Chapter 4.3

Insecticide Resistance in Colombia and Ecuador

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Farmer interviews conducted as part of extensive on-farm surveys for the diagnostic phase of the Tropical Whitefly Integrated Pest Management (TWF-IPM) Project (Rodríguez and Cardona, 2001; Chapter 4.1, this volume) showed that insecticide use against whiteflies in Colombia and Ecuador is excessive. In the tropical highlands and mid-altitude valleys, farmers spray their crops 5 to 6 times on average to control *Trialeurodes vaporariorum* (Westwood). The mean number of applications against the B biotype of *Bemisia tabaci* (Gennadius) in the tropical lowlands of Colombia and Ecuador was estimated at 6.5. Over-reliance on insecticides for whitefly control is so widespread that 30% of 325 farmers interviewed reported that they make more than 10 applications per cropping season. The frequency of applications in many cases is as high as two to three times per week. Most farmers complained about the limited control achieved by conventional insecticides and many farmers are now using novel insecticides such as buprofezin, pyriproxyfen, diafenthiuron and imidacloprid reportedly with better results. However, the 10 most widely used insecticides identified in the surveys comprised nine conventional products—dimethoate, carbofuran, chlorpyriphos, methamidophos, methomyl, profenofos, monocrotophos, cypermethrin and malathion—and only one of these novel insecticides, imidacloprid. The high toxicity of several of the conventional products in widespread use raises concerns over both human and environmental health, underlining the need for alternative approaches based on IPM.

Insecticide resistance in whiteflies is well documented as a widespread phenomenon in those countries in which monitoring of resistance has been conducted (Horowitz and Ishaaya, 1996). Recent reviews on the subject are those of Dittrich et al. (1990), Cahill et al. (1996b), Denholm et al. (1996) and Palumbo et al. (2001). In the Andean zone, Buitrago et al. (1994) detected high levels of resistance to cypermethrin and deltamethrin in populations of *T. vaporariorum* from the central highlands of Colombia. Resistance to organophosphates and carbamates was not as high, and ranged from low to moderate. Cardona et al. (1998; 2001) reported that the B biotype of *B. tabaci*, the main whitefly species in the lowlands of Colombia, was highly resistant to methamidophos and methomyl.

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A more general account of whitefly problems in the high Andes, mid-altitude valleys and lowlands of Colombia and Ecuador is presented elsewhere (Chapter 4.2, this volume), as are the results of biological surveys and farmer questionnaires, intended to characterize whitefly problems in these areas. Here, a summary is presented of findings on the resistance of whiteflies to conventional insecticides in the survey areas. Also included are baseline responses of *T. vaporariorum* adults to monocrotophos, lamda-cyhalothrin, bifenthrin, carbosulfan, carbofuran, thiamethoxam and imidacloprid. Baseline responses of *T. vaporariorum* nymphs to buprofezin, diafenthiuron and imidacloprid are included. The knowledge of whitefly problems synthesized in these studies is intended to provide a sound basis for pursuing IPM approaches in the Andean region of Latin America.

Methods and Materials

Experimental approach and locations

Field surveys and insecticide resistance testing were carried out between October 1997 and May 1999. In accordance with the common research methods agreed among the project partners, the vial technique was adopted to establish baseline data in the laboratory and for field assessment of resistance to conventional insecticides. The technique involves assessing the rate of mortality of a sample of whiteflies within a vial coated with a known concentration of insecticide (Plapp et al., 1990; Cahill and Hackett, 1992). Baseline data for imidacloprid were obtained following the methodology developed by Cahill et al. (1996a).

Laboratory work to establish baseline data and select diagnostic dosages was conducted at the headquarters of the Centro Internacional de Agricultura Tropical (CIAT) in Cali, Colombia. Average laboratory conditions were 24 °C and 75%-80% relative humidity (RH).

Field monitoring extended from northern Ecuador (Imbabura and Carchi provinces) to the north-eastern department of Guajira in Colombia. With the exception of coastal areas in Ecuador, all major areas affected by whiteflies were covered. Sites were chosen according to previous data on heavy insecticide use but care was taken not to perform tests in areas with mixed populations of whitefly species, as indicated in biological surveys carried out in parallel (Chapter 4.2, this volume). Thus, no insecticide resistance work was conducted in mid-altitude valleys where mixed populations of *B. tabaci* and *T. vaporariorum* had been recorded. In total, insecticide resistance was measured at 40 sites in Colombia and Ecuador. Between two and four insecticide resistance tests were conducted in each target area. Resistance of *T. vaporariorum* collected from common bean (*Phaseolus vulgaris* L.), tomato (*Lycopersicon esculentum* L.) and potato (*Solanum tuberosum* L.) was measured in highland areas along the Andean corridor from northern Ecuador to northern Colombia. Resistance of *B. tabaci* biotype B from tomato, eggplant (*Solanum melongena* L.), squash (*Cucurbita moschata* Duchesne) and cotton (*Gossypium hirsutum* L.) was measured in lowland areas of the region known as the “northern coast” of Colombia and in the Cauca Valley (a mid-altitude valley).

Baseline data and diagnostic doses

Adult whiteflies obtained from separate mass rearings of *T. vaporariorum* and *B. tabaci* biotype A, maintained at CIAT for over 10 years (without exposure to insecticides) were used to obtain baseline data. Technical grade insecticides were dissolved in acetone and 250 µL samples of the solution were pipetted into each 20-mL vial. The acetone was allowed to evaporate completely before introducing the test insects. Insecticides and dosages tested with *T. vaporariorum* were methamidophos (16.0, 8.0, 4.0, 2.0, 1.0 and 0.5 µg/vial), methomyl (2.5, 0.5, 0.1 and 0.02 µg/vial), cypermethrin (100.0, 50.0, 25.0, 12.5 and 6.25 µg/vial) and carbofuran (10.0, 3.3, 1.1, 0.33 and 0.11 µg/vial). Insecticides and dosages tested with the A biotype of *B. tabaci* were methomyl (25.0, 6.25, 1.56, 0.39 and 0.10 µg/vial), methamidophos (16.0, 4.0, 2.0, 1.0 and 0.5 µg/vial) and cypermethrin (450.0, 150.0, 50.0, 16.7 and 5.6 µg/vial). Five replicates of 20 insects were tested for each dosage. Acetone-coated vials were used as checks (controls). Mortality was recorded 6 hours after the adults were placed in the vials.

To generate baseline data for imidacloprid and thiamethoxam (Rodríguez et al., 2003), petioles of excised common bean leaves were immersed in aqueous solutions of commercial grade imidacloprid (10.0, 5.0, 2.5, 1.25, 0.625 and 0.312 ppm) for 48 hours to allow the leaves to take up the insecticide solution. Leaf discs were then cut and placed on agar in petri dishes, one leaf disk per petri dish (Cahill et al., 1996a) and infested with 20 *T. vaporariorum* adults per unit. Discs from leaves whose petioles had been immersed in distilled water were used as checks. Five replicates (units) per dosage were used. Mortality was recorded 6 hours after introduction of the adults into the units.

To generate baseline data with nymphs, we collected common bean leaves infested with pupae in each of the test sites. The infested material was then confined to cages. Adults emerging from these pupae were collected with an aspirator and introduced in clip cages attached to leaves of common bean seedlings. The adults (10 per clip cage) were allowed to oviposit for 24 hours. After hatching of the eggs, the area of the leaf infested with first instar nymphs was marked and the number of individuals thus obtained was recorded. Infested seedling leaves were then dipped for 5 s in 100 mL of the desired concentration of each of the insecticides tested. Formulated material was used in all tests and the desired concentrations were prepared by dilution with distilled water. Controls were dipped in distilled water. Treated plants were kept in a rearing chamber at 24 °C, 75%-80% RH and left undisturbed until adult emergence occurred. The number of surviving individuals in each treatment was recorded. Insecticides and dosages tested were buprofezin (100, 25, 6.2, 1.5, 0.3, 0.09 and 0.02 ppm), diafenthiuron (1000, 300, 100, 30, 10, 3, 1 and 0.3 ppm) and imidacloprid, (1000, 300, 100, 30, 10, 3 and 1 ppm). Four replicates were tested for each dosage.

Percentage mortality was corrected using the Abbott formula (Busvine, 1971). Tests in which check mortality was higher than 10% were not included in the analysis. Results were subjected to probit analysis (SAS Institute, 1988). Slopes, LC_{50} and LC_{90} values, as well as 95% confidence limits were tabulated. Baseline data were then used to choose diagnostic doses. Four doses were chosen empirically so as to obtain mortality ranging from 5% to 95%. After several tests, a single dose for each insect species and insecticide was chosen as the diagnostic dose (as defined by Halliday and Burnham, 1990) to be used in extensive monitoring of resistance under field conditions.

Field assessment of resistance

Resistance was assessed in the field using vials coated in the laboratory with diagnostic dosages of methomyl, methamidophos and cypermethrin, and transported to the field in ice-chests maintained at about 20 °C. These insecticides are representative of the conventional insecticides most widely used in the study area. At each site, adult whiteflies were collected with a mouth aspirator from the foliage of infested plants and transferred to the vials. Twenty adults per vial, replicated five times, were used for each test. Acetone-coated vials were used as controls. Infested vials were kept in the ice-chests for 6 hours before mortality was recorded. Any test in which mortality in control vials was higher than 10% was discarded. Percentage mortality was corrected using the Abbott formula (Busvine, 1971) and transformed to arcsine square root of proportion. Data were then analysed by a 1-way analysis of variance (ANOVA) (SAS Institute, 1988). When the *F* test was significant, comparison of means was by the least significance difference (LSD). Untransformed means are presented.

Results and Discussion

Baseline data and diagnostic doses

Table 1 presents baseline toxicology data for reference strains of *T. vaporariorum* and Table 2, the A biotype of *B. tabaci*. The LC_{50} and LC_{90} values reflect toxicities of the test

Table 1. Toxicological responses of laboratory strains of *Trialeurodes vaporariorum* to five insecticides.[a]

Insecticide	n	LC_{50} (95% FL)[b]	LC_{90} (95% FL)[b]	Slope ± SEM	X^2
Adults					
Methomyl	457	0.25 (0.15-2.6)	0.95 (0.76-14.7)	2.19 ± 0.55	4.05
Methamidophos	600	5.30 (2.5-7.6)	22.50 (15.6-48.7)	2.05 ± 0.49	0.08
Monocrotophos	710	9.70 (6.7-13.4)	175.40 (115.5-299.8)	1.00 ± 0.08	4.80
Cypermethrin	480	37.00 (22.0-55.7)	400.00 (232.7-953.5)	1.24 ± 0.18	2.95
Lambda-cyhalothrin	605	9.40 (6.1-13.5)	264.90 (170.9-455.3)	0.90 ± 0.06	0.10
Bifenthrin	380	2.40 (1.6-3.1)	6.70 (5.2-9.8)	2.90 ± 0.49	0.90
Carbofuran	504	1.97 (1.5-2.5)	6.80 (5.4-9.7)	2.37 ± 0.31	4.31
Carbosulfan	712	1.80 (1.5-2.1)	19.90 (16.3-24.9)	1.20 ± 0.05	3.30
Imidacloprid	921	5.70 (4.6-6.9)	28.40 (20.9-44.9)	1.90 ± 0.22	7.80
Thiamethoxam	670	8.60 (6.0-11.7)	101.00 (68.8-170.4)	1.20 ± 0.12	5.90
First instar nymphs					
Buprofezin	907	0.80 (0.6-1.1)	9.20 (6.9-13.0)	1.20 ± 0.09	7.30
Diafenthiuron	904	3.20 (2.3-4.4)	60.10 (41.8-92.8)	1.00 ± 0.06	10.50
Imidacloprid	483	16.50 (10.7-23.4)	171.50 (115.6-290.9)	1.21 ± 0.10	3.60

a. Conventional insecticides were tested using insecticide-coated glass vials. Imidacloprid tests were conducted using the technique developed by Cahill et al. (1996a). Nymphs were tested using a modification of the methodology described by Prabhaker et al. (1985).
b. Imidacloprid, thiamethoxam, buprofezin and diafenthiuron concentrations in ppm. All others in µg/vial. LC, lethal concentration; FL, fiducial limits.

Table 2. Toxicological responses of laboratory strains of *Bemisia tabaci* biotype A to three insecticides, using insecticide-coated glass vials.

Insecticide	n	LC_{50} (95% FL)[a]	LC_{90} (95% FL)[a]	Slope ± SEM	X^2
Methomyl	500	1.7 (1.1-2.3)	9.1 (6.7-13.7)	1.76 ± 0.21	1.73
Methamidophos	517	1.4 (0.9-1.6)	6.6 (5.3-14.7)	1.86 ± 0.47	14.89
Cypermethrin	502	14.4 (5.8-27.2)	202.9 (122.5-352.4)	1.12 ± 0.14	3.27

a. Insecticide concentration: µg/vial. LC, lethal concentration; FL, fiducial limits.

insecticides to susceptible strains of whiteflies that have not been exposed to insecticides for at least 10 years. As discussed by ffrench-Constant and Roush (1990), establishing baseline data for different insecticides is a fundamental step in resistance studies because these data will serve as a basis for future comparisons, which will allow researchers to detect any changes in insecticide resistance levels. In addition, as explained by Sanderson and Roush (1992) and Denholm et al. (1996), calculation of baseline data permits the selection of diagnostic doses, which can be used more conveniently in extensive efforts to detect and monitor resistance such as the one reported here that was carried out over numerous sites in the Andean zone of Ecuador and Colombia. Carbofuran was included because granular formulations of this insecticide are still effective against *T. vaporariorum* in Colombia (Cardona, 1995). Imidacloprid is a highly efficient insecticide that is becoming popular in the region. Careful monitoring of the efficiency of this product will be needed for future resistance management and IPM work to be undertaken by the second phase of the TWF-IPM Project. It is interesting to note that the LC_{50} value for imidacloprid for our reference strain of *T. vaporariorum* (2.4 ppm) was similar to that obtained by Cahill et al. (1996a) using susceptible strains of *B. tabaci* from Sudan and Pakistan.

The main purpose of the preliminary tests was to select diagnostic doses for methomyl, methamidophos and cypermethrin (Table 3) that were then used to compare the insecticide resistance of test samples of whiteflies from Colombia and Ecuador with that of the fully susceptible laboratory strains. We did not calculate diagnostic doses by statistical means. Instead, diagnostic doses were chosen empirically to produce >95% mortality.

Field monitoring of resistance in biotype B of B. tabaci

Responses to insecticides in our studies were arbitrarily classified as follows on the basis of mean percentage mortality:

- 0%-50% mortality: high resistance;
- 50%-80% mortality: intermediate resistance; and
- 80% mortality: low resistance.

According to this scale, the B biotype of *B. tabaci* recently introduced

Table 3. Response (corrected percentage mortality) of *Trialeurodes vaporariorum* and *Bemisia tabaci* adults to three insecticides.[a]

Whitefly species	Methomyl (2.5 µg/vial)	Methamidophos (32 µg/vial)	Cypermethrin (500 µg/vial)
T. vaporariorum	97	99	88
B. tabaci biotype A	99	100	98

a. Diagnostic dosages were tested using insecticide-coated glass vials.

into Colombia showed high levels of resistance to methomyl and methamidophos and, in some places, moderate levels of resistance to cypermethrin (Table 4). Considerable inter-population variation was detected. Variability in the response of the B biotype to insecticides has been detected also in Arizona (Sivasupramaniam et al., 1997), in Hawaii (Omer et al., 1993) and in California (Prabhaker et al., 1996). In general, insecticide resistance levels reflected insecticide use patterns. For example, the very high levels of resistance to acetylcholine esterase inhibitors (exemplified here by methomyl and methamidophos) in different sites in the departments of Atlántico, Córdoba and Sucre may reflect the extensive use of organophosphates on cotton, tomato and vegetables in the region. Lower levels of resistance to the test pyrethroid (cypermethrin) may be due to the less frequent use of this type of insecticide for whitefly control (Table 4 in Chapter 4.2, this volume). However, further interpretation of the data is hampered by the lack of knowledge of exactly where the B biotype whitefly that invaded Colombia originated and of the pattern of insecticide use in the area of origin. It is likely that the frequency of insecticide resistance was already high in the pest population at the time it was introduced.

Field monitoring of resistance in **T. vaporariorum**

The responses of *T. vaporariorum* to insecticides were arbitrarily classified on the same basis as those of *B. tabaci* (0%-50% mortality, high resistance; 50%-80% mortality, intermediate resistance; and > 80% mortality, low resistance). In the northern Ecuador-southern Colombia region (Table 5), *T. vaporariorum* populations were susceptible to methomyl in seven out of eight sites tested. Generalized resistance to methamidophos (the most widely used insecticide) was found. Extreme cases were those in northern Ecuador (Turquisal, Ibarra) and in southern Colombia (El Tambo, Gualmatán, Funes). Intermediate to low resistance to cypermethrin was detected in west-central Colombia (Table 6).

Table 4. Response (corrected percentage mortality) of *Bemisia tabaci* biotype B adults to three insecticides at sites on tropical lowland areas of the northern coast of Colombia.[a]

Site (department)[b]	Methomyl (2.5 µg/vial)	Methamidophos (32 µg/vial)	Cypermethrin (500 µg/vial)
Repelón (Atlántico)	42.8 c	2.0 d	92.0 ab
Cereté 1 (Córdoba)	54.0 b	1.5 d	87.8 abc
Cereté 2 (Córdoba)	12.4 de	0.8 d	68.8 c
Ciénaga de Oro (Córdoba)	41.8 bcd	3.0 d	94.6 a
Cotorra (Córdoba)	6.8 e	4.2 d	78.2 bc
Montería (Córdoba)	5.4 e	2.4 d	87.2 abc
Manaure (Guajira)	100.0 a	25.4 b	91.5 ab
Corozal 1 (Sucre)	9.8 e	14.0 bc	85.4 abc
Corozal 2 (Sucre)	30.0 bcde	4.8 cd	65.2 c
Sampués (Sucre)	15.4 cde	16.2 bc	88.8 abc
"CIAT" (reference strain)	100.0 a	100.0 a	100.0 a

a. Diagnostic dosages were tested under field conditions using insecticide-coated glass vials. Twenty adults per vial replicated five times were used for each test. Means within a column followed by the same letter are not significantly different at the 5% level (LSD test).
b. "CIAT" indicates a laboratory population of *B. tabaci* biotype A maintained for at least 10 years without exposure to insecticides.

Table 5. Response (corrected percentage mortality) of *Trialeurodes vaporariorum* adults to three insecticides in the southern Colombia-northern Ecuador tropical highland region.[a]

Site (country)[b]	Methomyl (2.5 µg/vial)	Methamidophos (32 µg/vial)	Cypermethrin (500 µg/vial)
El Tambo (Colombia)	100.0 a	1.8 d	95.2 a
Gualmatán (Colombia)	100.0 a	1.8 d	35.2 c
Funes (Colombia)	84.2 a	9.0 d	78.0 b
Ibarra (Ecuador)	100.0 a	4.0 d	100.0 a
Pimampiro (Ecuador)	87.4 a	46.4 bc	100.0 a
San Vicente (Ecuador)	55.6 b	59.0 b	100.0 a
Turquisal (Ecuador)	100.0 a	14.0 cd	100.0 a
"CIAT" (reference strain)	96.6 a	99.0 a	87.7 b

a. Diagnostic dosages were tested under field conditions using insecticide-coated glass vials. Twenty adults per vial replicated five times were used for each test. Means within a column followed by the same letter are not significantly different at the 5% level (LSD test).
b. "CIAT" indicates a laboratory population of whiteflies of the same species as the test populations maintained for at least 10 years without exposure to insecticides and used here as a susceptible check for toxicological studies.

Table 6. Response (corrected percentage mortality) of *Trialeurodes vaporariorum* adults to three insecticides at sites in the Antioquia-Cauca-Valle tropical highland region of west-central Colombia.[a]

Site (department)[b]	Methomyl (2.5 µg/vial)	Methamidophos (32 µg/vial)	Cypermethrin (500 µg/vial)
Carmen de Viboral (Antioquia)	96.0 a	55.0 c	69.8 d
El Peñol (Antioquia)	90.0 ab	76.4 b	80.2 cd
Rionegro (Antioquia)	100.0 a	43.4 c	84.2 bcd
La Unión (Cauca)	100.0 a	99.0 a	98.2 a
Pescador (Cauca)	62.0 c	2.6 e	67.0 d
Cerrito (Valle)	100.0 a	8.2 e	89.0 abc
La Cumbre (Valle)	100.0 a	100.0 a	98.0 a
Pradera (Valle)	79.4 bc	2.8 e	64.8 d
Tenerife (Valle)	99.6 a	3.0 e	72.4 cd
"CIAT" (reference strain)	96.6 a	99.0 a	87.8 bcd

a. Diagnostic dosages were tested under field conditions using insecticide-coated glass vials. Twenty adults per vial replicated five times were used for each test. Means within a column followed by the same letter are not significantly different at the 5% level (LSD test).
b. "CIAT" indicates a laboratory population of whiteflies of the same species as the test populations maintained for at least 10 years without exposure to insecticides and used here as a susceptible check for toxicological studies.

Whiteflies were susceptible to methomyl in all but two of the sites studied. Extreme resistance to methamidophos occurred in the Cauca Valley (Cerrito, Pradera, Tenerife) and in Cauca (Pescador). These are places where insecticide use on common bean and tomato is heaviest. Again, low to intermediate resistance to cypermethrin was detected. In the eastern mountain ranges of Colombia (Table 7), *T. vaporariorum* was susceptible to methomyl and less resistant to methamidophos than in other parts of the country, possibly because of lower insecticide use in that area.

Table 7. Response (corrected percentage mortality) of *Trialeurodes vaporariorum* adults to three insecticides at sites in the central-eastern tropical highland region of Colombia.[a]

Site (department)[b]	Methomyl (2.5 µg/vial)	Methamidophos[c] (32 µg/vial)	Cypermethrin (500 µg/vial)
Boyacá (Boyacá)	100.0 a	95.3 a	89.0 a
Tinjacá (Boyacá)	100.0 a	85.6 ab	75.2 abc
Bojacá (Cundinamarca)	100.0 a	40.0 c	57.9 cd
Pasca (Cundinamarca)	100.0 a	-	81.0 ab
Fosca (Cundinamarca)	100.0 a	71.6 bc	41.4 d
Garzón (Huila)	100.0 a	52.2 bc	88.8 a
Rivera (Huila)	100.0 a	40.0 c	88.7 a
Abrego (Norte de Santander)	98.4 a	60.3 bc	71.3 abc
Lebrija (Santander)	91.5 b	3.0 d	64.4 bc
"CIAT" (reference strain)	96.6 a	98.8 a	87.8 a

a. Diagnostic dosages were tested under field conditions using insecticide-coated glass vials. Twenty adults per vial replicated five times were used for each test. Means within a column followed by the same letter are not significantly different at the 5% level (LSD test).
b. "CIAT" indicates a laboratory population of whiteflies of the same species as the test populations maintained for at least 10 years without exposure to insecticides and used here as a susceptible check for toxicological studies.
c. Test was not conducted at Pasca location.

Conclusions

In summary, the B biotype of *B. tabaci*, recently introduced into Colombia, showed high levels of resistance to methomyl and methamidophos and, in some places, moderate levels of resistance to cypermethrin. In highland and mid-altitude areas of Colombia and Ecuador, *T. vaporariorum* showed high resistance to methamidophos, intermediate resistance to cypermethrin and low resistance to methomyl. In general, levels of insecticide resistance in whiteflies seem to be related to the intensity of use of the particular kind of insecticide. Baseline responses of *T. vaporariorum* to carbofuran and imidacloprid are presented.

These results provide a basis for future monitoring of resistance and for the development of insecticide resistance management strategies. Novel insecticides such as diafenthiuron, buprofezin, pyriproxyfen and imidacloprid are still effective for whitefly control in Colombia and Ecuador (Rodríguez et al., 2003). Because whiteflies can also develop resistance to novel insecticides (Denholm et al., 1996; Horowitz and Ishaaya, 1996; Elbert and Nauen, 2000; Palumbo et al., 2001) monitoring of resistance and development of baseline data should continue in order to facilitate management of whitefly populations. The results are further discussed and proposals for further action based on these results are presented in Chapter 4.4 (this volume).

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