A Five-Year Study of Insecticide Resistance in Whitefly[1] *Bemisia Argentifolii* Bellows & Perring from the Yaqui Valley, Mexico.

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Abstract. A five-year study to evaluate resistance of whitefly, *Bemisia argentifolii*, populations to four insecticides that are commonly used in northwestern Mexico (cypermethrin, endosulfan, methamidophos, and methyl parathion) was undertaken. Whitefly populations were collected from different crops in the Yaqui Valley in Sonora. Resistance levels were obtained, comparing LC_{50} of field populations with a susceptible whitefly strain that was maintained free of selection pressure from insecticides for nine years. Whitefly populations have increased resistance to cypermethrin (a pyrethroid), methyl parathion and methamidophos (both organophosphates). For endosulfan (an organochlorine), no significant differences were detected. Soybean summer crops in the Yaqui Valley used to be reservoirs of susceptible whiteflies when no insecticides were used in summer season. However, current use of insecticides no longer allows alternative summer crops in this region because whitefly infestations are uncontrollable. We demonstrated that the vial technique is reliable for identifying changes in seasonal and annual susceptibility patterns in whitefly populations.

Introduction

Relevance of silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring, also known as B biotype, as direct pest and geminivirus vector, has increased in the last few decades worldwide (Rao et al. 1990; Sparks et al. 2002). Silverleaf whitefly invaded the northwestern region of Mexico from the Imperial Valley of California into the Mexicali Valley and spread southward (Martínez 1994; Torres et al. 1996). This species was first detected in the Yaqui Valley in 1992. In 1993, the whitefly populations were ten times higher than the previous year (Martínez et al. 1998). Since its arrival in the Yaqui Valley, this pest has caused serious damage and losses to a wide variety of crops, including cotton, soybean, sesame, watermelon, squash, cantaloupe, tomato, potato, and pepper (Georghiou and Lagunes 1991; Pérez and Montes 1992; Fishpool and Burban 1994).

In the Yaqui Valley, this insect was listed as a key pest in 1993. Damage to soybeans was so serious during the years 1993 to 1995 that this crop could no

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longer be cultivated here at the same scale during the subsequent years, disappearing totally in season 1996-1997 (Table 1). Studies conducted under the Head Officer of Plant Health of the Ministry of Agriculture reported that, in 1996, whitefly had expanded into 17 Mexican states (Montealegre 1996; Servín et al. 1999, 2001).

Since the pest entered the Yaqui Valley, regional production shifted to vegetable and fruit crops, including citrus. However, despite implementing different agriculture practices, such as changes in cotton planting cycles, mandatory destruction of crop waste, and host-free periods that have reduced the problem to some degree, the silverleaf whitefly is still a leading pest of many crops (Martínez et al. 1998).

Chemical insecticides are not considered the ideal control strategy, but as no better alternatives are currently available, they have been extensively used for this purpose (Prakash et al. 1999; Dufour 2001). Consequences expected from this action, over the medium and long term, include increased resistance to insecticides, extermination of beneficial insects, outbreaks of secondary pests, and environmental contamination (Bi et al. 2002).

Table 1. Surface of Preferred Whitefly Crops in the Yaqui Valley from Where Whitefly Populations to Bioassays were Obtained.

Crop	1993-1994	1994-1995	1995-1996	1996-1997	1997-1998
Potato	778	1013	892	1190	1158
Watermelon	1274	1267	2403	3449	3065
Cantaloupe	160	37	71	75	167
Tomato	1041	1294	649	1159	417
Squash	437	193	428	573	799
Cotton	19198	33032	39650	24326	32383
Soybean	120127	24864	81	0	406

Many authors reported that whitefly populations developed resistance to several insecticides (Prabhaker et al. 1985; Dittrich et al. 1989; Cahill et al. 1996a, 1996b; Dennehy et al. 1996; Servín et al. 1997; Orozco et al. 2000). Authors have reported different techniques for monitoring whitefly resistance. Methods diverge mainly in the way the insecticide is applied to insects; there are, for example, approaches using polystyrene petri dishes (Cahill et al. 1986; Li et al. 2003), approaches using whitefly exposition to insecticide-immersed seedlings (Horowitz et al. 2004), approaches with direct spraying (Toscano et al. 1998; Toscano et al. 2001), techniques using sticky traps (Wool and Greenberg 1990; Prabhaker et al. 1992, 1996), and glass vial techniques (Campanhola and Plapp 1987; Staetz et al. 1992). In spite of several reports, no data have been gathered for northwestern Mexico. Therefore, a resistance-monitoring study of whitefly was started in 1994 in the Yaqui Valley. We discuss results obtained in a five-year study. In addition, since different approaches have been used in monitoring whitefly insecticide-resistance with variable response, we aim to confirm the glass vial approach as one of the best resistance-monitoring techniques.

Materials and Methods

Susceptible Whitefly Strain. A wooden cage was prepared to maintain a colony of *B. argentifolii* free of insecticide pressure. The 5.0 × 2.5 × 2.0 m cage has walls and ceiling covered by an anti-aphid mesh (BioNet screen 0.26 mm, Klayman Meteor Ltd., Petach-Tikva, Israel) to maintain isolation. A permanent cantaloupe crop was planted within the enclosure as a food substrate for whitefly. In spring 1993, adult silverleaf whiteflies were collected from cabbage, tomato, hot pepper, cantaloupe, and watermelon growing in La Paz, B.C.S., Mexico. Whiteflies were collected from commercial fields. Specimens were put into the cage for reproduction, and designated the free-from-selection-pressure strain (attenuated strain). Initial evaluations of increased susceptibility were performed throughout 1994-95; the last evaluation was made in 2002.

Field Whitefly Strains. Whitefly field populations were collected from 1994 to 1998. They were obtained from commercial fields of potato, cantaloupe, tomato, cotton, squash, and soybean (Table 1). Every population was obtained with a mouth aspirator made from 9-ml glass pipettes with a cloth screen and rubber hose to facilitate handling.

Bioassay Procedure and Resistance Estimates. Whitefly resistance was assessed with a modified vial bioassay (Campanhola and Plapp 1987; Staetz et al. 1992) that had proved to be more practical than the sticky trap and leaf disk methods (Simmons and Dennehy 1996; García-Marí and Soto 2001). The inner surface of 20-ml glass scintillation vials were coated with 1 ml of insecticide-acetone solution at different concentrations. Technical grade insecticides were used in these studies and were supplied by Bayer (Bayer CropScience, Santa Clara, Ecatepec, México). Cypermethrin (Rs)-alpha-cyano-3-phenoxybenzyl (IRS)-cistrans-3-3(2,2-dichlorovinyl)-2-2-diemthyl cyclopropane carboxylate); methyl parathion (O,O-dimethyl O-4-nitrophenyl phosphorothioate); endosulfan (6,9-methano-2,4,3-benzodioxathiepin,6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexaydro-3-oxido); and methamidophos (O,S-dimethyl phosphoramidothioate) were chosen because they are commonly used insecticides in this area. To ensure homogeneous coatings, vials were rolled for 15 minutes on a commercial hot dog roller until the acetone evaporated. The vials were then placed in front of a fan for 15 minutes to ventilate and eliminate all traces of acetone. Each treated vial was covered with a plastic cap with two perforations. One was covered with fabric screen for ventilation and the other blocked with a piece of paper. Twenty whiteflies were placed in each vial through this perforation and the vial was plugged again.

Vials were transported in boxes isolated from light and high temperatures to the lab and placed on a bench at normal room temperature (~25°C). According to Staetz et al. (1992) methodology, mortality was determined after 3 h by opening vials and tapping them over a black cloth. All individuals were softly touched with a small camel's brush, and those showing no response were counted as dead. A minimum of five concentrations of each insecticide and five replicates were used to run a regression analysis of the lines. Dosage-mortality values (LC_{50} and LC_{95}) were estimated using probit analysis (Raymond 1985). Resistance ratios (RR) for all bioassays were calculated by dividing the LC_{50} of every field strain of each crop (Table 3 to Table 7) by the lowest LC_{50} (obtained in 2002) of the correspondent insecticide of the attenuated strain that appears in the Table 2.

Results and Discussion

Susceptible Strain. The attenuated strain was used to determine the base line of each pesticide. Initial bioassays were performed in 1994-95 and repeated in 2002. Table 2 shows the LC_{50} and LC_{95} values, confidence limits, slopes, and χ^2 obtained for the attenuated strain. For each insecticide at the first-time bioassay made from March to November 1994, LC_{50} was 48.3, 198.6, 237.8, and 293.6 µg/ml for cypermethrin, endosulfan, methamidophos, and methyl parathion, respectively. For these insecticides, the attenuated strain showed a negative differential response over time, that is, a decline in the LC_{50}. In the July 1995 assay of the ≈15th generation of the attenuated strain, susceptibility had increased 2.8-, 3.8-, 3.1-, and 2.4-fold, respectively.

Bioassays were repeated seven years later in May 2002, approximately 70 generations later. The LC_{50} for these insecticides declined further. In 2002 the levels were 10.8, 15.2, 42.1, and 101.0 µg/ml, respectively. Susceptibility increased 4.4-, 13-, 5.7-, and 2.9-fold over the initial bioassay of 1994 (Table 2).

Table 2. Dose-Mortality Responses of an Attenuated Strain of Silverleaf Whitefly Maintained from 1993 to 2002 Without Selection Pressure from Agricultural Insecticides Used in Baja California Sur.

Insecticide	Date Month/Day/Year	LC_{50} (µg/ml)	Confidence limits (95%)	LC_{95} (µg/ml)	Slope	χ^2
Cypermethrin	04/21/94	48.3	9.3 - 252.4	361.5	1.88	0.18
	11/22/94	30.7	24.6 - 37.8	606.7	1.26	6.6
	12/14/94	28.3	10.4 - 76.4	640.2	1.21	1.42
	07/12/95	18.1	14.0 - 23.4	643.5	1.06	3.61
	07/13/95	17.1	13.7 - 21.2	268.2	1.37	9.37
	05/30/02	10.8	8.3 - 14.4	661.8	0.92	5.45
Endosulfan	03/23/94	198.6	166.0 - 231	910.9	2.48	4.85
	04/21/94	186.0	132.0 - 243.0	4080.9	1.22	0.13
	11/22/94	192.6	157.0 - 233.0	2000.3	1.61	3.09
	07/12/95	58.2	46.0 - 72.0	774.1	1.46	1.61
	07/13/95	51.9	41.0 - 65.0	988	1.29	3.87
	05/30/02	15.2	12.3 - 18.4	212.8	1.43	2.68
Methamidophos	11/22/94	237.8	206.0 - 271.0	1332.3	2.19	1.84
	12/13/94	93.6	79.0 – 109.0	435.0	2.46	0.35
	12/14/94	91.0	76.0 – 106.0	469.1	2.31	7.00
	07/12/95	75.6	60.0 - 91.0	630.2	1.78	1.23
	05/30/02	42.1	34.9 - 11.7	354.6	3.01	0.55
Methyl parathion	04/21/94	293.6	259.0 - 329.0	912.1	3.34	0.10
	11/22/94	224.2	154.0 - 325.0	950.0	2.19	10.24
	12/13/94	114.1	90.0 - 139.0	964.9	1.77	1.08
	12/14/94	105.9	86.0 - 127.0	1191.2	1.56	7.46
	07/14/95	118.9	99.0 - 141.0	838.5	1.93	7.06
	05/30/02	101.0	91.2 - 111.7	354.6	3.01	4.14

Resistance Estimates in Field Strains. Table 3 presents bioassay data for 1994 in the field strains. The LC_{50} detected in the evaluation of 3 March was the highest for all insecticides throughout the study period; such values remained stable or decreased. In terms of LC_{50}, cypermethrin was the most toxic material, followed by endosulfan, and least by methamidophos and methyl parathion. The highest LC_{95}s for endosulfan, methamidophos, and methyl parathion were also detected in the first evaluation conducted on specimens collected from potato crops on 3 March. For cypermethrin, the highest LC_{95} was detected in specimens collected from soybean crops on 13 July. The most significant slope, thus the most homogeneous response, was detected in specimens collected from cotton crops sprayed with endosulfan, methamidophos, and methyl parathion; the slope for cypermethrin was most significant in whitefly populations collected from soybean crops. The highest resistance ratios in 1994 occurred in the 3 March test with methamidophos and whitefly populations from potato crops. The next highest value was observed on the same date with endosulfan with populations from potato crops.

Table 3. Probit Analysis for Silverleaf Whitefly Populations in the Yaqui Valley, Sonora, Mexico in 1994.

CROP	DATE Month/day	I[a]	LC_{50}	FL[b] (95%)	LC_{95}	b	RR[c]	χ^2	p
Potato	03/03	C	25.0	18.8-32.8	352.0	1.43	2.31	2.67	0.55
Potato	03/25	C	4.4	3.3-5.7	63.0	1.43	0.4	7.50	0.94
Cotton	07/13	C	25.5	19.6-33.5	436.0	1.33	2.36	5.32	0.93
Soybean	07/13	C	43.1	34.1-52.3	262.0	2.10	3.99	0.23	0.10
Potato	03/03	E	195.1	145.7-259.2	3674.0	1.29	12.83	0.94	0.18
Potato	03/25	E	86.3	68.4-107.8	1133.0	1.47	5.67	0.79	0.14
Cotton	07/13	E	73.4	58.7-90.8	420.0	2.17	4.82	1.26	0.46
Soybean	07/13	E	83.7	69.9-99.0	482.0	2.16	5.50	4.99	0.82
Potato	03/03	M	571.3	449.9-710.4	7185.0	1.50	13.57	7.84	0.90
Potato	03/25	M	172.0	143.5-200.2	638.0	0.79	4.08	1.85	0.60
Cotton	07/13	M	193.3	169.5-217.3	456.0	4.41	4.59	2.35	0.69
Soybean	07/13	M	98.5	82.9-116.5	821.0	1.79	2.33	9.37	0.94
Potato	03/03	Mp	579.4	433.6-752.7	9734.0	1.34	5.73	7.71	0.94
Potato	03/25	Mp	117.2	73.2-186.3	785.0	1.99	1.16	8.54	0.96*
Cotton	07/13	Mp	147.4	130.8-165.8	402.0	3.78	1.45	1.99	0.63
Soybean	07/13	Mp	111.6	97.2-128.8	426.0	2.83	1.10	1.80	0.59

[a]I = Insecticide: C = Cypermethrin, E = Endosulfan, M = Methamidophos, Mp = Methyl parathion.
LC_{50} and LC_{95} in µg/ml.
b = slope.
[b]FL = Fiducial limits.
[c]RR = Resistance ratio.

In 1995 (Table 4), results showed variation when compared to 1994, observing in general decreases in LC_{50}s, LC_{95}s, and RRs. In 1995, the LC_{50} for all insecticides showed the highest value in cotton fields. LC_{95} values for cypermethrin and methyl parathion were highest in populations collected in cotton. The highest LC_{95}s for endosulfan and methamidophos were found in soybean and cantaloupe whiteflies, respectively. The most significant slopes for each compound were also related to crops, as follows: cypermethrin and methyl parathion for cantaloupe whiteflies, endosulfan from cotton, and methamidophos from soybean. The highest RRs in 1995 occurred in the 23 July test for all crops and insecticides. The highest RRs (over three-fold) were observed with methamidophos and endosulfan with populations from cotton, soybean, and cantaloupe in the warmest season (May to August).

In 1996, for cypermethrin, samples from soybean at the end of the season showed the highest LC_{50} (Table 5). This probably reflected migration movements of selected populations from nearby cotton crops, where the second highest LC_{50} was measured. For endosulfan, LC_{50}s ranged slightly higher than in 1995, but lower than in 1994. Endosulfan and methyl parathion showed the highest LC_{50} value also in whiteflies from soybean. For methamidophos, the highest LC_{50} was found in whiteflies from cotton at the end of season. In general, LC_{50}s for cypermethrin and endosulfan increased during 1995 and 1996 as the planting season progressed. This is true for samples from potatoes (16 April) to soybeans (6 August). There is an apparent trend that reflects the selection pressure of the insecticides from applications on different crops as the season progressed. In 1996, almost all the resistance ratios increased relative to the previous year. The highest RRs were observed in tests with methamidophos (cotton populations) and endosulfan, up to 4.38 and 5.47 (cotton and soybean populations, respectively). Also, cypermethrin had a high resistance ratio of 5.26 (cotton population). The maximum LC_{50} values of 1996 occurred in the warmest months (July and August).

Table 4. Probit Analysis for Silverleaf Whitefly Populations in the Yaqui Valley, Sonora, Mexico in 1995

CROP	DATE Month/day	I[a]	LC_{50}	FL[b] (95%)	LC_{95}	b	RR[c]	χ^2	*p*
Potato	04/10	C	12.8	10.1-16.1	264.0	1.25	1.18	1.32	0.14
Watermelon	04/25	C	8.4	6.4-10.8	156.0	1.30	0.77	4.57	0.79
Cantaloupe	05/26	C	12.4	10.3-14.7	102.0	1.80	1.14	0.87	0.07
Cotton	07/23	C	30.5	23.9-38.6	780.0	1.17	2.82	4.82	0.56
Soybean	08/07	C	29.4	23.6-36.5	494.0	1.34	2.72	2.21	0.30
Potato	04/10	E	17.7	14.8-21	143.0	1.82	1.16	5.58	0.86
Watermelon	04/25	E	12.2	9.6-15	136.0	1.57	0.80	3.95	0.73
Cantaloupe	05/26	E	10.7	8.9-12.5	57.0	2.25	0.70	0.74	0.13
Cotton	07/23	E	50.1	48.9-56.5	173.0	3.06	3.29	0.11	0.09
Soybean	08/07	E	46.1	39.7-53.7	282.0	2.10	3.03	3.78	0.71
Potato	04/10	M	100.7	88.8-113.1	365.0	2.94	2.39	0.68	0.12
Watermelon	04/25	M	34.6	28.1-41.4	319.0	1.71	0.82	0.42	0.64
Cantaloupe	05/26	M	128.8	109.4-152.5	1100.0	1.77	3.05	2.88	0.42
Cotton	07/23	M	155.6	136.1-175.5	661.0	2.62	3.69	5.86	0.79
Soybean	08/07	M	126.8	109.8-143.6	457.0	2.96	3.01	7.44	0.94
Potato	04/10	Mp	87.0	71.4-103.8	462.0	2.27	0.86	3.32	0.49
Watermelon	04/25	Mp	79.6	49.3-128.5	284.0	2.98	0.78	39.70	0.99**
Cantaloupe	05/26	Mp	91.3	65-128.2	291.0	3.27	0.90	14.16	0.99**
Cotton	05/23	Mp	93.7	74.9-113	513.0	2.23	0.92	2.37	0.50
Soybean	08/07	Mp	93.6	81-166	419.0	2.53	0.92	1.11	0.22

[a]I = Insecticide: C = Cypermethrin, E = Endosulfan, M = Methamidophos, Mp = Methyl parathion.
LC_{50} and LC_{95} in µg/ml.
b = slope.
[b]FL = Fiducial limits.
[c]RR = Resistance ratio.

Table 6 displays results from 1997. Cypermethrin and endosulfan showed their highest LC_{50} levels in populations from squash. In general, endosulfan LC_{50}s were lower than levels recorded in the previous three years. For methamidophos, LC_{50}s were similar to those previously observed, except in 1994. The highest LC_{50} for methyl parathion was observed in whiteflies from cotton fields.

In 1997, the highest LC_{95} value for cypermethrin occurred in populations from squash fields. The highest LC_{95} for endosulfan and methamidophos were detected in populations from cotton. For methyl parathion, the highest LC_{95} was detected in populations from squash. In general, RRs were lower in 1997, possibly as an effect by the elimination of soybean crops. However, the highest RR was observed in the warmest month (July), and again with methamidophos from the cotton population.

From Table 7, in 1998, the final year, the highest LC_{50} for cypermethrin was found in a population from soybean in August. This was the highest level of resistance to this insecticide in the Yaqui Valley since the monitoring program started in 1994; it probably reveals a population selected for pyrethroids. Similarly, in 1996, the LC_{50} increased two-fold on bioassays performed on populations from soybean fields when compared to those from cotton fields (Table 5). However, in 1997, LC_{50} values did not reach the level of 1996. The most probable explanation for the general decline in resistance ratios in 1997 is almost total elimination of soybean crops in the Yaqui Valley.

Table 5. Probit Analysis for Silverleaf Whitefly Populations in the Yaqui Valley, Sonora, Mexico in 1996

CROP	DATE Month/day	I[a]	LC_{50}	FL[b] (95%)	LC_{95}	b	RR[c]	χ^2	*p*
Potato	04/16	C	12.9	10.1-16.4	374.0	1.13	1.19	1.51	0.17
Watermelon	04/23	C	20.2	15.7-25.9	592.0	1.12	1.87	1.94	0.25
Cantaloupe	05/21	C	19.3	14.7-25.2	766.0	1.03	1.78	1.26	0.13
Cotton	07/02	C	56.9	42.2-74.0	2402.0	1.01	5.26	2.49	0.35
Soybean	08/06	C	110.3	87.1-138.0	2729.0	1.18	10.2	2.64	0.38
Potato	04/16	E	17.6	14.4-21.6	293.0	1.35	1.15	7.51	0.88
Watermelon	04/23	E	21.3	17.4-26.2	370.0	1.33	1.40	5.40	0.75
Cantaloupe	05/21	E	29.4	24.6-35.2	269.0	1.71	1.93	6.33	0.82
Cotton	07/02	E	66.7	54.9-80.3	909.0	1.45	4.38	3.67	0.54
Soybean	08/06	E	83.2	68.5-100.7	1221.0	1.41	5.47	2.78	0.40
Potato	04/16	M	100.9	82.7-122.3	1517.0	1.40	2.39	4.90	0.70
Watermelon	04/23	M	135.5	112-164	1935.0	1.42	3.21	3.73	0.55
Cantaloupe	05/21	M	143.1	118-174	1957.0	1.44	3.39	1.34	0.14
Cotton	07/02	M	181.8	140-236	6676.0	1.05	4.31	2.71	0.39
Soybean	08/06	M	152.3	116-199	7591.0	0.97	3.61	4.98	0.71
Potato	04/16	Mp	96.6	76.4-120.4	2001.0	1.25	0.95	3.75	0.56
Watermelon	04/23	Mp	114.9	91.9-142.6	2567.0	1.22	1.13	2.93	0.43
Cantaloupe	05/21	Mp	132.9	107-164	2646.0	1.27	1.31	2.16	0.29
Cotton	07/02	Mp	165.2	134-203	2999.0	1.31	1.63	8.83	0.93
Soybean	08/06	Mp	180.2	146-223	3756.0	1.25	1.78	9.18	0.95*

[a]I = Insecticide: C = Cypermethrin, E = Endosulfan, M = Methamidophos, Mp = Methyl parathion.
LC_{50} and LC_{95} in μg/ml.
b = slope.
[b]FL = Fiducial limits.
[c]RR = Resistance ratio.

The methamidophos highest LC_{50} in 1998 was detected in a population from cotton. In general, for this insecticide, the LC_{50} values of 1998 were the lowest in the entire five-year study. The highest LC_{50} and LC_{95} values were recorded in a soybean population for methyl parathion, cypermethrin, and endosulfan, while both highest lethal concentrations for methamidophos were from a cotton population.

The most significant slopes for cypermethrin, methamidophos, and methyl parathion were detected in populations from cantaloupe fields. The highest slope for endosulfan was from soybean field populations. In the same year, resistance ratios of tests with endosulfan increased significantly, showing the highest ratios (5.44, 4.12, and 7.84) with populations from soybean, tomato, and cotton. However, the most significant increase in resistance ratios occurred with cypermethrin, which had not shown similar values in the previous years. Cypermethrin showed the highest value of the five-year study (22.68) in August 1998, with a whitefly population from soybean fields.

In general, the results show the evolution of acquired resistance and susceptibility. The sole insecticide that increased the resistance level in a sustained manner was cypermethrin. However, for each insecticide, an intra-annual increase in resistance occurred, but was not sustained in following years. All insecticides showed their highest LC_{50} values and resistance ratios at the last bioassay of each year, except for the initial year (Tables 3 and 8). This illustrates a regular trend in whitefly resistance in areas of intermittent production. Continued application of insecticides during a crop season results in selection for more resistant individuals, but this level of resistance declines during the crop-free period after the harvest and before the next crop is planted. In this region, this fallow period occurs in summer (July-September) when temperature are greater than the tolerances of the crops, but still tolerable to breeding whiteflies that live on many species of wild plants (Cano-Ríos et al. 2001).

Table 6. Probit Analysis for Silverleaf Whitefly Populations in the Yaqui Valley, Sonora, Mexico in 1997

CROP	DATE Month/day	I[a]	LC_{50}	FL[b] (95%)	LC_{95}	b	RR[c]	χ^2	*p*
Potato	04/10	C	27.67	21.9-34.4	485.0	1.32	2.56	8.49	0.92
Squash	04/24	C	58.92	33.4-103.7	1841.0	1.10	5.45	10.60	0.96*
Cantaloupe	05/29	C	12.50	9.80-15.9	264.0	1.24	1.15	7.05	0.86
Cotton	07/16	C	19.00	9.8-37.0	796.0	1.01	1.75	12.61	0.98*
Potato	04/10	E	8.95	7.5-10.6	59.8	1.99	0.58	0.58	0.34
Squash	04/24	E	19.45	16.11-23.5	194.1	1.64	1.27	7.83	0.90
Cantaloupe	05/29	E	10.10	5.10-20.1	239.6	1.19	0.66	18.09	0.99
Cotton	07/16	E	12.50	9.50-16.3	463.6	1.05	0.82	4.83	0.69
Potato	04/10	M	136.19	115.2-160.9	851.0	2.07	3.23	3.58	0.53
Squash	04/24	M	157.72	131.0-189.7	1447.0	1.71	3.74	3.72	0.55
Cantaloupe	05/29	M	94.90	59.5-150.8	1043.0	1.58	2.25	14.05	0.99**
Cotton	07/16	M	171.30	70.0-423.9	4915.0	1.12	4.06	13.52	0.99**
Potato	04/10	Mp	120.61	99.0-146.0	1101.0	1.71	1.19	3.83	0.57
Squash	04/24	Mp	95.76	75.2-120.5	1950.0	1.26	0.94	5.96	0.79
Cantaloupe	05/29	Mp	122.90	108.6-139.1	632.0	2.31	1.21	1.70	0.20
Cotton	07/16	Mp	147.70	124.1-175.4	1624.0	1.58	1.46	5.36	0.62

[a]I = Insecticide: C = Cypermethrin, E = Endosulfan, M = Methamidophos, Mp = Methyl parathion.
LC_{50} and LC_{95} in µg/ml.
b = slope.
[b]FL = Fiducial limits.
[c]RR = Resistance ratio.

A new cycle of application starts in October, which initiates the process of selection for increased resistance. Many pest control advisors and this investigation support the conclusion that recovery of susceptibility occurs with endosulfan, methamidophos, and methyl parathion. In spite of the high toxicity of pyrethroids, cypermethrin is very commonly applied in the Yaqui Valley, usually at lower doses than most other insecticides. Nevertheless, from 1996 to 1998, the highest cypermethrin's LC_{50} was higher than the highest endosulfan LC_{50}. Also, in 1998, the highest cypermethrin LC_{50} was higher than the LC_{50} of all the other insecticides. This is most likely a consequence of differences in the stability level of the resistance mechanisms of whiteflies for each type of insecticide (Denholm et al. 2003) and the popularity of pyrethroids among the advisors in the 1990s.

In Fig. 1, LC_{50} values were plotted to detect trends over the five-year resistance assessment period. The organophosphate insecticides methamidophos and methyl parathion showed a negative trend over time. This suggests that whitefly resistance to these insecticides has been almost steady.

Table 7. Probit Analysis for Silverleaf Whitefly Populations in the Yaqui Valley, Sonora, Mexico in 1998

CROP	DATE Month/day	I[a]	LC_{50}	FL[b] (95%)	LC_{95}	b	RR[c]	χ^2	*p*
Potato	04/23	C	66.00	54.10-80.60	487.0	1.61	6.11	5.39	0.85
Watermelon	04/17	C	92.90	75.70-114.0	911.0	1.66	8.60	4.89	0.82
Cantaloupe	05/13	C	80.50	65.70-99.10	1005.0	1.50	7.45	6.17	0.89
Cotton	07/07	C	56.80	46.80-68.30	741.0	1.47	5.25	7.50	0.95*
Soybean	08/19	C	245.00	184-325	4608.0	1.29	22.68	1.50	0.52
Potato	04/23	E	49.30	36.90-65.60	3066.0	0.92	3.24	3.30	0.49
Watermelon	04/17	E	71.90	54.40-95.90	4341.0	0.92	4.73	4.46	0.65
Cantaloupe	05/13	E	82.80	63.60-109.0	3749.0	0.99	5.44	2.07	0.27
Cotton	07/07	E	62.70	45.70-83.40	3560.0	0.94	4.12	5.65	0.77
Soybean	08/19	E	119.20	91.1-154.5	4647.0	1.03	7.84	4.89	0.70
Potato	04/23	M	27.80	22.80-33.60	344.0	1.51	0.66	1.00	0.09
Watermelon	04/17	M	30.70	25.60-36.60	329.0	1.60	0.72	2.31	0.32
Cantaloupe	05/13	M	30.40	24.90-36.70	429.0	1.43	0.72	3.17	0.47
Cotton	07/07	M	56.80	46.80-68.30	741.0	1.47	1.34	2.93	0.43
Soybean	08/19	M	55.80	44.00-69.50	720.0	1.48	1.32	3.71	0.84
Potato	04/23	Mp	28.20	21.80-35.90	760.0	1.15	0.27	8.42	0.92
Watermelon	04/17	Mp	35.10	27.70-44.00	786.0	1.22	0.34	4.67	0.67
Cantaloupe	05/13	Mp	36.70	28.50-46.70	947.0	1.17	0.36	2.19	0.29
Cotton	07/07	Mp	89.70	70.20-112.7	2301.0	1.17	0.88	0.75	0.05
Soybean	08/19	Mp	101.50	78.90-128.7	3161.0	1.10	1.00	2.43	0.34

[a]I = Insecticide: C = Cypermethrin, E = Endosulfan, M = Methamidophos, Mp = Methyl parathion.
LC_{50} and LC_{95} in µg/ml.
b = slope.
[b]FL = Fiducial limits.
[c]RR = Resistance ratio.

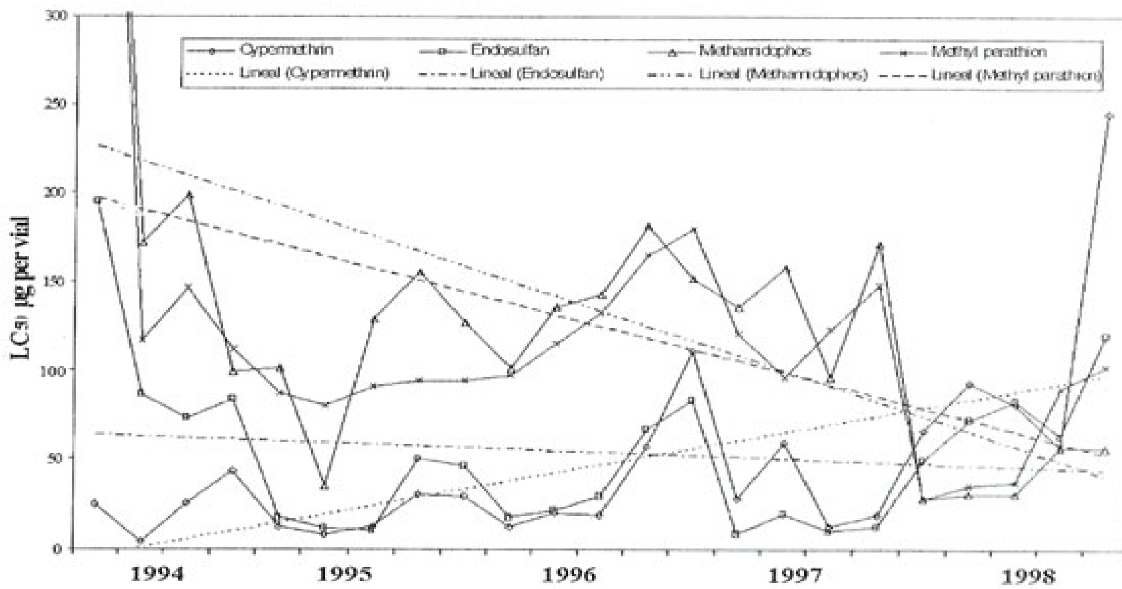

Fig. 1: Trends of LC_{50} Values of Four Insecticides Applied to Whitefly Populations in the Yaqui Valley, Sonora, Mexico from 1994-1998.

Data from the last year had the lowest values, compared to previous years. This supports the view that insecticide pressure is important for selecting resistant individuals, and that resistance mechanisms of these populations are not stable (Table 7), drifting up and down, depending on selection pressure.

Endosulfan did not show significant slope over time. However, in the last year of this study, its LC_{50} was higher than values observed earlier, suggesting that a selection process is developing. To offset this process, this chemical should be used with caution and rotated with other insecticides in a resistance management program (Prabkaker et al. 1998; Nava and Cano 2000).

Silverleaf whitefly populations in the Yaqui Valley have been selected for resistance to pyrethroid insecticides, as shown by cypermethrin data. Resistance values of this chemical (Fig. 1, Table 8) have an increasing trend over time. This was more evident with populations from soybean fields in 1996 (110.3 µg/ml) versus 1998 (245 µg/ml). These data will be valuable for designing a program for controlling whitefly populations in the Yaqui Valley. Our results support previous studies that claim that pyrethroid insecticides select for higher whitefly resistance faster than organophosphate or organochlorine insecticides (Garza 1994; Horowitz and Ishaaya 1995).

Table 8. LC_{50} and LC_{95} for Silverleaf Whitefly Populations in the Yaqui Valley, Sonora, Mexico from 1994 to 1998.

a	1994		1995		1996		1997		1998	
	LC_{50}	LC_{95}	LC_{50}	LC_{95}	LC_{50}	LC_{95}	LC_{50}	LC_{95}	LC_{50}	LC_{95}
C	25.0	352.0	12.8	264.0	12.9	374.0	27.67	485.0	66.00	487.0
C	4.4	63.0	8.4	156.0	20.2	592.0	**58.92**	**1841.0**	92.90	911.0
C	25.5	**436.0**	12.4	102.0	19.3	766.0	12.50	264.0	80.50	1005.0
C	**43.1**	262.0	**30.5**	**780.0**	56.9	2402.0	19.00	796.0	**161.9**	**2260**
C			29.4	494.0	**110.3**	**2729.0**			**245.00**	**4608.0**
E	**195.1**	**3674.0**	17.7	143.0	17.6	293.0	8.95	59.8	49.30	3066.0
E	86.3	1133.0	12.2	136.0	21.3	370.0	**19.45**	194.1	71.90	4341.0
E	73.4	420.0	10.7	57.0	29.4	269.0	10.10	239.6	82.80	3749.0
E	83.7	482.0	**50.1**	173.0	66.7	909.0	12.50	**463.6**	62.70	3560.0
E			46.1	**282.0**	**83.2**	**1221.0**			**119.20**	**4647.0**
M	**571.3**	**7185.0**	100.7	365.0	100.9	1517.0	136.19	851.0	27.80	344.0
M	172.0	638.0	34.6	319.0	135.5	1935.0	157.72	1447.0	30.70	329.0
M	193.3	456.0	128.8	**1100.0**	143.1	1957.0	94.90	1043.0	30.40	429.0
M	98.5	821.0	**155.6**	661.0	**181.8**	6676.0	**171.30**	**4915.0**	**56.80**	**741.0**
M			126.8	457.0	152.3	**7591.0**			55.80	720.0
Mp	**579.4**	**9734.0**	87.0	462.0	96.6	2001.0	120.61	1101.0	28.20	760.0
Mp	117.2	785.0	79.6	284.0	114.9	2567.0	95.76	**1950.0**	35.10	786.0
Mp	147.4	402.0	91.3	291.0	132.9	2646.0	122.90	632.0	36.70	947.0
Mp	111.6	426.0	**93.7**	**513.0**	165.2	2999.0	**147.70**	1624.0	89.70	2301.0
Mp			93.6	419.0	**180.2**	**3756.0**			**101.50**	**3161.0**

a = Insecticide, C = Cypermethrin, E = Endosulfan, M = Methamidophos, Mp = Methyl parathion. LC_{50} and LC_{95} in µg/ml. Bold-face indicates highest LC_{50} and LC_{95} values (resistance) for each insecticide in each year.

Some authors claim that the best way to generate resistance is to use pyrethroids (Liu et al. 1981; Forrester et al. 1993). We confirmed that there is no decline in the LC_{50} of cypermethrine, unlike the trend in organophosphate compounds. The general observation is that the first bioassays of a new season had lower LC_{50} and LC_{95} values than the LC_{50} and LC_{95} values of the last bioassay of the previous season (Table 7). In 1994, despite a number of insecticide applications, the whitefly outbreak caused serious damage and loss to soybean crops, which whiteflies prefer to other crops. In 1995, soybean cultivation was significantly reduced, and insecticides were not extensively used, which probably favored reduction of resistance to this insecticide. In the 1997 season, soybeans nearly disappeared from the Yaqui Valley, and whitefly was not as resistant as before (Table 6). Reduction in insecticide selection pressure occurred in 1997, and LC_{50} values declined. In 1998, new and larger whitefly populations invaded cotton and other crop fields sprayed with endosulfan and pyrethroids. The selection pressure exerted was demonstrated by the higher LC_{50} values recorded for the tested insecticides (Table 6).

In summary, from 1994 to 1998 (Table 8), testing revealed that whitefly populations in the Yaqui Valley increased their resistance to the pyrethroid insecticide cypermethrin. This was more evident in data from 1996 and 1998, where the LC_{50} increased significantly in populations collected from soybean fields. The organophosphate insecticides methamidophos and methyl parathion showed a negative trend. Endosulfan, an organochlorine insecticide, showed no significant trend over time. However, in 1998, LC_{50} values were higher than those recorded in bioassays of 1995, 1996, and 1997. This suggests selection for resistant individuals is developing.

We recommend that farmers avoid pyrethroid applications at the beginning of the agricultural season and limit application to one per season (Plapp et al. 1987; Forrester et al. 1993; Lagunes and Villanueva 1994). Our data supports the vial technique to evaluate insecticide resistance as a fast, practical, and inexpensive method for testing resistance in whitefly populations.

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