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Note

Adult vial bioassays of insecticidal toxicity against cotton fleahopper, *Pseudatomoscelis seriatus* (Hemiptera: Miridae)

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Glass vials coated with several technical insecticides were used to determine the contact toxicity of insecticides on adult laboratory-reared and field-collected cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter). For the 17 insecticides evaluated for laboratory-reared cotton fleahoppers, bifenthrin (pyrethroid), dicrotophos (organophosphate), thiamethoxam (neonicotinoid), and methomyl (carbamate) were the most toxic insecticides in their respective insecticidal classes based on LC_{50} values. There were significant differences between the LC_{50} values for the insecticides tested within each of the four insecticidal classes. There were 13-, 46-, 58-, and 31-fold differences between LC_{50} values for the insecticides within the pyrethroid, organophosphate, neonicotinoid, and carbarmate classes, respectively. Among fleahoppers collected from horsemint in May/June, adult vial testing showed increased susceptibility in males versus females. This difference can be attributed, at least in part, to differences in insect weights between the males and females since the females weighed significantly more than the males. Data presented herein provide a measure of acute potency of various insecticides against *P. seriatus* and serve as a measure of inherent relative differences between the insecticides. Baseline data will be useful for future comparison should suspicion of tolerance to these insecticides develop in field populations. These data are also important in comparing results from laboratory and field studies with cotton fleahoppers.

Keywords: *Pseudatomoscelis seriatus*, cotton fleahopper, technical insecticide, insecticide toxicity, adult vial technique.

Introduction

Cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter) (Hemiptera: Miridae) is an important pest of cotton during early fruiting stages of plant growth in south-central United States causing an estimated crop loss of $18 million in 2001 in Texas alone.[1)] During early season and until about first bloom, fleahoppers feed on terminals of cotton plants causing blasting and shedding of young squares, reduced fruiting branches, and production of whip-like growth.[2)] Pectinases in the fleahopper saliva have been found to destroy plant cells and subsequent production of ethylene in squares,[3)] which caused square abscission. Crop maturity was delayed by *P. seriatus* feeding on early season floral structures increasing vulnerability to lepidopteran pests, such as *Helicoverpa zea* (Boddie) and *Heliothis virescens* (F.).

Because of the success of boll weevil eradication in many areas of the Cotton Belt, where cotton fleahoppers are primary pests, insecticidal control directed solely at the cotton fleahopper is necessary. Previously, broad spectrum insecticidal control of both pests was possible because of the synchrony between the initiation of boll weevil and fleahopper damage. Fleahoppers caused extensive damage and subsequent lint loss in Texas where cotton was not protected with insecticide during the critical early fruiting stage.[4),5)] Traditional field tests that evaluate formulated insecticides for control of cotton fleahopper in small plots can be expensive and require spatial and temporal validation of the data to obtain meaningful results. Alternatively, a more rapid method of assessing toxicity of insecticides may be bioassaying using glass vials with the interior coated with varying levels of technical insecticides, which is referred to as the adult vial test (AVT) procedures. Accordingly, the toxicity of technical dicrotophos, acephate, imidacloprid, thiamethoxam and indoxacarb to cotton fleahopper have been analyzed using the AVT[6)]. AVT studies measure the inherent relative differences in contact toxicity between insecticides, while, field studies can measure both the contact and systemic properties of an insecticide. Because toxicity is related to concentration, determining systemic concentrations within plants and differentiating systemic toxicity from contact toxicity is difficult and does not facilitate testing of insecticidal activity for products that are not systemic.

In various growing areas of Texas, one to four insecticide applications for cotton fleahoppers are made per growing season with organophosphates being the dominant class of insecticide.[7),8)] The factor that most affects the number of applications is the number of cotton fleahoppers moving from senescing wild hosts into cotton during the early season. The introduction of the neonicotinoid class of insecticides has increased the number of products available for controlling cotton fleahopper. Most of the neonicotinoids are applied as seed treatments. The effectiveness of these treatments is affected by the systemic persistence of neonicotinoids in the cotton plant. There is some exposure of fleahoppers to the carbamates, especially aldicarb, applied in-furrow.

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The objectives of this work were to evaluate the contact toxicity of 17 labeled insecticides to laboratory-reared adult *P. seriatus* using AVT, establish a baseline of contact toxicity for these insecticides, and determine if the susceptibility of fleahoppers collected from horsemint was different from those reared in the laboratory for selected insecticides. Cotton fleahoppers were collected from horsemint because adults developing on horsemint are an important source of those dispersing into early season cotton. Cotton fleahoppers were collected from croton late in the growing season because these adults produce the overwintering generation. The baseline data for technical insecticides labeled for use against fleahoppers on cotton will be useful in detecting resistance to formulated or commercial insecticides and determining insecticide efficacy between years.

Note: Mention of a commercial or proprietary product does not constitute an endorsement for its use by the U. S. Department of Agriculture.

Materials and Methods

1. Laboratory-reared insects

Cotton fleahoppers were collected as diapausing eggs during the winter of 2003–2004 from woolly croton, *Croton capitatus* (Michaux) in the Brazos River Valley near College Station, TX (Latitude: 30°30′29N, Longitude: 96°25′03W). The croton branches were placed in burlap sacks and stored in a walk-in refrigerator maintained at 15°C. The eggs were reared to adults following the methods described by Breene et al. (1989). Croton stems harvested in the winter were broken into small pieces and placed inside 1-L coffee cans and exposed to 26–27°C temperatures in the laboratory room used for insect rearing. Periodically, the stems were soaked in tap water and returned to the rearing room after drying. Once nymphal emergence started, the cans were shaken vigorously daily into a funnel placed over a large plastic container to collect the nymphs. Young nymphs were transferred to a 9-L rectangular plastic Rubbermaid® container with shredded paper that was placed snugly in position. The container lid had the inner portion cut out and a piece of organdy cloth placed over the top to seal the container. During the 1st week, fleahoppers were fed green beans, *Phaseolus vulgaris*, and small pieces of potatoes, *Solanum tuberosum*, placed inside the containers. During the second week and thereafter, fleahoppers were fed green beans and potato pieces and artificial diet[10] placed over the organdy cloth. At the time of each test, fleahopper adults were collected from the containers using an aspirator.

2. Field-collected insects

Using gently swung sweep nets, fleahoppers were collected from croton in the fall of 2004 and 2005 and from green horsemint (*Monarda punctata* L.) during May and June of 2005 and 2006. Fleahoppers captured from the green horsemint were released inside a plastic cage and kept in a screened, well ventilated room. Fleahoppers were collected with an aspirator and tested for contact toxicity using the adult vial test. Fleahopper mortality was determined by sex for each insecticide. Also, a subset of the insects were sorted by gender and live weights were determined using a Sartorius balance (Model # CT225D) sensitive to 0.01 of a milligram.

3. Insecticides

A total of 17 insecticides were tested. These insecticides fell into four insecticidal classes: pyrethroids (esfenvalerate, bifenthrin, deltamethrin, lambda-cyhalothrin and zeta-cypermethrin), organophosphates (OPs) (dimethoate, methamidophos, methyl parathion, dicrotophos, acephate, and oxydemeton-methyl), neonicotinoids (acetamiprid, thiamethoxam, imidacloprid and thiacloprid), and carbamates (methomyl and oxamyl). Technical insecticides were purchased from Chem Service, Inc. (West Chester, PA 19380, USA) for these studies.

4. Determination of contact toxicity

Adult vial test procedures were similar to those described in previous literature.[11),12)] Briefly, stock solutions of technical grade insecticides were dissolved in acetone (assay 99.5% min.). Various concentrations of insecticides were prepared from 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 tested during the same day they were prepared.

Green beans purchased from local grocery stores were placed in a colander and washed. Baking soda (household bicarbonate of soda) was sprinkled over the beans 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 used in the bioassays were sliced into 1–2 cm 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. Controls treated only with acetone were maintained for all tests. Vials were kept in an environmental room maintained at 26.7°C, 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. Each test was replicated 3–6 times.

5. Data analysis

Dosage mortality equations [lethal concentrations (LCs)] and associated statistics were computed using POLO-PC.[13)] Statistical differences between LCs were determined using the presence or absence of overlap in the 95% confidence limits (CLs). Live weights of fleahoppers were analyzed using PROC GLM procedure and means were separated using LSMEANS procedure with an adjust=Tukey option.[13)]

Table 1. Lethal concentration (LC) (μg/vial) data (24 h) for contact toxicity of technical insecticides to unsexed laboratory reared cotton fleahoppers determined using adult vial bioassay

Insecticides	N[a)]	χ^2 (df)	Slope±SE[b)]	LC_{50}[c)]	95% CL
			Pyrethroids		
Bifenthrin	486	1.72 (3)	5.69±0.886	0.156a	0.135–0.171
λ-Cyhalothrin	322	2.02 (3)	3.27±0.42	0.635b	0.504–0.763
Esfenvalerate	336	1.46 (3)	2.80±0.31	0.692b	0.58–0.81
zeta-Cypermethrin	474	0.45 (3)	3.58±0.44	1.195c	1.021–1.356
Deltamethrin	483	1.99 (3)	2.50±0.304	2.056d	1.69–2.45
			Organophosphates		
Dicrotophos	491	6.08 (4)	7.14±0.75	0.189a	0.17–0.21
Methamidaphos	332	0.21 (1)	5.76±0.81	0.456b	0.38–0.52
Methyl parathion	437	1.34 (3)	2.28±0.28	0.931c	0.76–1.14
Dimethoate	441	0.91 (2)	2.00±0.32	1.030c	0.80–1.26
Acephate	570	6.68 (4)	3.86±0.443	7.663d	5.93–9.08
Oxydemeton-methyl	314	0.70 (2)	2.15±0.300	8.725d	6.45–10.89
			Neonicotinoids		
Thiamethoxam	690	8.26 (5)	1.84±0.173	0.385a	0.272–0.515
Imidacloprid	393	6.51 (5)	2.10±0.22	0.658a	0.442–0.916
Acetamiprid	348	2.97 (3)	1.13±0.15	2.349b	1.528–3.393
Thiacloprid	564	10.74 (5)	1.38±0.153	22.361c	12.20–34.21
			Carbamates		
Methomyl	532	3.71 (4)	4.76±0.50	0.413a	0.377–0.447
Oxamyl	610	1.43 (4)	1.92±0.21	12.952b	10.02–15.79

[a)] N = number of insects used. [b)] Calculated using POLO-PC (LeOra Software 1987). [c)] LC_{50} values for each chemical class in the same column are not significantly different based upon the presence of overlap in the 95% confidence limits.

Results and Discussion

1. Laboratory-reared fleahoppers

For the laboratory-reared fleahoppers, dosage mortality equations for all technical insecticides for 24 h responses provided good fit with significant χ^2 values (Table 1). The data are presented by insecticidal classes based on LC_{50}s (Lower-upper 95% Confidence Limits).

1.1. Pyrethroids

Bifenthrin with an LC_{50} of 0.156 (0.135–0.179: 95% CLs) μg/vial at 24 h was the most toxic pyrethroid to cotton fleahopper. The LC_{50} of bifenthrin was the lowest in value and was significantly different from all other LC_{50} values among pyrethoids. The next most toxic SPs were λ-cyhalothrin and esfenvalerate with LC_{50} values of 0.635 (0.504–0.763) and 0.692 (0.581–0.807) μg/vial, respectively. Zeta-cypermethrin with an LC_{50} of 1.195 (1.021–1.356) μg/vial was the fourth most toxic SP, followed by deltamethrin, which was the least toxic pyrethoid, with an LC_{50} of 2.056 (1.690–2.451) μg/vial. The LC_{50} of zeta-cypermethrin was significantly different from that of deltamethrin. In summary, the ranking of the pyrethoids from most toxic to least toxic was bifenthrin, λ-cyhalothrin, esfenvalerate, zeta-cypermethrin, and deltamethrin. There was a 13-fold difference in LC_{50} values in this insecticidal class.

1.2. Organophosphates (OPs)

Dicrotophos with an LC_{50} of 0.189 (0.170–0.207) μg/vial was most toxic to *P. seriatus*. Dicrotophos was significantly different in toxicity from all other OP compounds tested. Methamidophos with an LC_{50} of 0.456 (0.376–0.524) μg/vial was the second most toxic OP to fleahoppers. The LC_{50} values for methyl parathion and dimethoate were 0.931 (0.762–1.138) and 1.030 (0.797–1.261) μg/vial, respectively, and not significantly different from each other. Acephate and oxydemeton-methyl had LC_{50} values of 7.663 (5.930–9.080) and 8.725 (6.451–10.887) μg/vial, respectively, were the lowest in toxicity and not significantly different from each other. The rankings of the OPs from most toxic to least toxic were dicrotophos, methamidophos, methyl parathion, dimethoate, acephate, and oxydemeton-methyl. There was a 46-fold difference in LC_{50} values in this insecticidal class.

1.3. Neonicotinoids

Although not significantly different from each another, thi-

amethoxam and imidacloprid with LC_{50} values of 0.385 (0.272–0.515) and 0.658 (0.442–0.916) μg/vial, respectively, were the most toxic to cotton fleahopper. Acetamiprid with an LC_{50} of 2.349 (1.528–3.393) μg/vial was the next most toxic neonicotinoid. Thiacloprid, with the highest LC_{50} of 22.361 (12.197–34.211) μg/vial was the least toxic to *P. seriatus*. There was a 58-fold difference in LC_{50} values in this insecticidal class. It is important to note that AVT primarily tests contact toxicity. Neonicotinoids also have translaminar activity, which has proven difficult to assess realistically and repeatedly in the laboratory.

1.4. Carbamates

Methomyl with an LC_{50} of 0.413 (0.377–0.447) μg/vial was significantly more toxic than oxamyl with an LC_{50} of 12.952 (10.022–15.793) μg/vial. There was a 31-fold difference in LC_{50} values in this insecticidal class.

2. Field-collected insects

Table 2 contains the live weights of female and male fleahoppers collected on horsemint during the spring and croton during the fall and an aliquot of the laboratory-reared insects. In all cases, female fleahoppers weighed significantly more compared to male. Regardless of sex, fleahoppers collected in the spring on horsemint weighed significantly more than those captured in the fall on woolly croton. In the spring, the females weighed 20.6% more than the males. While the overall weights of the fleahoppers decreased in the fall collections for both males and females, the females weighed 53% more than the males. The laboratory reared females weighed an average of 36% more than their male counterparts. The implications of the weight differences between the sexes were revealed in the LC_{50} values. It is adult fleahoppers that are emerging from senescing horsemint and moving into cotton during early season that are of concern from a control standpoint. Although some indirect inferences could be made about the relationship between weight and toxicity to cotton fleahoppers, determining this experimentally would require weighing individuals before exposure and then correlating this weight to dead fleahoppers. Since fleahoppers tend to defecate and dehydrate prior to death, the usefulness of this line of research is questionable and was not pursued.

Table 2. Live weights±SD of field-collected female and male fleahopper collected on horsemint (spring) and croton (fall)

	Insect Weights (mg)[a)]		
Source	Female[b)] (mean±SD)	Male[b)] (mean±SD)	% Difference
Spring	1.11±0.025 aX (n=49)	0.92±0.025 aY (n=51)	20.6
Fall	0.95±0.035 bX (n=47)	0.62±0.014bY (n=65)	53.0
Laboratory	1.21±0.027 aX (n=47)	0.89±0.014 aY (n=65)	36.0

[a)] Means within each column followed by the same lower case letter (a, b) and means within each row followed by the same upper case letter (X, Y) are not significantly different ($P>0.05$) based on LSMEANS procedure with adjust=Tukey option. [b)] n=number of insects.

The dosage mortality equations for acephate, esfenvalerate, methomyl and thiamethoxam for female and male fleahoppers collected in the field are presented in Table 3. The data for 24-h responses provided good fit with significant χ^2 values. The LC_{50} values for acephate, esfenvalerate and thiamethoxam were almost always numerically higher for female fleahopper compared to male fleahopper, but not significantly so. However, the LC_{50} for methomyl was significantly higher for female fleahopper [0.352 (0.257–0.420) μg/vial] compared to male fleahopper [0.190 (0.137–0.248) μg/vial] for the field-collected fleahoppers. The higher female LC_{50} values for all four compounds were likely a result of the higher body weights of the females.

Table 3. Lethal concentration (LC) (μg/vial) data (24 h) for contact toxicity of technical insecticides to field-collected female and male cotton fleahoppers collected from green horsemint during May/June of 2004 and 2005 using adult vial bioassay

Chemical (Insecticidal Class)	Gender	N[a)]	χ^2	Slope±SE[b)]	LC_{50}[c)]	95% CL
Esfenvalerate	♀	126	1.10 (2)	2.65±0.502	0.477a	0.333–0.644
(Synthetic Pyrethroid)	♂	199	0.30 (2)	2.44±0.495	0.312a	0.219–0.403
Acephate	♀	255	2.61 (3)	5.89±0.789	1.667a	1.459–1.842
(Organophospate)	♂	310	0.54 (2)	6.52±0.83	1.375a	1.230–1.497
Thiamethoxam	♀	213	7.98 (4)	2.38±0.353	1.530a	0.977–2.875
(Neonicotinoid)	♂	244	7.50 (4)	1.94±0.294	0.903a	0.467–1.592
Methomyl	♀	130	0.71 (1)	4.76±1.265	0.352a	0.257–0.420
(Carbamate)	♂	148	0.10 (1)	3.86±0.710	0.190b	0.137–0.248

[a)] N=number of insects used. [b)] Calculated using POLO-PC (LeOra Software 1987). [c)] LC_{50} values for each chemical in the same column are not significantly different based upon the presence of overlap in the 95% confidence limits.

Table 4. Comparison of LC_{50}s for laboratory and field collected fleahoppers for selected insecticides

Chemical (Insecticidal Class)	LC_{50} [a)]		
	Laboratory Reared (Sexes Combined)	Field Collected Males	Field Collected Females
Esfenvalerate (Synthetic Pyrethroid)	0.692 (0.58–0.81) a	0.312 (0.219–0.403) b	0.477 (0.333–0.644) ab
Acephate (Organophospate)	7.663 (5.93–9.08) a	1.375 (1.23–1.497) b	1.667 (1.459–1.842) b
Thiamethoxam (Neonicotinoid)	0.385 (0.272–0.515) b	0.903 (0.467–1.592) ab	1.53 (0.977–2.875) a
Methomyl (Carbamate)	0.413 (0.377–0.447) a	0.190 (0.137–0.248) b	0.352 (0.257–0.42) ab

[a)] Means within each row followed by the same lower case letter are not significantly different ($P>0.05$).

3. Laboratory-reared versus field-collected insects

Toxicity results in laboratory-reared versus field-collected insects are compared by examining the LC_{50}s among the different insects for esfenvalerate, acephate, thiamethoxam, and methomyl (Table 4). The laboratory-reared fleahoppers had a significantly higher tolerance to the esfenvalerate (pyrethoid) and acephate (OP) compounds than the field collected males and females. The field-collected males and females had higher LC_{50} values than the laboratory-fleahoppers in response to exposure to thiamethoxam (neonicotinoid). The laboratory-reared fleahoppers were slightly more tolerant to methomyl (carbamates) than the field-collected females and significantly more tolerant than the field-collected males. The similarity in the responses between the laboratory-reared and the field-collected cotton fleahoppers to selected insecticides reflects the fact that laboratory-reared insects were actually field-collected as diapausing eggs.

Conclusions

Data presented herein provide a measure of acute potency of 17 selected insecticides against *P. seriatus*. Since field performance is dictated by use rate and exposure, the LC_{50} values reported in this study may be different for field populations of *P. seriatus*. However, baseline data may be useful for comparison should suspicion of tolerance to these insecticides develop in field populations. The main conclusions of this work were:

- There were significant differences between the LC_{50} values for the insecticides tested within each of the four insecticidal classes. There were 13-, 46-, 58-, and 31-fold differences between LC_{50} value for the insecticides in the pyrethroids, organophosphates, neonicotinoids, and carbarmates, respectively. Cotton fleahoppers are highly susceptible to the different classes of insecticides.
- Female fleahoppers weighed more than male fleahoppers with the difference ranging from 20.6–53%. Field fleahoppers captured in the spring weighed more than those captured in the field in the fall. These results may reflect differences between the two hosts, croton and horsemint, as well as possible growing conditions during the spring and fall when the fleahoppers were collected.
- For field-collected fleahoppers, females had higher LC_{50} values than their male counterparts; however, this difference was only significant for one of the carbamates (methomyl) tested with field-collected insects.
- Generally, there were significant differences in susceptibility to the insecticides between laboratory-reared and field-collected insects. These differences lead the authors to recommend that field-collected insects be used in resistance-type studies. Laboratory-reared insects can still be used when evaluating toxicity of different or new insecticides.

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