks in our study. Any other type of injury might produce a higher level of induced resistance and protection that lasted longer than two weeks because Lin *et al.*, (1990 a, b) demonstrated that the level of induced soybean resistance varied with the type of injury. Thus, if the level of injury is higher and stronger it might offer longer protection.

Induction enhances soybean resistance to insects and pathogens (Lin and Kogan 1990, Dann *et al.*, 1998, Thaler 1999). Induced responses produce volatiles that act as a direct defense against the herbivores (Karbon and Baldwin 1997) and as an



**Figure 1.** Mean feeding preference (PI) indices ( $\pm$  S.E.) of bean leaf beetles in dual-choice feeding preference tests 5, 10, 12, 14, 16, 20 and 25 days after herbivory on PI 227687 leaflets from control plants and plants exposed to bean leaf beetle feeding. Mean PI values were derived from 10 replicates of each test combination. Means followed by the same letter are not significantly different ( $p < 0.05$ ) by protected LSD.

attractant to parasitoids in the form of indirect defense (Turlings et al. 1995, Dicke and Loon 2000). Moreover, induced responses were also shown to affect the herbivore's feeding preference, growth rate and fecundity that would influence the dynamics of the population (Karban and Baldwin 1997). This may have an indirect effect on population dynamics of natural enemies in the crop ecosystems. The longevity of the induced response offers longer protection against herbivory in pest management. The presence of induced resistance for a longer time extends the possibility of any direct or indirect effects on natural enemies and other non-target arthropods/herbivores. Thus these possible long-term effects of induced resistance should be studied at a tritrophic and possibly multitrophic level of plant-insect interactions.

If induced resistance reduces pest damage to plants, then chemical elicitors such as Actigard™, can be used to induce resistance as a preventive practice and extend the duration of plant protection. However, cross-resistance may act as an important plant protectant in addition to application of chemical elicitors. If a pest population can induce resistance to other pests in the field, a useful management tool could be to monitor the insect population that has the potential of inducing cross-resistance to other pests emerging either at the same time or later. Future research should focus on defining the longevity of soybean induced responses in the field, its effects on the population dynamics of pest insects and also to study the possibilities of extending this longevity using chemical elicitors.

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