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ADULT VIAL TECHNIQUE FOR EVALUATING INSECTICIDAL TOXICITY TO COTTON FLEAHOPPER

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Abstract

An adult vial test was used to determine contact toxicity of technical dicrotophos (Bidrin ® 8), acephate (Orthene ®), imidacloprid (Provado ® 1.6), thiamethoxam (Centric™ 40 WG) and indoxacarb (Steward™) to cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter). These data are the first published for this species. Lethal concentrations [LC_{50}s (95% CLs)] at 24 h for dicrotophos and acephate were 0.1886 (0.1696-0.2066) and 7.6630 (5.9272-9.0815) µg/vial, respectively. These values were significantly different. For imidacloprid and thiamethoxam, LC_{50}s (95% CLs) at 24 h were 0.6583 (0.4422-0.9157) and 0.3855 (0.2724-0.5149) µg/vial, respectively. These values were not significantly different. Both imidacloprid and thiamethoxam were significantly less toxic than dicrotophos but significantly more toxic than acephate. Contact with indoxacarb did not significantly influence fleahopper mortality. Systemic toxicity of imidacloprid and thiamethoxam was studied by soaking green beans, *Phaseolus* spp. in water containing different concentrations of Provado 1.6 and Centric 40 WG. LC_{50}s (95% CLs) at 24 h for Provado and Centric were 74.8052 (54.9742-97.0045) and 36.0169 (20.8745-53.2132) µg/ml, respectively. These values were significantly different and were much higher than those for the same chemicals by contact.

Introduction

Cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter) (Hemiptera: Miridae) is an important pest of cotton during early stages of plant growth in southcentral United States and caused an estimated crop loss of more than $36 million in 1997 (Williams 1998). Fleahoppers feed on terminals of cotton plants and cause blasting and shedding of young squares, reduce fruiting branches and produce whip-like growth (Reinhard 1926). More recently, Parker (1996) reported that fleahoppers caused extensive damage and subsequent lint loss in Texas where cotton was not protected with insecticide during the critical early fruiting stage.

Many insecticides including organophosphates are suggested by the Texas Extension Service for control of fleahoppers on cotton (Moore et al. 2003). However, insecticides less harmful to the environment are being sought for controlling fleahoppers on cotton because organophosphates are likely to be banned in the near future by the U. S. Environmental Protection Agency in accordance with the Food Quality Protection Act of 1996. The neonicotinoid class of insecticides appears to be a likely alternative to organophosphates (Sansone et al. 2002; Parker and Norman 2003). To assess efficacy of insecticides for control of fleahoppers in cotton, it is useful to obtain information on the toxicity of these pesticides using the adult vial test. Moreover, these data provide information for monitoring development of insecticide resistance in fleahopper populations. In this paper, we report on a study designed to determine the toxicity of organophosphate and neonicotinoid insecticides as well as indoxacarb to adult *P. seriatus*.

Materials and Methods

Insects

Cotton fleahoppers were collected as diapausing eggs during 2002-2003 from its overwintering host, woolly croton, *Croton capitatus* (Michaux) in the Brazos River Valley of Texas and were reared in accordance with the methods described by Breene et al. (1989). Briefly, croton stems harvested in the winter and kept refrigerated were broken into small pieces and placed inside 39-oz coffee cans. Periodically, the stems were soaked in tap water and shaken vigorously into a funnel placed over a large plastic container to collect the nymphs as they hatched. Young nymphs were transferred to a 2.4-gallon rectangular plastic Rubbermaid® container in which shredded paper was placed snugly in position. The top of the container from which the inner portion had been cut was used to hold a piece of organdy cloth over the top to seal the container. During the 1st week, fleahoppers were fed with green beans and cut pieces of potatoes placed inside the containers and during the 2nd week and thereafter, fleahoppers were fed with green beans, cut pieces of potatoes and artificial diet (Cohen 2000) placed over the organdy cloth. At the time of each test, adult fleahoppers were collected from the containers using an aspirator.

Insecticides

Technical insecticides used were: dicrotophos (Bidrin® 8) 86% (Amvac Chemical Corp.), acephate (Orthene ®) 99.2% (Valent Chemical Corp.,), imidacloprid (Provado ® 1.6) 99.5%, thiamethoxam (Centric ™ 40 WG) 99% and indoxacarb (Steward ™) 98% (Chem Service, West Chester, PA.). Commercially-formulated insecticides used were: Provado ® 1.6 and Centric™ 40 WG (UAP, Snook, TX and Syngenta Corp., respectively).

Determination of Contact Toxicity

Adult vial test procedures were similar to those described earlier (Plapp et al. 1987; Snodgrass 1996). Briefly, stock solutions of technical grade insecticides were mixed in acetone (assay 99.5% min.). Various concentrations of insecticides were prepared from the stock solutions and stored in a refrigerator. At the time of each test, insecticide solutions were warmed to ambient temperature in the laboratory. One-half ml of each concentration was pipetted into a 20-ml scintillation vial. The vials were then placed on a hot dog roller (heating elements removed) and the roller was operated until the acetone completely evaporated leaving behind insecticidal residues inside the vials. Vials were used for testing during the same day they were prepared.

Green beans, *Phaseolus* spp. purchased from local grocery stores were placed in a colander, washed in a sink and baking soda (household bicarbonate of soda) was sprinkled over them to neutralize any pesticide residues. Baking soda was completely washed off and the beans were dried by blowing air over them with an electric fan. Green beans were cut with a razor blade into ½ to ¾ in. long pieces. Moisture on the cut ends was dried with a paper towel and one piece was placed inside each vial. Five to 10 adult fleahoppers were aspirated into each insecticide-treated vial and the mouth of the vial was closed with a ball of cotton. An untreated control treated only with acetone was maintained for all tests. Vials were kept in an environmental room maintained at 80º F, RH >60% and a photoperiod of 14:10 h L:D. Mortality was checked 24 h thereafter. Fleahoppers were considered dead when they could not right themselves after the test vials were emptied into a container.

Determination of Systemic Toxicity

Stock solutions of Provado and Centric were prepared by mixing 100 mg of each chemical (511 µl of Provado and 0.25 gm of Centric) in RO water (reverse osmosis, purity ~25 ppm) to a total volume of 100 ml. Various concentrations of the insecticides were then prepared and stored in 120 ml wide mouth amber glass bottles. At the time of each test, green beans were individually soaked in the desired concentration of the insecticide for 1-min and dried at ambient temperature in the laboratory, placed on a paper towel and cut into ½ to ¾ in. long pieces. The cut ends were dried with a paper towel and placed individually in test vials. Test procedures to determine mortality were similar to those described earlier.

A test was conducted in which green beans soaked in Provado solution at 300 µg/ml for 1-min were compared to similarly treated beans washed in tap water and an untreated control. The intent was to differentiate between systemic and contact toxicity.

Data Analysis

Dosage mortality equations [lethal concentrations (LCs)] and associated statistics were computed using POLO-PC (LeOra software 1987). Statistical differences between LCs were determined using the presence or absence of overlap in the 95% confidence limits (CLs). Regression statistics were computed using PROC REG (SAS 2001).

Results and Discussion

Contact Toxicity

Dosage mortality equations provided good fit for the mortality data with $\chi^2 = 6.08$ and 6.68 with 4 *df* for 24 h responses, respectively, for dicrotophos and acephate (Table 1). The LC_{50} (95% CLs) values for dicrotophos and acephate after 24 h were 0.1886 (0.1696-0.2066) and 7.6630 (5.9272-9.0815) µg/vial, respectively. Based upon the lack of overlap in the 95% CL values, dicrotophos was significantly more toxic to fleahoppers than acephate. Also, the slope of the regression line for dicrotophos was significantly greater than that for acephate ($t = 3.76$; $df = 8$; $P < 0.05$). To our knowledge, the work we report here is the first use of glass-vial assay for fleahopper mortality. By comparison, Snodgrass (1996) reported that LC_{50} for acephate varied between 6.05 to 8.48 µg/vial for a related species, adult tarnished plant bugs (Hemiptera: Miridae) collected in Mississippi and Arkansas farm lands. The LC_{50} (7.66 µg/vial) for acephate reported in this study for fleahoppers is comparable.

Dosage mortality equations also provided good fit for the mortality data with $\chi^2 = 6.51$ and 8.26 with 5 *df* for 24 h responses, respectively, for imidacloprid and thiamethoxam (Table 2). The LC_{50} (95% CLs) values for imidacloprid and thiamethoxam after 24 h were 0.6583 (0.4422-0.9157) and 0.3855 (0.2724-0.5149) µg/vial, respectively. Based upon presence of overlap in the 95% CL values, contact toxicity of imidacloprid and thiamethoxam for fleahoppers was comparable. The slopes of the regression line for imidacloprid and thiamethoxam were comparable as well ($t = 0.86$; $df = 10$; $P > 0.05$). Both imidacloprid and thiamethoxam were significantly less toxic than dicrotophos but significantly more toxic than acephate.

Dosage mortality equation for contact toxicity of indoxacarb for fleahoppers could not be computed as the data revealed no dosage response (Fig.1). Regression of percentage mortality of fleahoppers on indoxacarb concentrations in µg/vial was highly significant ($F = 9.58$; $df = 1, 87$; $P < 0.01$) with the slope of the regression coefficient being significantly different from 0 ($t = 3.10$; $P < 0.01$). However, the regression model accounted for only 10% of the variation ($R^2 = 0.0992$) with percentage mortality of fleahoppers increasing by a small amount ($b = + 0.01834$). These data suggest that indoxacarb is not very toxic to fleahoppers.

Systemic Toxicity

Dosage mortality equations provided good fit for the mortality data with $\chi^2 = 2.14$ and 2.00 with 3 *df* for 24-h responses, respectively, for Provado and Centric (Table 3). The LC_{50} (95% CLs) values for Provado and Centric after 24 h were 74.8052 (54.9742-97.0045) and 36.0169 (20.8745-53.2132) µg/ml, respectively. Based upon lack of overlap in the 95% CL values, systemic toxicity of Centric for fleahoppers was significantly greater than that for Provado. However, the slope of the regression coefficient for Provado was not significantly different from that for Centric ($t = 0.28$; $df = 6$; $P > 0.05$).

It is likely that fleahopper mortality attributed to green beans treated with Provado and Centric may be due not only to systemic toxicity but contact toxicity as well. Figure 2 shows that green beans unwashed after treatment with Provado at 300 µg/ml provided significantly greater fleahopper mortality compared to similarly treated washed beans ($P < 0.05$). Fleahopper mortality for treated washed beans did not significantly differ from the untreated control. Data suggest that systemic toxicity reported here is confounded with contact toxicity. Note that the research reported here is based upon limited data and further research is needed to fully understand and elucidate these mortality components.

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Table 1. Lethal concentration (LC) (µg/vial) data (24 h) for contact toxicity of technical dicrotophos (Bidrin® 8) and acephate (Orthene ®) to cotton fleahoppers when exposed to insecticides prepared in acetone and placed inside 20 ml scintillation vials [a/].

Regression Statistics	Dicrotophos	Acephate
Slope (±SE)	7.1372±0.7499	3.8588 (±0.44301)
χ^2 (df)	6.08 (4)	6.68 (4)
LC_{10} (95% lower-upper limits)	0.1247a (0.0939-0.1443)	3.5668b (1.7379-4.9092)
LC_{50} (95% lower-upper limits)	0.1886a (0.1696-0.2066)	7.6630b (5.9272-9.0815)
LC_{90} (95% lower-upper limits)	0.2852a (0.2518-0.3598)	16.4631b (13.2405-25.6483)

[a/] Based upon 491 and 570 adults for dicrotophos and acephate, respectively. Calculated using POLO-PC (LeOra Software, 1987). LC values in the same row followed by the same lower case letter are significantly different based upon lack of overlap in the 95% confidence limits.

Table 2. Lethal concentration (LC) (µg/vial) data (24 h) for contact toxicity of technical imidacloprid (Provado ® 1.6) and thiamethoxam (Centric™ 40 WG) to cotton fleahoppers when exposed to insecticides prepared in acetone and placed inside 20 ml scintillation vials [a/].

Regression Statistics	Imidacloprid	Thiamethoxam
Slope (±SE)	2.0788±0.2195	1.8372±0.1735
χ^2 (df)	6.51 (5)	8.26 (5)
LC_{10} (95% lower-upper limits)	0.1592a (0.0664-0.2638)	0.0773a (0.0310-0.1293)
LC_{50} (95% lower-upper limits)	0.6583a (0.4422-0.9157)	0.3855a (0.2724-0.5149)
LC_{90} (95% lower-upper limits)	2.7224a (1.8332-5.1045)	1.9211a (1.2751-3.8647)

[a/] Based upon 393 and 690 adults for imidacloprid and thiamethoxam, respectively. Calculated using POLO-PC (LeOra Software 1987). LC values in the same row followed by the same lower case letter are not significantly different based upon the presence of overlap in the 95% confidence limits.

Table 3. Lethal concentration (LC) (µg/ml) data (24 h) for systemic toxicity of Provado® 1.6 and Centric™ 40 WG to fleahoppers when green beans soaked in solutions of the insecticides for 1-min and placed inside 20 ml scintillation vials [a/].

Regression Statistics	Provado 1.6	Centric 40 WG
Slope (±SE)	1.3608±0.2012	1.3978±0.2555
χ^2 (df)	2.136 (3)	2.00 (3)
LC_{10} (95% lower-upper limits)	8.5539a (3.0272-15.4745)	4.3617a (0.9262-9.4433)
LC_{50} (95% lower-upper limits)	74.8052a (54.9742-97.0045)	36.0169b 20.8745-53.2132)
LC_{90} (95% lower-upper limits)	654.1811a (402.1267-1509.6690)	297.4114a (173.5166-813.0308)

[a/] Based upon 477 and 281 adults for Provado and Centric, respectively. Calculated using POLO-PC (LeOra Software, 1987). LC values in the same row followed by the same lower case letter are not significantly different based upon the presence of overlap in the 95% confidence limits.

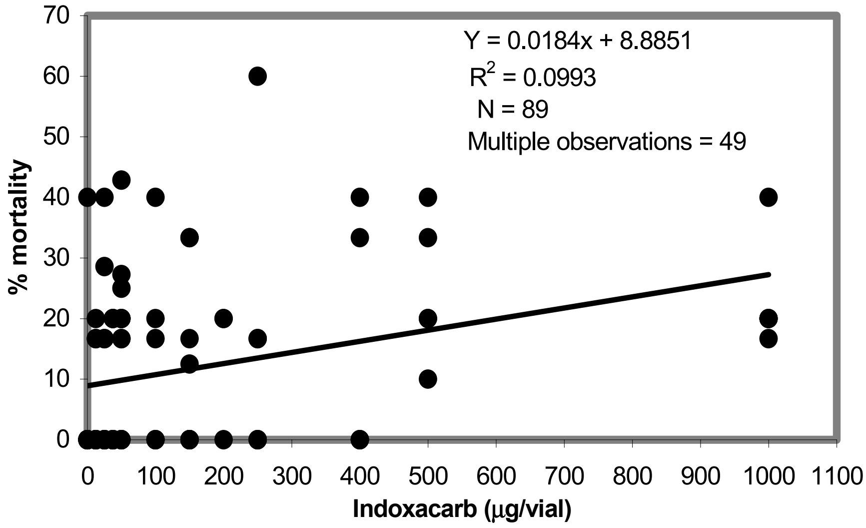

Figure 1. Relationship between contact toxicity of indoxacarb and % mortality of cotton fleahoppers when insects were aspirated into scintillation vials coated with insecticide in µg/vial. Number of fleahoppers used in the study = 503.

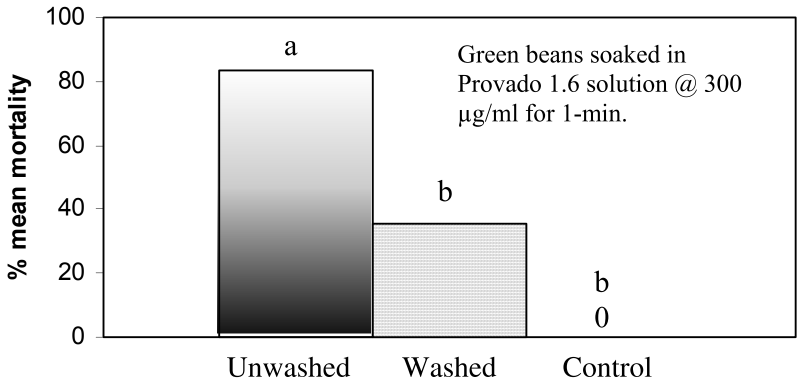

Figure 2. Comparison of percentage mean fleahopper mortality for washed and unwashed green beans soaked in Provado 1.6 solution at 300 µg/ml for 1-min and compared to an untreated control. Means followed by the same lower case letter are not significantly different ($P < 0.05$; Protected LSD test).