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Laboratory Toxicity Profile of an Organic Formulation of Spinosad against the Eggplant Flea Beetle, *Epitrix Fuscula* Crotch

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Abstract

The eggplant flea beetle, *Epitrix fuscula* Crotch, is a major insect pest of eggplant, *Solanum melongena* L. var. *esculentum* (Solanaceae), produced in the south central United States and often requires management with insecticides immediately following setting of transplants into the field. Few insecticides approved for use in organic production have provided effective management of the beetles. The study reported herein demonstrates the effectiveness of Entrust, a new organic approved formulation of spinosad. Following exposure on eggplant foliage, Entrust was shown to be toxic to adult flea beetles with LC_{50} and LC_{95} values of 42.9 and 296.7 ppm, respectively. Although mortality following exposure was sometimes delayed until six days, the effects on flea beetle behavior were observed in as little as six hours following exposure. Feeding was significantly reduced one day after exposure to both the highest recommended field rate and to a 25% concentration of the high rate. Persistence in the field was short. No significant differences in mortality were detected for beetles transferred to treated and water-treated eggplant foliage taken three days after application. Of the insecticides approved for use in organic production, Entrust likely offers an excellent choice for management of eggplant flea beetles on eggplant

Keywords: Spinosad; Entrust; Eggplant flea beetle; *Epitrix fuscula*, Toxicity

Introduction

The eggplant flea beetle, *Epitrix fuscula* Crotch, is one of the major insect pests of eggplant, *Solanum melongena* L. var. *esculentum* (Solanaceae), produced in the south central United States. The insect overwinters as an adult and attacks eggplant seedlings as soon as they are transplanted into the field in early spring. The beetles feed on both the upper and lower leaf surfaces. Initial feeding results in a "shot-hole" appearance and, as feeding continues, the small holes can coalesce into large areas of necrotic leaf tissue [1]. Also, at the time of initial attack, the transplanted seedlings are generally weak from being produced during late winter in the greenhouse. The transplanting process can further weaken the plants and as temperatures are low in the spring, plant growth may be slow. If beetle population level is high, injury can be severe and seedling death may occur. This is especially common along field edges [2]. To limit this injury, insecticides are often applied to eggplant foliage at, or immediately after, transplanting. Although traditional synthetic insecticides have provided acceptable flea beetle management [3], insecticides approved for organic production have only provided marginal levels of management. Ellis and Bradley [4] recommended several botanical insecticides for flea beetle management including neem, rotenone, pyrethrin and sabadilla. Although each of these materials may possess some toxicity to eggplant flea beetle, their benefits under field conditions are limited [5]. Patton et al. [6] reported on the effectiveness of Surround [Kaolin clay] + Trilogy [neem oil extract] and Diatect [diatomaceous earth] against eggplant flea beetle. Although adult numbers were reduced, damage levels were only reduced by about 50% despite four applications. Another organic approved product, Nature's Glory [citric acid], was tested by Sorensen and Cooke [7]. Flea beetle damage was significantly reduced in only two of six observations. To our knowledge, no other organically approved insecticide has been shown to provide acceptable management of the eggplant flea beetle.

McLeod and Diaz [3] demonstrated the effectiveness of Spintor (Dow AgroSciences, Indianapolis, IN), a formulation of spinosad not approved for organic use, against the eggplant flea beetle. After a single application following transplanting, both flea beetle numbers and feeding injury were significantly reduced in field studies. Spinosad is a fermentation product produced by the soil bacterium, *Saccharopolyspora spinosa*, and is low in toxicity to non-target organisms including beneficial insects [8,9]. In recent years a new organic approved formulation of spinosad, Entrust (Dow AgroSciences, Indianapolis, IN), has become available. Because of the previously reported effectiveness of Spintor against flea beetles, studies were undertaken to determine the toxicity of Entrust against the eggplant flea beetle.

Materials and Methods

Insect source

Eggplant flea beetles used in the study were collected from field-grown "Black Beauty" eggplant grown at the University of Arkansas Main Experimental Station, Fayetteville. During late winter of 2010, eggplant transplants were produced in 5.5 x 5.5 x 7.6 cm peat cups in a greenhouse and set into the field on 16 April. Plants were produced following local recommendations including fertilization [300kg 13-13-13 per ha], weed management [1.5kg S-metolachlor per ha along with hand weeding] and weekly watering through drip irrigation. No insecticides were applied. Transplants grew and produced fruit throughout the production season until freezing temperatures that occurred in late October. Adult eggplant flea beetles used in the bioassays were collected in the field from eggplants using an aspirator.

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Test foliage

Eggplant cv 'Black Beauty' plants were grown in 3.8 l plastic pots containing potting soil (Scott's Moisture Advantage, Scott's Company, Marysville, OH) from seed obtained from the Green Seed Co., Inc., Springfield, MO. Plants were produced from February until December 2010 in the vegetable insect greenhouse located at the University of Arkansas Main Experimental Station, Fayetteville, Arkansas. Temperature was maintained between 25 and 29°C with natural light.

Dosage-mortality response

The test insecticide was Entrust, a commercial formulation containing 80% spinosad. The material is listed by the Organic Materials Review Institute (OMRI) for use in organic production. The spinosad powder was weighed and mixed with 500 ml water containing 0.005 % crop oil surfactant. The resulting concentration was 1088 ppm spinosad:water. This was further serially diluted to 544, 54, 27, and 7 ppm. Each spinosad mixture along with a water check was placed in a hand operated sprayer (Gilmour Manufacturing Co., Somerset, PA) that was calibrated to deliver 257.4 l/ha. Each concentration was applied to the upper leaf surface of eggplant foliage that had been cut with a razor from the greenhouse-grown plants. After drying, leaves were placed with the treated side to the inside over a 28-ml plastic cup (Solo Cup Co., Lake Forest, IL) that had the cup bottom replaced with saran mesh [10]. A loose fitting cardboard cap was placed over the leaf and five field-collected eggplant flea beetle adults were aspirated into the cup. The cap was quickly pushed into the cup rim. Leaf edges were trimmed with a razor blade and the cup was inverted over moist blotter paper in a plastic tray. Four cups each containing five eggplant flea beetles were used for each concentration and the test was repeated eight times. Thus, 160 beetles were tested for each spinosad concentration. Trays were placed in an environmental chamber set at 23°C and 12:12 L:D photoperiod. Mortality was assessed five days after exposure by tapping the test arena and looking for beetle movement. Lack of movement was used as the criterium for judging death. Data were analyzed with Proc Probit which corrects for control mortality (SAS 9.1, SAS Institute, Cary, NC).

Influence of exposure time on feeding and mortality

A similar study was undertaken to determine when mortality occurred, i.e., the time-mortality response, and the level of feeding activity following exposure to spinosad. The rate used was 544 ppm. This was equivalent to the highest recommended field rate of Entrust on eggplant, i.e., 0.125 lb ai/A or 140 g ai/ha. This concentration was again applied to greenhouse produced eggplant foliage as described above. Beetles were collected from the field and five were transferred to each of 16 test arenas as was done in the dosage-mortality study. Five beetles were also transferred to each of 16 cups with water-treated foliage which served as a control. Cups were held as in the dosage-mortality bioassay. During the initial day of exposure, mortality was assessed every six hours by tapping the test arena and looking for beetle movement. Lack of movement was used as the criterium for judging death. The number of feeding pits were also counted. After the first day, mortality and the number of feeding pits were determined each day until the test was terminated on day seven. Data were analyzed by Proc GLM and means were separated by LSD (SAS 9.1, SAS Institute, Cary, NC).

Spinosad persistence on field-grown eggplant foliage

A study to determine the length of activity of spinosad on field grown eggplant was undertaken. 'Black Beauty' eggplant seedlings were produced to the six true leaf stage in the greenhouse described above and hand planted in a small plot at the University of Arkansas Main Experimental Station, Fayetteville on 10 May 2010. The experimental design was a randomized complete block with three treatments and five replications. Each plot consisted of a single row containing 15 plants, with plants spaced every 0.5 m. Rows were separated by 1 m. Plants were grown as described above. No insecticides were applied prior to the test. One week after transplanting four leaves on each of 10 plants in each plot were marked on the leaf edge with a felt tip pen. Treatments consisted of Entrust (544 and 136 ppm) and a water only check, applied at 257.4 l/ha with the hand sprayer described above. Care was taken to apply the spray to each marked leaf, and sprays included crop oil at 0.005%. Immediately after the sprays had dried, eight randomly selected marked leaves were cut from each plot and transferred to the lab for bioassay. Each leaf was placed with the top side toward the cup interior, five eggplant flea beetle adults were introduced, and caps were pushed into the cup as described above. Cups were held as in the previous experiments. Beetle mortality (lack of movement) and the number of feeding pits were determined five days later. The test was repeated one, two, three and four days after the field application. Data were analyzed by Proc GLM and means were separated by LSD (SAS 9.1, SAS Institute, Cary, NC).

Results and Discussion

Dosage-mortality response

The spinosad formulation, Entrust, was toxic to eggplant flea beetle adults held on treated eggplant leaf disks. Percent mortality ranged from 3.1 at the low dose of 7 ppm to 97.5 at the high dose of 544 ppm (Figure 1). The parameters of the dosage-mortality response are listed in Table 1. The highest recommended rate of Entrust on eggplant is 140

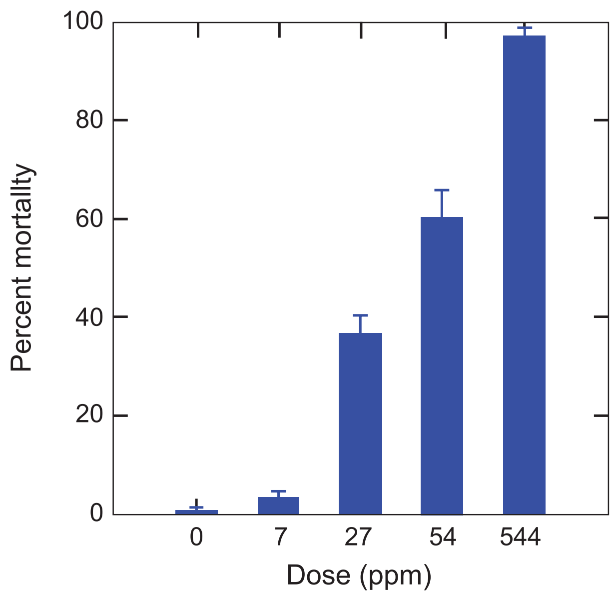

Figure 1: Dosage-mortality response of adult eggplant flea beetles to spinosad applied to eggplant foliage in the laboratory.

	n	slope ± SE	LC_{50} (95%FL)	LC_{95} (95%FL)	X^2
Entrust	640	1.959 ± 0.149	42.9 (36.9-50.2)	296.7 (219.4-440.9)	171.73

Table1: Dosage-mortality response of eggplant flea beetle adults to Entrust, an organically acceptable formulation of spinosad. Values are in ppm spinosad:water.

g ai/ha (0.125 lb ai/A). When this rate is applied at 257.4 l/ha, the spray volume used in the study, the equivalent rate is 544 ppm. The estimated LC_{95} was 296.7 ppm (Table 1). This value was approximately half the recommended field rate. Although many factors including spray coverage, persistence and insect behaviour may reduce the effectiveness of an insecticide applied under field conditions, the dosage-response data indicate that Entrust applied at the highest recommended field rate should be sufficiently toxic to effectively reduce eggplant flea beetle levels on eggplant.

Influence of exposure time on feeding and mortality

Spinosad rapidly affected the eggplant flea beetles placed on treated eggplant foliage. In as little as six hours, a loss of coordination was observed. Beetles on treated surfaces were generally able to stand upright but movement of antennae and legs appeared much slower than for beetles held on water-treated foliage. One day after exposure 20% of the beetles on treated foliage were unable to move and were considered dead (Figure 2). This percentage increased to 50% on day 2, a significant increase (F=83.5; p< 0.001). Mortality further increased significantly to 71.2% on day 3 and this was not significantly different from the 76.2% on day 4. On day 5, mortality was significantly increased to 95.0%. This was not significantly different from that for the 100% on day 6 and 7. No beetles held on water treated foliage died.

Although beetle death often required several days, the impact of spinosad on reducing feeding was more rapid (Figure 3). Non-treated beetles produced a mean of 12.7 feeding pits per leaf disk during the initial day of exposure while beetles on spinosad treated foliage produced only 2.1 pits, a significantly lower number (F=62.3; p=0.0002). During the second day, the mean number of pits on treated leaf disks was only 0.6 while a mean of 6.4 pits was observed on water-treated disks, again a significant difference (F=66.7; p<0.0001). This trend continued for the duration of the study.

Spinosad persistence on field-grown eggplant foliage

Eggplant leaves collected immediately after Entrust was applied in the field was toxic to adult eggplant flea beetles (Figure 4). The highest recommended Entrust rate of 544 ppm produced 98.5% mortality. Although the lower rate of 136 ppm produced significantly lower mortality, 90% of the beetles were killed (F=472.45; p<0.0001). No mortality was observed with beetles placed on water-treated foliage. One day after application 90.5% of the beetles placed on foliage treated with the high rate were killed. Mortality at the low rate was only 13.0%, significantly lower than that for the high rate (F=638.62; p<0.0001). Control mortality was 0.5%. Two days after application mortality declined to 29.5% for the high rate and 4.5% for the low rate. Only the high rate was significantly greater than that for the control (F=15.52; p<0.0001). Mortality was low at three and four days after application and no significant differences were detected among the three treatments

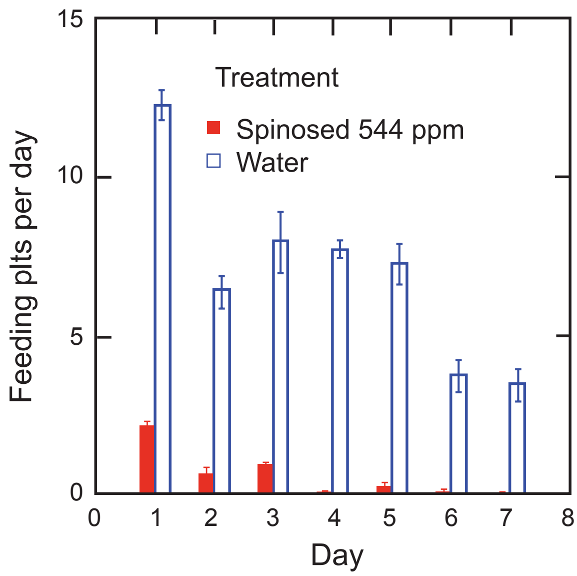

Figure 3: Influence of exposure time on eggplant flea beetle feeding following exposure on eggplant foliage treated with spinosad at 544 ppm in the laboratory.

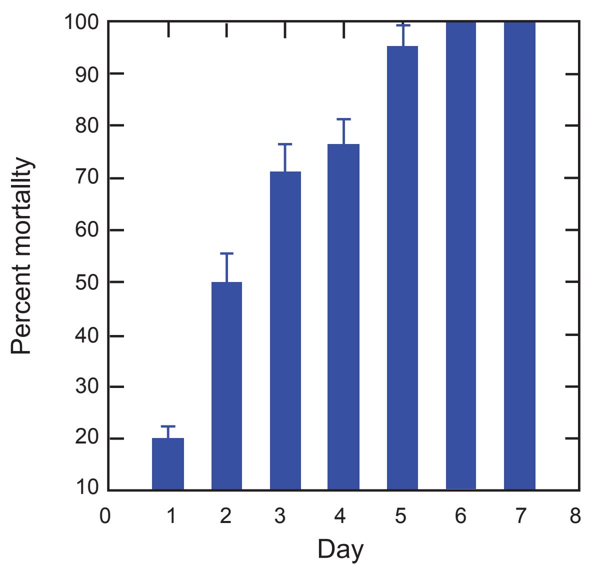

Figure 2: Influence of exposure time on eggplant flea beetle mortality following exposure on eggplant foliage treated with spinosad at 544 ppm in the laboratory.

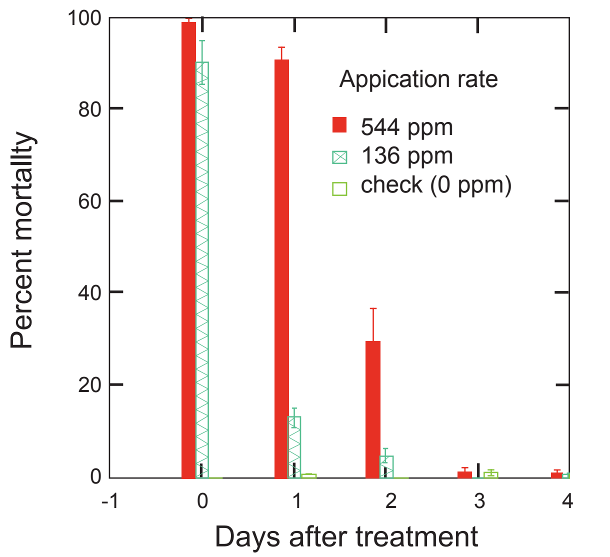

Figure 4: Residual toxicity of spinosad against eggplant flea beetles following application to eggplant leaves in the field.

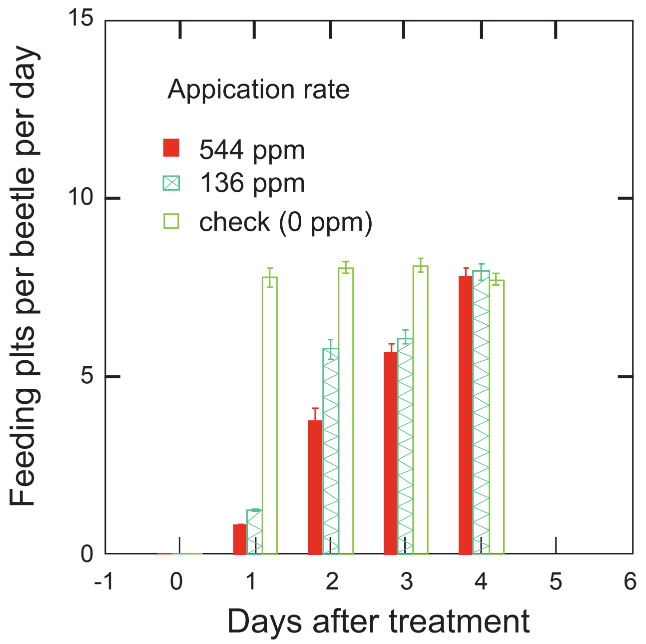

Figure 5: Effect of spinosad on eggplant flea beetle feeding following application to eggplant leaves in the field.

(F=1.70; p=0.2012).

The number of feeding pits on treated foliage also was reduced when compared to water-treated foliage one day after application (Figure 5). A mean of 7.8 pits was counted on check disks while 0.8 and 1.2 pits were counted on foliage with the high and low rate of Entrust, respectively. Both means for the treated foliage were significantly lower than on the check foliage (F=621.91; p<0.0001). Also, the number of pits did not significantly differ between the high and low rate on day 1. On day 2, the mean numbers of feeding pits were 3.7, 5.8 and 8.1 for the high rate, low rate and check, respectively. Each of the three treatments were significantly different from the others (F=73.9; p<0.0001). On day three, the mean number of feeding pits was 5.7, 6.1 and 8.1, for the low rate, high rate and check, respectively. Although no significant difference was detected between the two Entrust rates, both were significantly lower than for the check (P=45.03; p<0.0001). On foliage taken four days after application, no significant differences in the number of feeding pits were detected among the three treatments (F=0.27; p=0.7647). These data indicate that Entrust persistence is relatively short on field-grown eggplant. Rapid declines in toxicity were observed two and three days after application and no significant toxicity was detected four days after Entrust was applied. Thus, with eggplant transplants on which newly emerging eggplant flea beetles infest, additional applications of Entrust may be required.

Conclusions

Data from these studies indicate that Entrust, the organic formulation of spinosad, is toxic to eggplant flea adults when applied to eggplant foliage. An LC_{95} value of 296.7 ppm was established. The maximum recommended rate of 140 g ai/ha or 544 ppm is almost twice this LC_{95}. Although mortality was sometimes delayed until six days following exposure to spinosad, the insecticide rapidly affected flea beetle behavior. Feeding was significantly reduced one day after exposure. Further, exposure to a reduced rate of spinosad (equivalent to 35 g ai/ha) also resulted in significant mortality and reductions in feeding. Of the insecticides approved for use in organic production, Entrust likely offers a valuable choice for management of eggplant flea beetles on eggplant.

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