Response of Silverleaf Whitefly (Homoptera: Aleyrodidae) to Bifenthrin and Endosulfan by Vial Bioassay in Florida, Georgia and Texas[1]

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Abstract Median lethal concentration (LC50) values of bifenthrin and endosulfan were determined for dead and moribund + dead categories for adults of the silverleaf whitefly, *Bemisia argentifolii* (Bellows and Perring) (formerly sweetpotato whitefly, *B. tabaci* (Gennadius), strain B), in 3-h vial bioassays in Florida, Georgia, and Texas in 1991 and 1992. For the moribund + dead category in Florida, LC50 values for bifenthrin ranged from 0.00076 to 1.48 µg/vial, a 1,947-fold difference. An LC50 as high as 128.1 µg bifenthrin/vial for adults from cabbage in Texas was determined for the dead category. For endosulfan in Florida, LC50 values of the moribund plus dead categories for endosulfan ranged from 0.04 to 35.6 µg/vial, an 890-fold difference. An LC50 as high as 119.7 µg endosulfan/vial was determined for adults from cotton in Texas for the dead category. The wide ranges of LC50 values for both insecticides suggest the presence of resistant and susceptible whiteflies in all three states.

Key Words *Bemisia argentifolii*, insecticide resistance, bifenthrin, endosulfan

The silverleaf whitefly, *Bemisia argentifolii* (Bellows and Perring [previously sweetpotato whitefly, *B. tabaci* (Gennadius), strain B]), became a major crop pest in the southern United States in the late 1980's, and by 1991 caused a loss of a half billion dollars in Florida, California, Arizona, Texas, Georgia and other southern states in a single year (Perring et al. 1993). Whiteflies can rapidly attain high populations and can severely affect yields in various crops, especially cotton and vegetables (Riley 1996). Insecticide treatment has been the most important control tactic for this pest, but insecticide resistance has been a major constraint to this tactic (Denholm et al. 1996).

Vial bioassays of adult whitefly were conducted in 1991 by Staetz et al. (1992) and Gage et al. (1992) in the United States to determine the median lethal concentration (LC50) values for the insecticides, bifenthrin and endosulfan. At the same time, U.S. Department of Agriculture cooperative agreements were established as part of the Sweetpotato Whitefly: 5-Year Plan for Development of Management and Control Methodologies to evaluate insecticide efficacy in Florida, Texas, Arizona, and Cali-

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fornia (and, if possible, other areas) with bifenthrin and endosulfan as reference insecticides. As part of this effort, we bioassayed bifenthrin and endosulfan for toxicity against adults of silverleaf whitefly in Florida, Georgia, and Texas using the method of Staetz et al. (1992). Concurrent studies for California and Arizona have already been published (Prabhaker et al. 1996, Sivasupramaniam et al. 1997). Cahill and Hackett (1992) conducted similar bioassays with another vial method in the United Kingdom. The objective of our study was to document responses of whitefly populations in Florida, Georgia, and Texas to bifenthrin and endosulfan. Results are presented here as LC50 values for both insecticides against adults collected from leaves of various vegetable crops in the field and greenhouse and field-grown cotton to illustrate the range in responses.

Materials and Methods

Preparation of vials. The inner surfaces of 20-ml glass scintillation vials were coated with various concentrations of technical grade bifenthrin (97.1%) and endosulfan (95%) (FMC, Princeton, NJ) dissolved in acetone as described by Staetz et al. (1992). Concentrations of bifenthrin in µg/vial were 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, and 1.0; concentrations of endosulfan in µg/vial were 0.3, 1.0, 3.0, 10, 300, and 1000. All coated vials were furnished by FMC. No food was placed in the vials. Vials were kept at 25 ± 3°C in closed vial boxes for 4 months. Staetz et al. (1992) found that toxicity did not diminish during this time.

Whitefly collection. Ten to 70 adults were tapped from infested leaves of the various crops at each location into a yellow plastic funnel measuring 15 cm in diam and 20 cm in length, and then into a vial which was immediately covered with Parafilm M® (American National Can™, Neenah, WI) according to methods described by Staetz et al. (1992) and Gage et al. (1992). An aspirator was used when populations of adults were low. Age and sex of adults were not recorded.

Bioassay procedure. The basic procedure of Tan et al. (1996) was used. Each test dose was replicated 2 to 8 times on the indicated sample day, and a vial containing the same dose represented a replicate. Each vial was used once. Because the insects are phototactic, vials containing them were positioned so adults would alight and stay on as much of the treated surface as possible when exposed to the light positioned above the vials. We determined which insects were dead, moribund or alive after they had been in the vials for 3 h. Gage et al. (1992) observed no change in the status of the dead and moribund category after 3 h compared to 6 h after the insects were exposed to bifenthrin, so we used the 3-h exposure time. It should be noted that 24 h resulted in too high mortality in the control vials in our preliminary tests. The methods of Cahill and Hackett (1992) differed because they provided food for the insects during testing and determined mortality after 24 h. The dead and moribund adults were gently tapped from the vials onto a flat black paper surface. Whiteflies were considered dead if they did not move when prodded. Moribund insects could not coordinate the movement of their legs and wings nor right themselves if they were on their backs. Live insects remained on the glass surface and moved normally. The number in each category was determined for each vial. The number of insects used to test bifenthrin ranged from 54 to 1861 and number of insects tested with endosulfan ranged from 75 to 795.

The numbers of live, dead and moribund insects were counted for each dose or control vial. The numbers of dead and dead + moribund adults were analyzed sepa-

rately by probit analysis (SAS 1988) for LC50, slope, and 95% confidence interval (C.I.). Total numbers treated would be the same for both categories. Dead and moribund adults in control vials were counted and used to correct for natural mortality or morbidity. Regression with ratios of slope/standard error of $\leq 1.96\ t_{\infty\,\infty_{0.05}}$ were not significantly different from zero. LC50 values were considered not significantly different when their 95% C.I. values overlapped. LC50 values above and below the doses tested also were included when the regression model was significant ($P < 0.05$).

Results and Discussion

Bifenthrin. In 1991-1992, there were 39 significant regressions for bifenthrin, but only 33 were reported (Table 1) because of excessively large (∞) confidence intervals for six LC50 values. In addition, 17 other regressions for dead and dead + moribund (including the susceptible strain) were not significant from Florida, Georgia and Texas in 1991 and 1992 despite doses of bifenthrin that spanned four logarithm cycles. In 1992, the highest LC50 attained by bifenthrin for the category of dead + moribund adult was 1.48 µg/vial at Bradenton, FL, and the lowest was 0.00076 µg/vial for a laboratory-susceptible strain from Apopka, FL, a 1,947-fold difference. The high LC50 was obtained from whiteflies collected from tomato plants in a field that had been treated 10 times with 0.056 kg AI of bifenthrin/ha at Bradenton, FL. For 33 regressions of bifenthrin where 95% C.I. were determined, 84% of dead and dead + moribund categories were significantly greater than the susceptible strain. Fifteen [45%] dead and dead + moribund regressions showed overlapping 95% C.I. values with the most resistant strain. The average LC50 values (and their ranges) of adults in all categories collected in Florida, Georgia and Texas were 0.36 ± 0.58 (0.0022-1.48), 0.057 ± 0.064 (0.018-0.17), and 6.2 ± 28 (0.0019-128), respectively. The greatest LC50 value of bifenthrin for the dead category was 128.08 µg/vial for insects taken from cabbage on day 307 and 34.34 µg/vial for insects from squash on day 316 (Table 1). Adults on any one crop were not more resistant to bifenthrin than on any other.

Slope values of bifenthrin LC50 values, based on the available significant probit analysis of either the dead or dead + moribund categories, ranged from 0.58 to 2.65 with 64% greater than 1.0 (Table 1). Slope values (18) of bifenthrin with non-significant regressions of the same criteria ranged from 0.01 to 1.49; 39% ranged from 1 to 1.49. Slope values >1 were considered to be steep for bifenthrin in our evaluation and was similar to the range in slope values of Tan et al. (1996), that is, 0.43 to 2.29. However, steeper slope values have been obtained in similar studies in Arizona (range = 1.40 to 5.26, Sivasupramaniam et al. 1997) and California (range = 1.9 to 3.1, Prabhaker et al. 1996), suggesting less sensitivity to bifenthrin in the Florida, Georgia, and Texas populations.

Endosulfan. Doses of endosulfan spanned five logarithm cycles, yet 12 of the probit analysis of either the dead or dead + moribund categories were not significant from Florida, Georgia and Texas in 1991 and 1992 and only 28 significant regressions were reported which were all observed in 1992 (Table 2). For the 28 LC50 values, 88% were significantly greater than the LC50 value of the susceptible strain which was 0.04 µg/vial. The greatest LC50 for dead + moribund category was 35.61 µg/vial from tomato in Florida and 119.74 µg/vial for dead category from cotton in Texas (C.I. values overlapped). In Immokalee and Bradenton, FL, adults collected from tomato had slopes ± SE of 1.27 ± 0.08 and 10.48 ± 10.0 × 10^5 when 155 (day 214) and 181 (day 244) were tested, respectively. On day 230 the LC50 value was 35.6 ± 00, slope

Table 1. Toxicity of bifenthrin expressed as moribund + dead and dead adults of *B. argentifolii* after 3 h of exposure in a glass vial 1991-1992 sorted by location, host plant, and date

Site	Insect condition	Host plant	Day	No. of insects	Slope ± SE	LC50 [µg/vial]	[95% C. I.]
Florida laboratory susceptible							
Apopka	Dead + Moribund	Squash*	155	428	1.05 ± 0.11	0.00076	0.00044 – 0.0012
Florida field and greenhouse populations							
Zellwood	Dead + Moribund	Cantaloupe	162	180	0.92 ± 0.15	0.0022	0.00099 – 0.0040
Immokalee	Dead + Moribund	Tomato	190	307	0.94 ± 0.29	0.024	0.0017 – 0.19
Bradenton	Dead + Moribund	Tomato†	214	229	0.75 ± 0.27	1.48	$0.21 - 1.9 \times 10^{9}$
Bradenton	Dead + Moribund	Tomato**	215	197	1.07 ± 0.25	0.032	0.0069 – 0.11
Immokalee	Dead + Moribund	Tomato†	243	351	0.78 ± 0.19	0.56	0.15 – 18.35
Sanford	Dead + Moribund	Magnolia*	232	192	1.53 ± 0.53	0.065	0.0032 – 0.34
Georgia greenhouse populations							
Tifton	Dead + Moribund	Cotton*	167	229	1.20 ± 0.19	0.018	0.0076 – 0.055
Tifton	Dead + Moribund	Cotton*	168	189	0.70 ± 0.26	0.042	0.00055 – 9.03
Tifton	Dead	Cotton*	168	189	0.59 ± 0.18	0.17	0.031 – 24.35
Tifton	Dead + Moribund	Cotton*	169	398	1.31 ± 0.28	0.020	0.0061 – 0.048
Tifton	Dead + Moribund	Cotton*	175	445	1.39 ± 0.26	0.035	0.013 – 0.074
Texas field and greenhouse populations							
Weslaco	Dead + Moribund	Cabbage	267	392	0.92 ± 0.26	0.063	0.0068 – 0.63
Weslaco	Dead	Cabbage	267	392	0.93 ± 0.33	0.26	$0.04 - 2.92 \times 10^{15}$

Table 1. Continued.

Site	Insect condition	Host plant	Day	No. of insects	Slope ± SE	LC50 [µg/vial]	[95% C. I.]
Weslaco	Dead	Cabbage	272	395	1.90 ± 0.39	0.19	0.091 – 0.51
Weslaco	Dead	Cabbage†	307	502	0.76 ± 0.31	128.08	$8.02 - 2.0 \times 10^{12}$
Weslaco	Dead + Moribund	Cantaloupe	252	226	1.83 ± 0.20	0.088	0.061 – 0.12
Weslaco	Dead + Moribund	Cantaloupe	256	443	1.17 ± 0.27	0.079	0.043 – 0.13
Weslaco	Dead + Moribund	Cotton	212‡	255	–0.82 ± 0.25	0.0019	0.001 – 0.0039
Weslaco	Dead + Moribund	Cotton	222	354	1.25 ± 0.45	0.028	0.0024 – 0.070
Weslaco	Dead	Cotton	227	54	1.57 ± 0.46	0.43	0.21 – 1.81
Weslaco	Dead + Moribund	Cotton*	252	296	1.86 ± 0.41	0.043	0.012 – 0.11
Weslaco	Dead + Moribund	Cotton	258	865	0.82 ± 0.25	0.0041	2.50×10^{-7} – 0.022
Weslaco	Dead	Cotton	258	865	2.14 ± 0.76	0.26	0.0021 – 11.16
Weslaco	Dead	Cotton*	275	556	0.97 ± 0.25	0.20	0.056 – 6.87
Weslaco	Dead + Moribund	Cotton*	275	556	1.02 ± 0.36	0.033	0.00003 – 751.02
Weslaco	Dead + Moribund	Squash	231	344	2.25 ± 0.49	0.088	0.05 – 0.15
Weslaco	Dead + Moribund	Squash	246	467	1.05 ± 0.24	0.08	0.02 – 0.28

* Obtained from greenhouse plants.
** From Endosulfan treated plants in field plots.
† From Bifenthrin treated plants in field plots.
‡ Determined in 1991.

Table 2. Toxicity of endosulfan expressed as moribund + dead and dead adults of *B. argentifolii* after 3 h of exposure in a glass vial 1992 sorted by location, host plant, and date

Site	Insect condition	Host plant	Day	No. of insects	Slope ± SE	LC50 [µg/vial]	[95% C. I.]
Florida laboratory susceptible							
Apopka	Dead + Moribund	Squash*	178	207	0.72 ± 0.19	0.04	0.00063 – 0.17
Florida field populations							
Sanford	Dead + Moribund	Magnolia	238	95	0.99 ± 0.23	9.65	4.45 – 24.30
So. Florida	Dead + Moribund	Watermelon	190	151	1.57 ± 0.29	5.93	3.35 – 9.26
Georgia greenhouse populations							
Tifton	Dead + Moribund	Cotton*	167	133	1.03 ± 0.36	5.77	0.0035 – 1635.3
Tifton	Dead + Moribund	Cotton*	168	227	1.94 ± 0.66	4.36	0.020 – 36.58
Tifton	Dead + Moribund	Cotton*	169	393	6.46 ± 0.66	4.36	0.020 – 36.58
Tifton	Dead	Cotton*	175	228	2.77 ± 0.78	8.88	1.92 – 20.31
Texas field and greenhouse populations							
Weslaco	Dead + Moribund	Cantaloupe	256	383	4.27 ± 0.62	4.40	3.80 – 5.06
Weslaco	Dead + Moribund	Cabbage	306	250	4.37 ± 0.86	3.47	2.86 – 4.13
Weslaco	Dead	Cabbage	306	250	2.91 ± 0.46	4.50	3.51 – 5.59
Weslaco	Dead + Moribund	Cabbage	307	254	4.47 ± 0.65	4.50	3.80 – 5.34
Weslaco	Dead	Cabbage	307	254	3.45 ± 0.44	6.38	5.25 – 7.69
Weslaco	Dead + Moribund	Cotton	82	581	1.74 ± 0.14	0.91	0.74 – 1.10
Weslaco	Dead	Cotton	89	376	1.36 ± 0.16	0.74	0.48 – 1.03

Table 2. Continued.

Site	Insect condition	Host plant	Day	No. of insects	Slope ± SE	LC50 [µg/vial]	[95% C. I.]
Weslaco	Dead	Cotton	90	280	2.37 ± 0.46	1.75	1.09 – 2.46
Weslaco	Dead	Cotton	124	644	1.31 ± 0.40	3.62	0.93 – 16.54
Weslaco	Dead	Cotton	127	685	1.18 ± 0.29	1.36	0.064 – 4.30
Weslaco	Dead	Cotton	229	161	1.34 ± 0.41	119.74	$19.91 - 8.1 \times 10^{22}$
Weslaco	Dead	Cotton	229	323	3.27 ± 0.73	3.37	2.10 – 4.52
Weslaco	Dead + Moribund	Cotton*	261	540	5.72 ± 0.52	5.24	4.77 – 5.89
Weslaco	Dead	Cotton*	261	540	4.41 ± 1.19	6.61	3.28 – 49.13
Weslaco	Dead + Moribund	Cotton*	265	598	5.02 ± 0.41	5.75	5.21 – 6.35
Weslaco	Dead	Cotton*	265	598	3.30 ± 0.63	8.00	4.40 – 14.57
Weslaco	Dead + Moribund	Cotton*	275	795	2.39 ± 0.46	14.38	6.35 – 34.00
Weslaco	Dead	Cotton*	275	795	1.88 ± 0.32	22.69	9.38 – 53.89
Weslaco	Dead + Moribund	Cotton*	309	598	1.57 ± 0.11	9.71	7.87 – 11.74
Weslaco	Dead	Cotton*	309	598	1.75 ± 0.12	21.04	17.76 – 24.87
Weslaco	Dead	Squash	246	170	2.45 ± 0.48	4.27	2.53 – 5.99

* Obtained from greenhouse plants.

1.47 ± 0.67 (143 whiteflies tested) indicating a highly resistant population. A laboratory resistant colony from Apopka had a non-significant regression to endosulfan with a slope ± SE of 1.12 ± 0.8 for 298 adults (day 204). No more than 5% of adults of this strain died after 3 h at any dose when treated with either insecticide; thus, this strain is almost immune to these insecticides.

At Tifton, bioassays with endosulfan of dead adults collected from cotton showed a ratio of <1.96 for slope ± SE values of 19.0 ± 6.1 × 10^5 for 393 (day 169). At Weslaco, dead adults showed values of 2.0 ± 1.92, 0.52 ± 0.35, 1.10 ± 0.7, and 0.92 ± 0.53 for 286, (day 76), 1587 (day 131), 140 (day 215), and 352 (day 231) when collected from cotton, cotton, cantaloupes and squash, respectively. Regressions for both categories that did not differ from 0 were exhibited for whitefly on most crops bioassayed. In 1991, 893 adults collected from cotton in Texas and bioassayed with endosulfan showed slope values ± SE of 0.031 ± 0.12 and 0.12 ± 0.2 for dead and dead + moribund adults (day 212), respectively.

In 1992, the highest LC50 value for endosulfan for dead ± moribund was from treated plots at Bradenton, FL, and it was 890-fold greater than that for the susceptible laboratory strain from Apopka, FL (Table 2). On day 309, LC50 for dead was significantly greater than for dead + moribund whiteflies. For the other five LC50 values (14% of total determined in 1992) the 95% C.I. ranged from 0.1 to 13.0 µg/vial as shown by (Staetz et al. 1992) and they encompassed the 95% C.I. values of LC50 values from all three states. Response of this insect to endosulfan in 1991 and 1992 was about equal in Florida, Georgia and Texas. In this study, the average LC50 values (and their ranges) of adults in all categories collected in Florida, Georgia and Texas were 5.2 ± 4.8 (0.04-9.65), 5.8 ± 2.1 (4.36-9.65), and 12 ± 25 (0.74-120), respectively.

In 1991 and 1992 slope values (36) of endosulfan with significant regressions of dead versus dead + moribund ranged from 0.72 to 6.46; 8% had <1, 42% had 1 to 2, 17% had 2 to 3, 11% had 3 to 4, 19% had 4 to 5 and 3% had >6 slope values. Slope values for endosulfan were much steeper than shown for bifenthrin after the 3 h bioassay. Slope values (12) of endosulfan with non-significant regression ranged from 0.031 to 19.0; 50% had <1, 25% had 1 to 2, 16% had 2 to 3 and 8% >10. Slope values for endosulfan that were obtained in similar studies in Arizona (Sivasupramaniam et al. 1997) and California (Prabhaker et al. 1996) ranged from 0.79 to 7.67 and 1.4 to 3.0, respectively.

The bioassay technique described here was relatively simple to conduct; however, significant regressions were not obtained for a large percentage of the populations tested. Potential problems associated with the vial technique, such as fumigation effects and deterioration of chemicals were discussed by Prabhaker et al. (1996). However, the bioassay did provide a reasonably good measure of whitefly resistance to these contact insecticides, similar to the results of Sivasupramaniam et al. (1997), and has been used effectively for an inheritance study on bifenthrin resistance (Tan et al. 1996).

Significant differences were observed between several LC50 values for both insecticides across locations, crops, and dates. Several general observations can be made based on these data. First, high levels of variation were present across those populations tested. Non-significant regressions for both insecticides occurred because toxicity of all the doses was either >90% or <10%, extreme variation occurred across dosages, or a combination of both. Secondly, certain Weslaco, TX, whitefly populations exhibited remarkable high levels of resistance to both compounds, par-

ticularly late in the year. The extensive use of both compounds in this area in 1991 and 1992 could account for a shift in insecticide susceptibility similar to that reported for locations in California (Prabhaker et al. 1996). Third, the dead category was 28% and 46% of the significant regressions for bifenthrin and endosulfan, respectively. This suggests that the moribund category is very important in interpreting response of whiteflies to insecticides. Even so, we suspect that the presence or absence of resistance in populations of the silverleaf whitefly is best defined by the use of the dead category. Prabhaker et al. (1996) suggested that survivors 24 h after insecticide exposure may be a better indicator of resistance and moribund individuals at 3 h have a chance of being included in this group. Finally, where significant differences occur between dead and dead + moribund for the cyclodiene and pyrethroid insecticides, we believe that this is evidence for the mechanisms of resistance described by Byrne and Devonshire (1993), that is, insensitivity of the insecticide target site for response by this insect.

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