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## STABILITY OF INSECTICIDE RESISTANCE OF SILVERLEAF WHITEFLY (HOMOPTERA: ALEYRODIDAE) IN THE ABSENCE OF SELECTION PRESSURE

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**ABSTRACT.** The silverleaf whitefly is a major pest of more than 500 vegetable species. This insect transmits extremely damaging plant viruses, and feeding by nymphs and adults induce a systemic, irregular ripening disorder. In 1993, a large, genetically diverse pool of this species was collected from cabbage *Brassica oleracea* L., tomato *Lycopersicon esculentum* L., hot pepper *Capsicum annuum* L., cantaloupe *Cucumis melo* L., and watermelon *Citrullus vulgaris* L. A proportion of the mixed original population has been maintained free of selection pressure to attenuate the resistance processes. Susceptibility of the original freedom from selection pressure population has been evaluated in 1994, 1995, and 2002. Collections of new field strains of silverleaf whitefly were also done twice in 1994, and once in 1996. Each time, colonies of both strains (from field, and free of selection pressure) were established in the laboratory. Mortality (LC<sub>50</sub> and LC<sub>95</sub>), and resistance ratios with common insecticides methamidophos, methyl parathion, cypermethrin, and endosulfan were evaluated. Not all the field populations were resistant. The highest resistance ratios for endosulfan, methamidophos, cypermethrin, and methyl parathion were: 68x, 22x, 9.5x, and 4.5x respectively. The highest resistance level was found to endosulfan with a LC<sub>50</sub> value of 1034.6 µg/ml.

**KEY WORDS:** *Bemisia argentifolii*, insecticide resistance, selection pressure.

Servín-Villegas, R., J.L. García-Hernández, B. Murillo-Amador, A. Tejas y J.L. Martínez-Carrillo. Estabilidad de la resistencia a insecticidas de la mosca blanca de la hoja plateada (Homoptera: Aleyrodidae) en ausencia de presión de selección. *Folia Entomol. Mex.*, 45 (1): 27-34.

**RESUMEN.** La mosca blanca de la hoja plateada es una plaga principal de más de 500 especies de plantas. Este insecto transmite virus de plantas extremadamente dañinos, y el proceso de alimentación de las ninfas y adultos inducen desordenes de crecimiento irregular en forma sistémica. En 1993, se colectó un gran número de individuos de esta especie para formar un conjunto genéticamente diverso. Los cultivos de donde se obtuvieron los individuos fueron col *Brassica oleracea* L., tomate *Lycopersicon esculentum* L., chile *Capsicum annuum* L., melón *Cucumis melo* L. y sandía *Citrullus vulgaris* L. A partir de la mezcla de este conjunto original se ha mantenido una población libre de presión de selección con la finalidad de revertir el proceso de resistencia a insecticidas. La susceptibilidad de esta población libre de presión de insecticidas fue evaluada en 1994, 1995 y 2002. Asimismo, se obtuvieron colectas independientes de mosca blanca de los campos de producción comerciales dos veces en 1994 y una vez más en 1996. Para todos los casos, las colonias de ambos tipos de poblaciones (de campo o libre de presión de selección) se llevaron al laboratorio, para evaluar la mortalidad (CL<sub>50</sub> y CL<sub>95</sub>) y el radio de resistencia a los insecticidas metamidofos, paration metílico, cipermetrina y endosulfan. No todas las poblaciones fueron resistentes. Los mayores radios de resistencia para endosulfan, metamidofos, cipermetrina y paration metílico fueron 68x, 22x, 9.5x y 4.5x. El mayor grado de resistencia se encontró con endosulfan con un valor de CL<sub>50</sub> de 1034.6 µg/ml.

**PALABRAS CLAVE:** *Bemisia argentifolii*, resistencia a insecticidas, presión de selección.

Whiteflies belong to the family Aleyrodidae, and include a large insect group with more than 1200 species (Martínez-Carrillo, 1998a), some of which are notorious agricultural pests (Servín-Villegas *et al.*, 2001). In México, there are reported seven whitefly species as economically important (Ortega, 1992). The silverleaf whitefly *Bemisia argentifolii* (Bellows & Perring, 1994) has become a serious pest of many crops worldwide (Schuster, 2003; Cano-Ríos *et al.*, 2001a; Cano-Ríos *et al.*, 2001b). It causes extensive crop damage through direct feeding and transmission of plant viruses (Sivasupramaniam and Watson, 2000). Outbreaks of this insect are believed to be triggered by the development of insecticide resistance, due to an over-reliance on broad spectrum insecticides (Dittrich *et al.*, 1990; Wool and Greenberg, 1990; Mochizuki, 1994; Ortega, 1998).

The Baja California Peninsula has multi-cropping systems where various crops are grown throughout the year. Most insecticide applications targeting silverleaf whiteflies in vegetables, cabbage, *Brassica oleracea* L, tomato, *Lycopersicon esculentum* L., hot pepper, *Capsicum annuum* L., cantaloupe, *Cucumis melo* L., and watermelon, *Citrullus vulgaris* L., contain methamidophos, methyl parathion, endosulfan, and cypermethrin (Servín-Villegas *et al.*, 1997). Extensive reliance on chemicals for whiteflies control had resulted in resistance to many classes of insecticides and has become a serious constraint on effective control in many countries and in México (Ortega, 1998; Sivasupramaniam and Watson, 2000). Cypermethrin and endosulfan are chemically different; however, according to Mueller-Beilschmidt (2005); as a pyrethroid and organochlorine, respectively, they share mode of action in insects, and resistance to one is likely to convey resistance to the other (Ortega, 1998). Also, both organochlorine and pyrethroid insecticides lead to resistance in pests having several generations per year (Ortega, 1998; Martínez-Carrillo,

1998b).

Silverleaf whitefly has found favorable environment in the southern region of the Baja California Peninsula. In the last decade, many pest control advisors have reported that organophosphate (OP), organochlorine (OC), and pyrethroid insecticides (alone and combined) achieve commercially acceptable whitefly control (Servín *et al.*, 1997). However, some growers experience control failures, which have been attributed to insecticide resistance due to the continued use of broad-spectrum pesticides. With this respect, no supportive data have been published. Previous studies have demonstrated a large increase in the population of this pest, feeding on vegetable crops and sheltering in wild plants from 26 plant families across BCS (Servin-Villegas *et al.*, 2001). Toxicological bioassays are essential for the identification of insecticide resistance in insect pests (Byrne *et al.*, 2003). A whitefly colony with three years free of selection pressure has been regarded as susceptible to evaluate the susceptibility level of field populations (Ortega *et al.*, 1998). The objectives of this study were to investigate the capacity of *B. argentifolii* field strains from major vegetable crops of La Paz, B.C.S. to develop resistance to the following insecticides: methamidophos, methyl parathion, endosulfan, and cypermethrin, and the susceptibility in the isolated population in absence of selection pressure. Results of this study could be used as a basis when designing resistance management programs for *B. argentifolii*.

## MATERIALS AND METHODS

**Free Pressure Strain.** At the Experimental Field of the 'Centro de Investigaciones Biológicas del Noroeste'; which is located in La Paz, B.C.S., in Northwest México, a wooden cage was prepared to maintain a colony of *B. argentifolii* free of insecticide selection pressure. The cage, 5.0 × 2.5 × 2.0 m, had walls and ceiling covered by an anti-aphid mesh (BioNet Screen, 0.26 mm

hole size) for maintaining isolation. A permanent cantaloupe crop was planted within the enclosure as substrate for whitefly. In spring 1993, adult silverleaf whiteflies were collected from cabbage, tomato, hot pepper, cantaloupe, and watermelon growing in El Centenario; rural town 15 km from La Paz. Collections of whiteflies from fields were performed by using a microaspirator. Specimens were put in the cage for reproduction, and we referred to as the free of selection pressure strain (FRE). Initial evaluations of susceptibility were performed throughout 1994-95, and a new evaluation with this strain was made in 2002. Subsequently, this population has been maintained free of selection pressure.

**Field Strains.** Bioassays to evaluate susceptibility were performed on field populations. The collections of silverleaf whitefly from crop fields were analyzed for susceptibility twice in 1994 and once in 1996. Field populations (FP) were collected at the same locations as the FRE; where different combinations of endosulfan, cypermethrin, methyl parathion and methamidophos were applied three times per week. FP collecting methods were the same as those used for collecting the original pool of FRE. The most common crop for obtain the FP populations was cabbage, due to highest availability.

**Insecticides.** Technical grade insecticides were used in these studies. All of the insecticides were supplied by Bayer Company (Bayer Crop-Science, Santa Clara, Ecatepec, México). Cypermethrin, pyrethroid insecticide: (Rs)-alpha-Cyano-3-Phenoxybenzyl (IRS)-Cistrans-3-3(2,2-dichlorovinyl)-2-2-dimethyl cyclopropane carboxylate); endosulfan, organochlorine: (6,9-methano-2,4,3-benzodioxathiepin, 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-, 3-oxido); methamidophos, organophosphate: (O,S-Dimethyl phosphoramidothioate); and methyl parathion, organophosphate: (O,O-Dimethyl O-4-nitrophenyl phosphorothioate). were chosen because they are the commonly used insecticides in this

area.

**Bioassay Procedure and Susceptibility Estimates.** Assays were performed with adults of each strain. A modified procedure from two papers (Staetz *et al.*, 1992; Plapp *et al.*, 1987) glass vial techniques was used to determine susceptibility levels of adults to insecticides, as follows: Prepared concentrations of insecticides (1 ml with acetone as solvent) were placed in 20-ml glass vials. Vials were placed horizontally over a rotation device. Desired chemical residues were achieved by rotating the vial until the acetone evaporated, leaving a pesticide residue evenly distributed over the interior surface of the vial. Whitefly adults (20; unsexed) were aspirated and transferred to the treated vials. The vials were capped and held at  $20 \pm 5^\circ\text{C}$  for 3 h before making counts of mortality. The symmetric design recommended by Finney (1971) for precise estimation of the  $\text{LC}_{50}$  was used in all studies. Assays of the FR strain were performed between 1994 and 1995, and replicated in 2002. Assays of FP strain were performed twice in 1994 and once in 1996. A series of six to eight doses was tested for each of the four chemicals. The entire procedure was replicated three times. Controls were conducted with vials coated with acetone only.

**Statistical Analysis.** Dosage-mortality curves for all bioassays were estimated using probit analysis (Raymond, 1985) to estimate values of the equation  $Y = a + bX$ , where  $X$  corresponds to dosage logarithm, and  $Y$  to probit. Concentrations required to kill 50% ( $\text{LC}_{50}$ ) of the populations were estimated using probit regression, with 95% confidence limits to separate differences. Resistance ratios (RR) for all bioassays were calculated by dividing the  $\text{LC}_{50}$  of the field strain by the 2002  $\text{LC}_{50}$  of the Free strain.

## RESULTS AND DISCUSSION

**Susceptible Strain (FRE).** The population that has been isolated from insecticides was used to determine the base lines for the pesticides. Initial

bioassays were performed throughout 1994-95 with FRE and replicated in 2002. Table 1 shows the values of  $LC_{50}$  and  $LC_{95}$ , confidence limits, and slopes obtained for the FRE strain. At the first bioassay; performed from March to November 1994 for each insecticide, the  $LC_{50}$  were 123.9, 198.6, 237.8, and 293.6  $\mu\text{g/ml}$  for cypermethrin, endosulfan, methamidophos, and methyl parathion, respectively. For these insecticides, FRE showed a negative differential response over time, a decline in  $LC_{50}$ . In the assay of the FRE strain in July 1995 (approximately the 15th generation), 17.1, 51.9, 75.6, and 118.9  $\mu\text{g/ml}$  of the respective insecticides was needed to obtain  $LC_{50}$ . Susceptibility had increased 2.8, 3.8, 3.1, and 2.4-fold, respectively for these insecticides in the mentioned order.

Seven years later, the bioassays were replicated. In May 2002; after a theoretical approximation of seventy generations, the  $LC_{50}$  for every insecticide declined further. At the last bioassays, the levels were 10.8, 15.2, 42.1, and 101.0  $\mu\text{g/ml}$  for cypermethrin, endosulfan, methamidophos, and methyl parathion, respectively. By toxicological group, the organophosphate (OP) (represented by methamidophos and methyl parathion) showed the highest toxicity. In the other hand, the pyrethroid (cypermethrin) showed the lowest toxicity. Susceptibility increased to 4.4, 13, 5.7, and 2.9-fold for these insecticides relative to the initial bioassay.

The slope in the regression equation indicates the degree of homogeneity of population on its response to the toxic. There is a positive correlation slope-homogeneity. At the last bioassay in 2002 (Table 1), the most heterogeneous response to develop resistance was for cypermethrin (0.92), while the most homogeneous were for both OPs methamidophos and methyl parathion (3.01).

**Responses to these insecticides differed.** For cypermethrin, there was a gradual reduction in resistance at the theoretical 15th generation, with

further reduction seven years later (Table 1). For endosulfan, a rapid increase in susceptibility occurred from March 94 to July 95, more than 3-fold (Table 1). Between 1994 and 2002 tolerance declined 13-fold. It was the proportionately greatest increase in susceptibility among the pesticides.

In the evaluations performed during 1994-95, the fastest rate of initial recovering back susceptibility was the 2.6-fold increase of whitefly to methamidophos from November to December 1994 (Table 1). After this rapid transformation, changes in susceptibility were slower, seven years later; susceptibility had increased 5.7-fold with respect to the original  $LC_{50}$ .

These results are explained by differences in the stability level of the different resistance mechanisms of whiteflies for each type of insecticide (Denholm *et al.*, 2003). In the case of parathion, with the highest level of stability of resistance, there was a gradual delaying of resistance (Table 1). Susceptibility in 2002, 2.9-fold greater with respect to the original susceptibility, had the lowest change with respect to the earlier 15th generation susceptibility, with a 2.4-fold increase in susceptibility with respect to the original generation.

There are two major mechanisms conferring resistance to OPs and pyrethroids: target-site insensitivity and detoxification (Zalom *et al.*, 2005). OPs bind to and inhibit the activity of the synaptic enzyme acetylcholinesterase (AChE), resulting in disruption of the normal transmission of nervous impulses across the synapse. In resistant insects, insensitivity of the AChE to binding by OPs restores synaptic function even in the presence of OPs. Pyrethroids bind to sites on the sodium channel and disrupt the transmission of impulses along the nervous axon by holding the channels in an open position (Bradbury and Coats, 1989). Cross-resistance occurs in an insect when a resistance mechanism selected in response to exposure to one insecticide also confers re-

**Table 1**

LC<sub>50</sub> responses of silverleaf whitefly free of insecticide selection pressure to four common insecticides in Baja California Sur between 1994-2002

Insecticide	Date	LC <sub>50</sub> (mg/ml)	Confidence limits (95%)	LC <sub>95</sub> (mg/ml)	Slope
Cypermethrin	21/04/94	123.9	9.3 – 252.4	361.5	1.88
	22/11/94	30.7	24.6 – 37.8	606.7	1.26
	14/12/94	28.3	10.4 – 76.4	640.2	1.21
	12/07/95	18.1	14.0 – 23.4	643.5	1.06
	13/07/95	17.1	13.7 – 21.2	268.2	1.37
	30/05/02	10.8	8.3 – 14.4	661.8	0.92
Endosulfan	23/03/94	198.6	166.0 – 231	910.9	2.48
	21/04/94	186.0	132.0 – 243.0	4080.9	1.22
	22/11/94	192.6	157.0 – 233.0	2000.3	1.61
	12/07/95	58.2	46.0 – 72.0	774.1	1.46
	13/07/95	51.9	41.0 – 65.0	988	1.29
	30/05/02	15.2	12.3 – 18.4	212.8	1.43
Methamidophos	22/11/94	237.8	206.0 – 271.0	1332.3	2.19
	13/12/94	93.6	79.0 – 109.0	435.0	2.46
	14/12/94	91.0	76.0 – 106.0	469.1	2.31
	12/07/95	75.6	60.0 – 91.0	630.2	1.78
	30/05/02	42.1	34.9 – 11.7	354.6	3.01
Methyl parathion	21/04/94	293.6	259.0 – 329.0	912.1	3.34
	22/11/94	224.2	154.0 – 325.0	950.0	2.19
	13/12/94	114.1	90.0 - 139.0	964.9	1.77
	14/12/94	105.9	86.0 – 127.0	1191.2	1.56
	14/07/95	118.9	99.0 – 141.0	838.5	1.93
	30/05/02	101.0	91.2 – 111.7	354.6	3.01

sistance to a second insecticide to which the insect has not been exposed. Target-site cross-resistance is very common and expected with insecticides of the same toxicological group. However, cross-resistance among different classes, having different resistance mechanisms, is viewed as a more serious problem (Zalom *et al.*, 2005). Based on the historical management of whiteflies in BCS and the complexity of our

results, it is likely that *B. argentifolii* in BCS has achieved resistance across more than one class of insecticides.

The results obtained in 2002 were used as a reference to determine estimates of resistance reached by whitefly in field populations. This scenario should be considered by decision makers in whitefly management.

**Susceptibility Level for Field (FP) Strains.**

Most of the whiteflies used to evaluate susceptibility were collected from cabbage crops with a regimen of up to three applications of insecticide per week (different combinations of parathion methyl, cypermethrin, endosulfan, and methamidophos during at least 5 years). In FP strains, cypermethrin was the most toxic product, with LC<sub>50</sub> values of 98.0, 55.0, and 103.1 µg/ml. The lowest

toxic insecticide was endosulfan, with LC<sub>50</sub> values of 823.3, 507, and 1034.6 µg/ml (Table 2). The most heterogeneous response to develop resistance was for endosulfan, with slope values of 1.24, 1.55, and 0.81, while the most homogeneous were for both OPs, but even higher for methamidophos with slope values of 3.43, 3.95, and 2.26 (Table 2).

**Table 2**

LC<sub>50</sub> responses of silverleaf whitefly field strains to four common insecticides in Baja California Sur between 1994-2002

Insecticide	Date	LC <sub>50</sub> (µg / ml)	Confidence limits (95%)	LC <sub>95</sub> (µg / ml)	Slope	RR <sup>a</sup>
Cypermethrin	06/01/94	98.0	78.4 – 119.0	878.4	1.72	9.07
	07/02/94	55.0	43.0 – 70.0	558.0	1.63	5.09
	08/03/96	103.1	77.4 – 134.8	3901.7	1.04	9.54
Endosulfan	06/01/94	823.3	39.5 – 1047.0	17230.9	1.24	54.1
	07/02/94	507	401.0 – 627.0	5802.3	1.55	33.3
	08/03/96	1034.6	734.0 - 1420	110088.1	0.81	68.0
Methamidophos	06/01/94	918.9	827.0 – 1014.0	2773.4	3.43	21.8
	07/02/94	673.2	431.0 – 1042.0	1754.4	3.95	15.9
	08/03/96	954.6	827.0 – 1112.0	5095.5	2.26	22.6
Methyl parathion	06/01/94	457.6	400.0 – 519.0	2005.5	2.56	4.5
	07/02/94	349.7	280.0 – 425.0	4523.0	1.48	3.4
	08/03/96	377.4	286.0 – 486.0	8170.3	1.23	3.7

To determine susceptibility level of FP strains, the resistance ratio was calculated by dividing the LC<sub>50</sub> of FP by the LC<sub>50</sub> of FRE. Table 2 shows that field strains in BCS were most tolerant to endosulfan, and in decreasing order, methamidophos, cypermethrin, and methyl parathion. The resistance ratios for these insecticides were: 68x, 22x, 9.5x, and 4.5x, in the order listed in the previous sentence. Considering the levels of resistance reported to this whitefly in other regions in the world, it is notable that the values measured

in BCS were significantly lower. According to these results, the studied FP can just be considered resistant to endosulfan and methamidophos. Dittrich *et al.* (1989), and Martínez-Carrillo (1998b) reported levels of resistance ratios in the order of 54x to methyl parathion, 29x to permethrin, and 400x to methamidophos. Gunning (1997) reported resistance ratios of 2000x for pyrethroids, 500x for OPs, 500x for carbamates, and 100x for endosulfan in Australia.

Endosulfan is widely used in Mexico, espe-

cially in the northwestern region, because it has a relative low cost and it is regarded as a "friendly to beneficial insects" insecticide by some pest control advisors (Stevensen, 1993). Given this belief, this insecticide is generally used without restriction. Methamidophos is one of the most common insecticides in region, regarded as a "wide-spectrum" insecticide. Lab studies indicate that pyrethroid and OP resistance in silverleaf whitefly were very rapidly selected by insecticides, and highly resistant populations can be generated in two generations (Gunning, 1997). Martínez-Carrillo (1998b) mentioned that silverleaf whitefly developed resistance against every insecticide that has been used for control. Further, selection for resistance to most insecticides is very rapid in the field (Gunning, 1997).

Cypermethrin is now a very common insecticide in Mexico; but, in the 1990s, pyrethroids were hardly used because of its higher cost compared to OPs and OCs. This could explain the lower resistance. Methyl parathion is widely used in Mexico, but, it was the insecticide to which whiteflies developed the lowest resistance. No robust explanation has been found to understand the basis for the low level of resistance, especially considering that methyl parathion is one of the most toxic insecticides (Reigart and Roberts, 1999; Waxman, 1998). Results summarized in Table 2 indicate that silverleaf whitefly has developed resistance to endosulfan and methamidophos, and the values of RR close to 9 showed for cypermethrin in 1994 and 1996 indicate a very probably cross-resistance between endosulfan and cypermethrin (Mueller-Beilschmidt, 2005). The showed results indicate that at the time of this study, *B. argentifolii* of BCS still had a low frequency of resistance genes. The relatively low level of resistance that we found on field populations can be explained by the limited area of crop production and the many wild plant hosts for whitefly in BCS (Servín-Villegas *et al.*, 2001), which are a reservoir of genetic suscepti-

bility. Production areas in this state are limited by irrigation water and for most of the year; there is no cultivation, so that many whitefly individuals are living on wild plants (Cano-Ríos *et al.*, 2001b, Servín-Villegas *et al.*, 1997). In spite of the low level of resistance to pesticides, care in the use of every insecticide is necessary to prevent an expected increase in resistance. Overuse of any insecticide against silverleaf whitefly should be avoided in this region. Implementation of every other alternative should be the foundation of whitefly control in BCS. The combination of biological control agents, low-environmental-impact insecticides, and entomopathogenic organisms may offer an efficient alternative that is highly preferable over the use of conventional insecticides.

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