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Effect of the Chemical Inducer Actigard™ in Inducing Resistance to Bean Leaf Beetle, *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae), Feeding in Soybean[1]

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ABSTRACT Induced resistance in soybean was investigated in the greenhouse using different concentrations of the chemical inducer Actigard™ to determine the influence on feeding preference of bean leaf beetle adults. Treatments of 0 (control), 15, 25, 40, 60, and 80 ppm Actigard™, and artificial defoliation followed by 25 ppm Actigard™ were applied to V1 and V6 stages of soybean plants. Dual-choice feeding preference tests with the bean leaf beetle were used to assess induced resistance. The adult beetles were collected from soybean fields in east central Nebraska 2 to 5 days prior to the feeding preference tests. Pair-wise comparisons of leaflets from Actigard™-treated plants and control plant leaflets demonstrated that Actigard™ at 25 ppm concentration showed lower preference index (PI) when plants were treated at V1 stage. At V6, feeding preferences were not significantly different; however, all PI values for both V1 and V6 plants indicated that the bean leaf beetles preferred the untreated plants over the Actigard™-treated plants.

KEY WORDS soybean, bean leaf beetle, induced resistance, plant-insect interactions, chemical inducer, Actigard™

The induced response to various biotic and abiotic or chemical elicitors, attributed to the synthesis of defensive phytochemicals (Kogan & Paxton 1983) and plant pathogenesis-related proteins (Ebel & Cosio 1994) in tissues away from the site of prior damage, has been reported in a variety of host-pest systems (Kogan & Fischer 1991, Bodnaryk & Rymerson 1994, Hammerschmidt & Dann 1997, Stout et al. 1998). Resistance in soybean has been induced by different types of injuries involving insects such as soybean looper, *Pseudoplusia includens* (Walker), and Mexican bean beetle, *Epilachna varivestis* Mulsant (Smith 1985, Fischer et al. 1990a,b, Lin & Kogan 1990, Lin et al. 1990b, Kogan & Fischer 1991).

Induction of resistance using abiotic elicitors such as sodium azide (Chakraborty & Purkayastha 1987), ethylene (Yoshikawa et al. 1990), ozone (Lin et al. 1990a), and antibiotics (Purkayastha & Banerjee 1990) also has been reported. Specifically, the chemical inducer 2,6-dichloroisonicotinic acid (INA) has been demonstrated to induce resistance against pathogens of pear (*Pyrus* sp.),

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tobacco (*Nicotiana tabacum* L.), rice (*Oryza sativa* L.), and green bean (*Phaseolus vulgaris* L.) (Me'traux et al. 1991, Dann & Deverall 1996). Another chemical inducer, benzo (1,2,3)-thidiazole-7-carbothioic *S*-methyl ester (Actigard™, Novartis Crop Protection Inc., Greensboro, North Carolina), was reported as the first synthetic chemical that induced resistance to pathogens in cereals and tobacco (Ruess et al. 1996). It was reported to induce resistance against pathogens in wheat (*Triticum aestivum* L.), tomato (*Lycopersicon esculentum* Mill.), tobacco (*N. tabacum*), soybean (*Glycine max* [L.] Merrill), and cucumber (*Cucumis sativus* L.) (Kessmann et al. 1993, Friedrich et al. 1996, Gorlach et al.1996, Lawton et al. 1996, Benhamou & Belanger 1998, Dann et al. 1998). Neither INA nor Actigard™ exhibit any direct antimicrobial activity, but they do activate genes responsible for induced responses (Friedrich et al. 1996, Lawton et al. 1996). This has led to the conclusion that they are chemical inducers activating the plant's own defense system (Morris et al. 1998). Actigard™ was used by Inbar et al. (1998) to induce resistance in tomato, thereby reducing the incidence of bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*), early blight (*Alternaria solani*), and also leading to lower larval leafminer (*Liriomyza* sp.) densities. These authors also reported reduction of whiteflies (*Bemisia argentifolii* Bellows & Perring) and powdery mildew (*Oidium* sp.).

The bean leaf beetle, *Cerotoma trifurcata* (Forster), has been a sporadic pest of soybean in Nebraska and the midwestern region of the United States, but in recent times has increased in importance. Previous research by Srinivas et al. (2001) showed that Actigard™ induced resistance against bean leaf beetle, *C. trifurcata*, in soybean. The objectives of this study were to determine if applications of Actigard™ can induce resistance to *C. trifurcata* feeding, and to determine the optimum concentration of Actigard™ that induces resistance to *C. trifurcata* feeding in two early season growth stages (V1 and V6) of soybean.

Materials and Methods

This study was conducted using soybean, *G. max* (L.) Merrill, plant introduction 227687, at V1 and V6 growth stages (Fehr et al. 1971). This plant introduction exhibited induced resistance to *P. includens* and *E. varivestis* in earlier studies (Smith 1985, Chiang et al. 1987, Lin et al. 1990a,b, Kogan & Fischer 1991). 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°C ± 5°C, 70% ± 10% RH, and a 14:10 (L:D)photoperiod with supplementary metal halide illumination.

Adult beetles were collected 2 to 4 days prior to feeding preference tests from fields at University of Nebraska-Lincoln (UNL)-ARDC in Saunders County, Nebraska, and from fields at the UNL East Campus and Havelock Farms in Lancaster County, Nebraska. Actigard™ 50 WG [benzo(1,2,3-thiadiazole-7-carbothioic acid (*S*)-methyl ester] was obtained from Novartis Crop Protection Inc.

The experimental design was completely randomized with seven treatments of 0 (control), 15, 25, 40, 60, and 80 ppm Actigard™, and artificial defoliation (25%) by tearing leaflets with no. 1 insect pins followed by 25 ppm Actigard™. There were 14 replications for the study done on V1 stage plants and 12 replications on V6 stage plants. Each caged plant was considered an experimental unit. All the potted plants, including the control plants, were covered with cylindrical cages

(15.24 cm wide × 60.96 cm tall) constructed of clear lexan plastic fitted with vents for aeration to avoid insect damage. The apical trifoliates were used for dual-choice feeding preference tests 2 weeks after plants were treated. This procedure eliminated the possibility of Actigard™ directly affecting bean leaf beetle feeding because the apical trifoliates used in the preference tests emerged after the application of Actigard™. In these tests, the adult beetles were starved for 24 h prior to the tests and were supplied with water.

Dual-choice tests were conducted to assess bean leaf beetle feeding preferences in this study. Each test arena consisted of six excised leaflet disks from both untreated or control (C) and treated (T) experimental plants that were arranged in an alternating pattern around the bottom of a petri dish. Similar dual-choice feeding preference tests were used by Srinivas et al. (2001) and Lin et al. (1990b) in induced resistance studies in soybean. Four starved adult beetles were released into each petri dish, and were allowed to feed for 4 to 6 h. Leaflet disk areas were measured before and after feeding using a LICOR-3000 area meter (LICOR, Lincoln, Nebraska). The proportion of consumed disk area of T and C leaflets were used to calculate the feeding preference index (PI), where $PI = 2T/(T + C)$ (Kogan & Goeden 1970, Kogan 1972). The PI value ranges from 0 to 2, with a PI of 1 indicating no feeding preference for either C or T disks, a PI >1 indicating preference for T disks, and a PI <1 indicating preference for C leaflet disks.

The PI data were analyzed using a general linear model procedure (SAS Institute 1997) for completely randomized design and means were compared using the least significance difference (LSD) computed at $\alpha = 0.05$.

Results and Discussion

Results of this experiment confirmed the report of Srinivas et al. (2001) that Actigard™ could be used as a chemical elicitor in inducing resistance against bean leaf beetle adult damage. All PI values were less than 1.0, suggesting that all concentrations of Actigard™ tested elicited an induced response, which affected subsequent *C. trifurcata* feeding in both V1 and V6 stages of soybean (Table 1). The differences in feeding preference of leaflets from treated and untreated plants were considered as the effect of different concentrations of Actigard™ treatment on *C. trifurcata* feeding.

The average PI of *C. trifurcata* feeding varied with the concentration of Actigard™ used, and induced responses were significantly different for V1 stage plants ($F = 5.62$; df = 5, 78; $P = 0.0002$). The response induced by 25 ppm (PI = 0.30) was similar to the induced response produced using artificial defoliation plus 25 ppm (PI = 0.32). The PI (= 0.50) for 60 ppm concentration of Actigard™ spray was statistically similar to resistance induced at 40 ppm (PI = 0.44), 15 ppm (PI = 0.43), and 80 ppm (PI = 0.41) Actigard™ concentrations. The results from tests with V6 stage soybean were not statistically significant ($F = 1.64$; df = 5, 66; $P = 0.3060$) for the Actigard™ concentrations; however, the preference indices were less than 1.0, suggesting an elicitation of induced response and indicating that the beetles preferred the untreated (C) leaflet disks in this study.

Results of our experiment suggest that different concentrations of Actigard™ can be used to induce resistance to *C. trifurcata* feeding in soybean. The highest level of induction against *C. trifurcata* feeding was produced by 25 ppm and artificial defoliation plus 25 ppm applications made at V1 stage of soybean

Table 1. Mean feeding preference index (PI) ± SE values for bean leaf beetles fed soybean plant introduction 227687 leaflets treated with various concentrations of Actigard™.

	Mean PI index[a]	
Actigard™ treatment (ppm)	V1 stage	V6 stage
15	0.432 ± 0.012 a	0.543 ± 0.035 a
25	0.302 ± 0.014 c	0.470 ± 0.035 a
40	0.437 ± 0.019 a	0.454 ± 0.041 a
60	0.496 ± 0.022 a	0.596 ± 0.028 a
80	0.410 ± 0.020 ab	0.606 ± 0.047 a
AD + 25	0.324 ± 0.015 bc	0.479 ± 0.045 a
P value[b]	0.0002	0.3060

[a]Means followed by the same letter are not significantly different ($P < 0.05$) by LSD.
[b]Probability values for the F test of treatments in each test soybean. AD, artificial defoliation.

growth. The induction of resistance with Actigard™ demonstrates that it can be used as a chemical elicitor to stimulate a defensive mechanism against *C. trifurcata* feeding in soybean.

Inbar et al. (1998) used three foliar applications of Actigard™ to induce resistance in tomato against leafminer larvae and whiteflies. Dann et al. (1998) showed that multiple applications of Actigard™ were necessary in field and greenhouse soybean to decrease fungal disease incidence and severity. However, our study demonstrates that a single application of Actigard™ is sufficient to induce resistance in soybean to *C. trifurcata* feeding at 2 weeks post-application (samples for feeding preference were collected 2 weeks after treatment). This seems to be in corroboration with results from Gorlach et al. (1996) that showed that a single application of Actigard™ provided long lasting protection in wheat and rice against fungal diseases.

The PIs of different concentrations of Actigard™ were not significantly different at V6 growth stage, but all PIs being <1.0 indicates *C. trifurcata*'s preference for untreated versus Actigard™-treated plants. However, the response at V1 stage was quite pronounced and statistically significant. These results seem to agree with the findings of Alarcon & Malone (1995), who demonstrated induced resistance most pronounced in young tomato seedlings in the form of higher amounts of proteinase inhibitors. These endogenous proteinase inhibitors produced as a result of wound response have been found to be effective against insect pests (Hilder et al. 1987, Ryan 1990). We believe that further investigation should occur at the biochemical and molecular levels to understand the level of induced gene expression and pathways involved in induced responses to pests when Actigard™ is used. Future research should also focus on studying the optimization of Actigard™ dosage in the field against soybean insect pests and also the possibilities of using chemical elicitors like Actigard™ in combination with insecticides for insect pest management in soybean.

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