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Effects of Insecticide Residues on Adult Boll Weevils and Immatures Developing Inside Fallen Cotton Fruit

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ABSTRACT

The chief control tactic for the boll weevil, *Anthonomus grandis grandis* Boheman, during the cotton growing season in the Lower Rio Grande Valley of Texas is application of insecticides when squares first begin to develop, and when a threshold of 10% of randomly examined squares have oviposition punctures, which usually occurs around cut-out. Trap-collected boll weevils were used to assess direct contact and residual effects of cyfluthrin, befenthrin, azinphosmethyl, methyl parathion, and oxamyl, on adult mortality, and egg-punctured squares and bolls were sprayed to assess toxic effects on immature stages developing inside. Adults sprayed with the insecticides all died within 30 min. In a leaf residue assay, bifenthrin caused the fastest mortality on the day of application, but none of the insecticides were superior to the others when the residues were aged 1-4 days. The insecticides failed to cause mortality to the boll weevils developing within treated squares or bolls. The limitations of the insecticides are one reason for repetitive late-season spraying in the Lower Rio Grande Valley.

RESUMEN

La principal estrategia para el control del picudo del algodón, *Anthonomus grandis grandis* Boheman, durante la estación del cultivo del algodón en el Bajo Valle del Río Grande en Texas es la aplicación de insecticidas cuando las cápsulas apenas empiezan a desarrollarse, y cuando un umbral del 10% de las cápsulas examinadas tienen picaduras de oviposición, lo que usualmente sucede alrededor del tiempo del despunte. Los picudos colectados en las trampas fueron usados para estimar el efecto por contacto directo y residual del ciflutrín, befentrín, azinfosmetil y oxamil sobre la mortalidad de los adultos; además, las cápsulas y capullos con picaduras fueron asperjados para estudiar el efecto tóxico sobre los estados inmaduros desarrollándose en el interior. Todos los adultos asperjados con los insecticidas murieron en 30 minutos. Al relizar un estudio sobre el efecto residual, se encontró que el befentrín ocasionó la mortalidad mas rápida en el día de la aplicación, pero ninguno de los insecticidas fue superior a los otros después de 1 a 4 días de la aplicación. Los insecticidas no mataron a los picudos desarrollándose en las cápsulas y los capullos tratados. Las limitaciones de los insecticidas son una de las razones para el asperjado repetitivo en la fase tardía del cultivo en el Bajo Valle del Río Grande.

Keywords: Anthonomus grandis grandis, bolls, boll weevil, cotton, insecticides, squares

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The principal tactic for boll weevil, *Anthonomus grandis grandis* Boheman, control during the cotton, *Gossypium hirsutum* L. growing season in the Lower Rio Grande Valley of Texas is application of insecticides from early squaring almost until harvest. The late-season (post-match-head size) applications are generally initiated when a threshold of 10% of randomly examined squares have oviposition punctures (Loera-Gallardo et al. 1997, Showler 2002). Because pre-emptive early-season insecticides application, which usually begin at pinhead or match-head square size and are repeated 2-3 times at 5-d intervals (Heilman et al. 1979, Showler 2004b), is commonly practiced in the Lower Rio Grande Valley

(Showler 2002, 2004a), the duration of residual activity is important. Residual toxicity to boll weevils is advantageous during squaring because a substantial proportion of the population is developing inside squares or bolls on plants or on the soil surface (Showler et al. 2005) and might not exit that protected niche for ≈ 18 d (Bailey et al. 1969, Showler and Cantú 2004).

Different strains of boll weevils have different degrees of vulnerability to some insecticides (Kanga et al. 1995, Wolfenbarger et al. 1998, Foster et al. 2002a). Insecticide comparisons have been conducted against boll weevils before, but those tests used laboratory strains (Furr and Merkl 1967, Scott and Lloyd 1978, Martin et al. 1993). In the laboratory, Foster et al. (2000, 2001, 2002b) demonstrated that malathion provides residual activity for up to two weeks after treatment. A leaf disk insecticide bioassay used boll weevils native to the Lower Rio Grande Valley (Spurgeon and Raulston 1997), but of the tested insecticides only one, oxamyl, is commonly used at the present time in the Lower Rio Grande Valley. At this time, the LRGV is one of the only areas in the United States cotton belt that is not in the national boll weevil eradication program (Smith 1998) and although malathion is the insecticide used in the boll weevil eradication program (Allen and Kharboutli 2000), it is not commonly used in the Lower Rio Grande Valley. The purpose of this study is to assess the initial and residual activities of five insecticides to endemic boll weevils, both as adults and as immature stages developing inside fallen squares and bolls.

MATERIALS AND METHODS

All boll weevils used in the study were collected in the Lower Rio Grande Valley of Texas using Hercon (Hercon Environmental, Emigsville, PA) boll weevil traps with Grandlure (Hercon Environmental, Emigsville, PA) pheromone lures. All of the weevils were kept at the laboratory ≈ 24 h and fed nonsprayed 4-8-mm diameter cotton squares.

Contact Assay. Fifty boll weevils of undetermined sex ratio were sprayed using a manual Greenlawn (Gilmore, Somerset, PA) 3.8-liter capacity pump sprayer with the nozzle adjusted to a cone spray pattern at a pressure of 2.7 kg/cm^2 with cyfluthrin (Baythroid, Bayer, Kansas City, MO), 0.4 ml (AI)/liter; bifenthrin (Capture FMC, Philadelphia, PA), 1 ml(AI)/liter; azinphosmethyl (Guthion, Bayer, Kansas City, MO), 2.6 ml (AI)/liter; methyl parathion (Methyl parathion, Dupont, Wilmington, DE) 2.6 ml (AI)/liter; and oxamyl (Vydate, Dupont, Wilmington, DE), 2.6 ml (AI)/liter, all recommended rates, from a height of 50 cm for one second on a 50 cm x 1.5 m paper surface. The weevils were then placed individually in separate petri dishes, using forceps, with a nontreated cotton square for food, and mortality was recorded after 15 and 30 min.

Leaf Residue Assay. The cotton variety was Deltapine 5415, planted on 11 March 2002 at the USDA-ARS Kika de la Garza Subtropical Agricultural Research Center, Hidalgo Co., Weslaco, TX, on 25 plots, each 8.1 m wide (8 rows, row spacing = 1 m) by 15.2 m long (0.0125 ha) with a 1-m bare ground buffer between the plots arranged in a completely randomized design. On 12 March, pendimethalin (Prowl 3.3 EC, American Cyanamid, Parsippany, NJ) was applied by tractor immediately after planting at a rate of 924 g (AI)/ha, and weed control was thereafter conducted with a rolling cultivator and by hand roguing. Irrigation occurred at the start of bloom (late May). On 25 June cyfluthrin 45 g_(AI)/ha, bifenthrin at 112 g (AI)/ha, azinphosmethyl at 280 g (AI)/ha; methyl parathion at 280 g (AI)/ha, and oxamyl at 527 g (AI)/ha were applied to five plots each through 16 Teejet nozzles, two angled toward each row, at a pressure of 3.5 kg/cm^2 (1.6 liters/min/nozzle) on a tractor boom. The remaining five plots, the controls, were not treated with an insecticide over the course of the study. Applications were carried out on a morning without substantial wind (<3 km/h) to reduce the chance of cross contamination of the plots.

Two cotton leaves from the plant canopies in each plot were randomly taken from the upper six fully expanded leaves near the centers of each plot on the day the treatments were applied and on each of five consecutive post-application days. The leaves were collected using disposable gloves and freshly washed scissors to prevent cross-contamination, and placed in zip-lock bags for transport to the laboratory (Showler et al. 2002). Each pair of leaves was placed in a separate ventilated 100-mm x 15-mm plastic petri dish with five adult boll weevils. The petri dishes, each a treatment replicate, were maintained under 14:10 h L:D fluorescent lighting at 27-28ºC. Mortality was recorded daily for 5 d after the weevils were released into the dishes. One-way ANOVA was used to detect significant differences between mean mortalities on residues of the same age and assay day, and Tukey's HSD was used to separate means. Repeated measures analysis was used to assess the effects of treatment and time on boll weevil mortality (Analytical Software 1998).

Square Assays. Forty cotton squares, each 6-8 mm in diameter, were placed in each of four 40-cm^3 cages with 50 boll weevils (undetermined sex ratio). After 24 h squares that had oviposition punctures were removed, set 1 cm apart from each other on a flat surface and sprayed, using the Greenlawn sprayer, with the five insecticides and other squares were nontreated controls. Twenty-five squares from each treatment were kept singly in petri dishes under 14:10 h L:D fluorescent lighting at 27.5ºC. The squares were observed daily for 30 d and the numbers of emergent adult boll weevils were counted. On both 21 May and on 25 June, each insecticide was sprayed from the tractor-mounted rig with drop nozzles aimed downward 0.5 m over furrow centers for a distance of 5 m at one randomly selected location in each plot. After 3 h, 20 oviposition-punctured cotton fruit from treated or control furrows in each plot were collected in separate 0.12-m^3 cages, brought to the laboratory, and kept at room temperature for 20 d. Boll weevils that emerged from the fruit were counted daily. One-way ANOVA and Tukey's HSD were used to compare mean cumulative numbers of living adult boll weevils in both assays.

RESULTS

Contact Assay. Cyfluthrin, methyl parathion, and oxamyl caused 100% boll weevil mortality at 15 min after application,

Table 1. Mean (± SE)[a] cumulative mortality of boll weevils[b] exposed to treated or nontreated cotton leaves in petri dishes.

Assay Day	Treatment[c]	Residual Age 0	1	2	3	4
1	control	0.2 ± 0.2 bc	0	0	0.2 ± 0.2	0.4 ± 0.2
	cyfluthrin	2.2 ± 0.7 ab	1.2 ± 0.7	0.4 ± 0.2	0.8 ± 0.4	0.4 ± 0.2
	bifenthrin	2.8 ± 0.7 a	1.6 ± 0.7	1.0 ± 0.4	0	0.2 ± 0.2
	azinphosmeth.	0 c	0.4 ± 0.4	0	0.6 ± 0.4	0.4 ± 0.4
	m. parathion	0.2 ± 0.2 bc	0	0	1.2 ± 1.0	0.2 ± 0.2
	oxamyl	0.2 ± 0.2 bc	1.6 ± 0.5	1.2 ± 0.5	0.8 ± 0.5	0.4 ± 0.4
	F	12.09	2.43	2.41	0.76	0.35
	P	< 0.0001	0.064	0.067	0.589	0.878
2	control	0.4 ± 0.2 c	0.2 ± 0.2	0.2 ± 0.2 b	0.6 ± 0.4	0.6 ± 0.2
	cyfluthrin	2.8 ± 1.0 abc	2.6 ± 0.9	5.0 ± 0 a	0.8 ± 0.4	1.0 ± 0.3
	bifenthrin	3.8 ± 0.7 ab	3.0 ± 1.0	5.0 ± 0 a	1.4 ± 0.9	0.4 ± 0.2
	azinphosmeth.	1.6 ± 0.9 bc	2.8 ± 1.2	4.0 ± 0.4 a	0.6 ± 0.4	0.6 ± 0.4
	m. parathion	1.2 ± 0.6 bc	2.8 ± 1.0	3.8 ± 1.0 a	1.2 ± 1.0	0.6 ± 0.4
	oxamyl	4.8 ± 0.2 a	3.2 ± 0.7	5.0 ± 0 a	0.8 ± 0.5	0.6 ± 0.4
	F	6.25	1.61	17.61	0.26	0.33
	P	0.0008	0.196	< 0.0001	0.931	0.889
3	control	0.4 ± 0.2 c	0.2 ± 0.2 b	0.2 ± 0.2 b	0.8 ± 0.4	0.6 ± 0.2
	cyfluthrin	3.8 ± 0.8 ab	3.8 ± 0.6 a	5.0 ± 0 a	3.2 ± 1.0	1.8 ± 0.9
	bifenthrin	4.6 ± 0.2 ab	4.8 ± 0.2 a	5.0 ± 0 a	3.4 ± 1.0	1.2 ± 0.4
	azinphosmeth.	3.8 ± 0.5 ab	4.0 ± 1.0 a	4.4 ± 0.4 a	4.4 ± 0.6	1.0 ± 0.3
	m. parathion	2.8 ± 0.7 b	4.0 ± 0.4 a	4.2 ± 0.6 a	2.8 ± 1.0	0.8 ± 0.4
	oxamyl	5.0 ± 0 a	5.0 ± 0 a	5.0 ± 0 a	1.8 ± 0.7	1.8 ± 0.9
	F	11.40	11.34	39.19	2.49	0.80
	P	< 0.0001	< 0.0001	< 0.0001	0.059	0.561
4	control	0.4 ± 0.2 c	0.2 ± 0.2 b	0.6 ± 0.2 b	0.8 ± 0.4 b	0.6 ± 0.2
	cyfluthrin	4.6 ± 0.4 ab	4.0 ± 0.8 a	5.0 ± 0 a	3.6 ± 0.4 a	1.8 ± 0.9
	bifenthrin	4.8 ± 0.2 a	5.0 ± 0 a	5.0 ± 0 a	4.6 ± 0.4 a	1.4 ± 0.2
	azinphosmeth.	4.2 ± 0.5 ab	4.2 ± 0.8 a	5.0 ± 0 a	4.6 ± 0.4 a	1.0 ± 0.3
	m. parathion	3.2 ± 0.5 b	4.2 ± 0.4 a	5.0 ± 0 a	4.0 ± 0.6 a	0.8 ± 0.4
	oxamyl	5.0 ± 0 a	5.0 ± 0 a	5.0 ± 0 a	3.4 ± 0.8 a	1.8 ± 0.9
	F	24.49	13.68	322.67	5.49	0.86
	P	< 0.0001	< 0.0001	< 0.0001	0.0016	0.524
5	control	0.6 ± 0.2 b	0.6 ± 0.4 b	0.6 ± 0.2 b	0.8 ± 0.4 b	0.6 ± 0.2
	cyfluthrin	4.8 ± 0.2 a	4.2 ± 0.6 a	5.0 ± 0 a	4.2 ± 0.5 a	2.4 ± 0.8
	bifenthrin	5.0 ± 0 a	5.0 ± 0 a	5.0 ± 0 a	4.8 ± 0.2 a	1.6 ± 0.2
	azinphosmeth.	4.6 ± 0.4 a	5.0 ± 0 a	5.0 ± 0 a	4.8 ± 0.2 a	1.2 ± 0.2
	m. parathion	4.4 ± 0.2 a	4.4 ± 0.4 a	5.0 ± 0 a	4.2 ± 0.5 a	1.0 ± 0.3
	oxamyl	5.0 ± 0 a	5.0 ± 0 a	5.0 ± 0 a	4.2 ± 0.4 a	2.0 ± 0.8
	F	55.10	26.82	322.67	16.93	1.87
	P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.137

[a]Means in the same group of each column followed by different letters are significantly different ($P < 0.05$), one way ANOVA, Tukey's HSD.

[b]Five weevils per dish, $n = 5$. Weevils were collected in the Lower Rio Grande Valley of Texas.

[c]Cyfluthrin was applied at 45 g (AI)/ha, bifenthrin at 112 g (AI)/ha, azinphosmethyl at 280 g (AI)/ha, methyl parathion at 280 g (AI)/ha, and oxamyl at 527 g (AI)/ha. *F* and *P* values are given below each group of means.

and at 30 min, all of the weevils treated with azinphosmethyl and bifenthrin were dead. No weevil mortality occurred in the control.

Leaf Residue Assay. Cumulative boll weevil mortality was consistently greater ($P \leq 0.05$) in the bifenthrin treatment than in the control for all five assay days when the residue was not aged before weevils were released in the petri dishes (Table 1). Mortalities in the azinphosmethyl and methyl parathion treatments were lower ($P \leq 0.05$) than bifenthrin and oxamyl on assay days 1 and 2, respectively. Mortality caused by methyl parathion was less ($P \leq 0.05$) than in the oxamyl, and bifenthrin and oxamyl on assay days 3 and 4 (Table 1). Mortality in the oxamyl treatment was greater ($P \leq 0.05$) than in the control for the last four days, and cyfluthrin, azinphosmethyl, and methyl parathion were greater ($P \leq 0.05$) than the control for the last

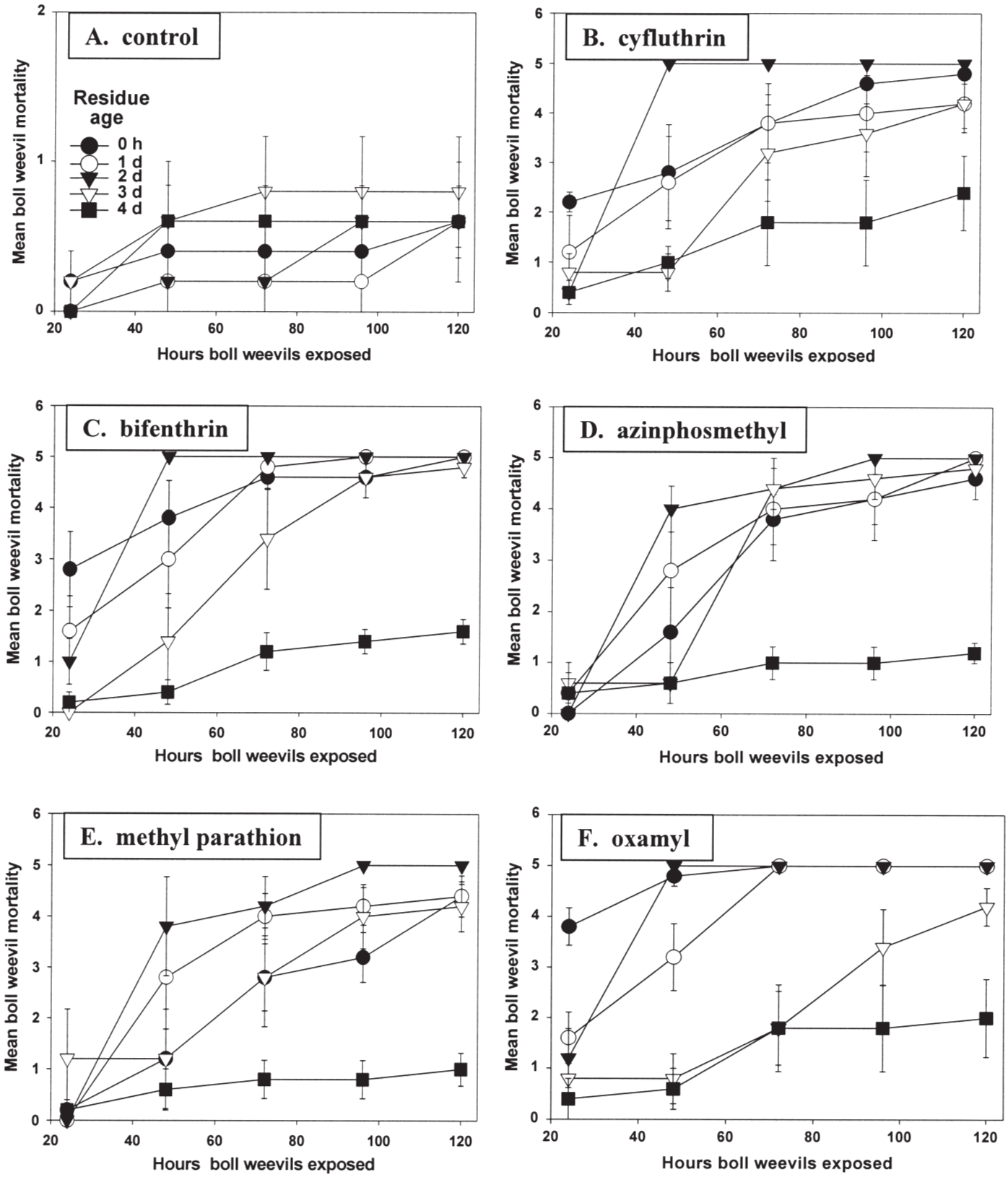

Fig. 1. Mean boll weevil mortalities caused by leaf residues of five insecticides commonly used in the Lower Rio Grande Valley, TX, and nontreated controls.

Table 2. Repeated measures analyses *F* and *P* values for comparing residual ages over time (5 d) of five insecticides used on Lower Rio Grande Valley boll weevils in leaf bioassays.

Treatment[a]	Effect[b]	*F*	*P*
Control	treatment	1.83	0.1295
	time	3.37	0.0124
	interaction	0.24	0.9989
Cyfluthrin	treatment	13.65	< 0.0001
	time	21.35	< 0.0001
	interaction	1.45	0.1349
Bifenthrin	treatment	46.08	< 0.0001
	time	42.41	< 0.0001
	interaction	2.88	0.0008
Azinphosmethyl	treatment	23.97	< 0.0001
	time	51.06	< 0.0001
	interaction	3.42	0.0001
Methyl parathion	treatment	19.22	< 0.0001
	time	30.69	< 0.0001
	interaction	2.23	0.0084
Oxamyl	treatment	49.54	< 0.0001
	time	28.15	< 0.0001
	interaction	2.86	0.0007

[a]Cyfluthrin applied at 45 g (AI)/ha, bifenthrin at 112 g (AI)/ha, azinphosmethyl at 280 g (AI)/ha, methyl parathion at 280 g (AI)/ha, and oxamyl at 527 g (AI)/ha.

[b]Treatments = insecticides, df = 4, 100; time = daily sampling for five days after boll weevils were released on the leaves, df = 4, 100; interaction = treatment by time, df = 16, 100.

three assay days. When the insecticide residues were aged for one, two, or three days before weevils were exposed to them, mortalities in each of the insecticide treatments were greater ($P \leq 0.05$) than the controls on the last three, four, and two assay days, respectively (Table 1). Differences ($P > 0.05$) were not detected between the insecticide treatments when the residues were aged for four days.

Repeated measures analyses showed that there were differences ($P < 0.0001$) between cumulative boll weevil mortalities when exposed to the different residue ages for each of the insecticides (Table 2). There were no significant differences between the controls (Fig. 1A, Table 2). By the fifth assay day, ≥80% of the boll weevils were killed by each insecticide in the assays for the 0-, 1-, 2-, and 3-d-old residues, but in the 4-d residue assay, cumulative mortalities were ≤52% (Fig. 1B-F, Table 2). Treatment by time interactions were detected ($P \leq 0.01$) in the bifenthrin, azinphosmethyl, methyl parathion, and oxamyl treatments (Table 2).

Square Assays. Adult boll weevils developed inside ≈ 90% of the squares treated with insecticides in the laboratory and in the control. In the experiment where fallen fruit were sprayed in the furrows, no significant differences were detected between treatments.

DISCUSSION

The contact assay demonstrated that all of the insecticides were lethal to boll weevils within 30 min of when the weevils were sprayed. Acute dermal toxicities induced by each of the five insecticides were similar. For reducing adult boll weevils directly exposed to any of the sprays, one insecticide was essentially as effective as the others.

The leaf residue assay showed that bifenthrin caused the most rapid mortality on the day of application, but by 2 d, oxamyl had caused the greatest cumulative mortality. None of the insecticides were superior to the others when the residues were aged 1-4 d. Because none of the fourth day residues caused substantial mortality, one insecticide does not appear to be better than another when used as pre-emptive sprays. The early toxic action by bifenthrin might conceivably be viewed as an advantage if the economic costs of the other insecticides were the same.

The interactions between treatment and time for the differently aged residues of bifenthrin, azinphosmethyl, methyl parathion, and oxamyl result from the greater rate of mortality induced by the 0-3-d-old residues than the 4-d-old residue. The lack of significant interaction for cyfluthrin is a reflection of the greater mortality rate caused by the 4 d-old residue compared to the lower rates of the other insecticides.

The square assays showed that populations of boll weevils developing inside squares survive insecticide applications, although adults that emerge within 72 h of the application and contact treated surfaces sufficiently to receive a lethal dose would also die under field conditions. Because the boll weevil's life cycle involves ≈ 18 d within squares (Showler and Cantú 2005) moderate residual insecticide (in this case ≈ 1 wk) would represent an improvement over the five insecticides assayed in this study. In the Lower Rio Grande Valley, where boll weevil pressure combined with the cost of early season pre-emptive and frequent, sometimes biweekly, insecticide applications just before and after cut-out erode economic profit margins. Although each of the insecticides in this study was

effective for three days, our study suggests that none of them contribute toward reducing the number of sprays. Also, because boll weevils developing within squares either on the plant or on the soil surface are not affected by the insecticides, only the adult portion of the population that is directly exposed to the spray or its residue would be suppressed. Furthermore, the existence of bracts on squares, whether attached to the plant or abscised, likely reduces the exposure of the square to the insecticide in field conditions. Insecticide sprays in the Lower Rio Grande Valley do not occur when boll weevils feed on large (5-mm-diameter) squares, which accelerate boll weevil fecundity and oviposition compared to smaller squares and bolls (Showler 2004a).

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