Assessment of Insecticide Resistance in Five Insect Pests Attacking Field and Vegetable Crops in Nicaragua

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J. Econ. Entomol. 93(6): 1779–1787 (2000)

ABSTRACT Field populations of *Hypothenemus hampei* (Ferrari), *Plutella xylostella* (L.), *Spodoptera exigua* (Hübner), *Helicoverpa zea* (Boddie), and *Bemisia tabaci* (Gennadius) were tested for resistance to several insecticides commonly used in Nicaragua. Assays were conducted to estimate the LD_{50}s or LC_{50}s and the corresponding resistance ratios. A diagnostic concentration was used to discriminate between susceptible and resistant strains of *H. hampei*. The tests with >6,000 *H. hampei* adults collected from six different sites indicate the absence of resistance to endosulfan. Resistance to cypermethrin, deltamethrin, chlorfluazuron, thiocyclam, and methamidophos was documented in six field populations of *P. xylostella*. High levels of resistance to cypermethrin and deltamethrin, but moderate levels of resistance to chlorpyriphos and methomyl, were also documented in two field populations of *S. exigua*. Moderate levels of resistance to cypermethrin, deltamethrin and chlorpyriphos were also documented in three field populations of *H. zea*. Moderate to high levels of resistance to bifenthrin, methamidophos and endosulfan were documented in four field populations of *B. tabaci*. The presence of significant correlations between LD_{50}s or LC_{50}s suggests the occurrence of cross-resistance or simultaneous selection for resistance by different insecticides with different modes of action. Our data could not differentiate between these two possibilities. Because insecticides will continue being used in Nicaragua, a resistance management program is urgently needed. The implementation of integrated pest management tactics must be accompanied by specific regulations for pesticide registration. In the future, pesticide registration regulations in Nicaragua should include periodic resistance monitoring. The mechanisms to cover the costs of resistance monitoring and resistance management should also be established.

KEY WORDS resistance, synthetic insecticides, Nicaragua

AGRICULTURE HAS BEEN the most important economic activity in Nicaragua. Since the introduction of cotton (*Gossypium hirsutum* L.) as a major export crop in the 1950s, pest management in cotton has primarily relied on synthetic insecticides. Insecticide use against cotton pests became so intense in the 1960s that Nicaragua and other Central American countries became a testing ground for many new insecticide products (Swezey et al. 1986).

Intense use of insecticides has resulted in the evolution of resistance in >440 insect species and mites worldwide (Roush and Tabashnik 1990). This inventory includes several insect species that occur in Nicaragua as pests of several important agricultural crops. For the foreseeable future, insecticides will continue to be heavily used for pest management in Nicaragua in a wide array of crops, despite the efforts undertaken by several national and international organizations to reduce insecticide use.

Efforts to document resistance in Nicaragua and other Central American countries have been scarce but in 1994 the government of Nicaragua created the Pesticide Management Program (PMP) within the Ministry of the Environment and Natural Resources. The main objectives of the PMP were to formulate scientific policies for pesticide registration and use in the country, conduct studies to determine the environmental effects of past insecticide use, and monitor resistance to insecticides in insect pests of economic importance.

In 1996, the PMP called on four institutions to conduct a study aimed at monitoring resistance to insecticides in selected insect species. The institutions participating in this study were Cornell University, Ithaca, NY; the Panamerican School of Agriculture (Zamorano) from Tegucigalpa, Honduras; The National Agriculture University, from Managua, Nicaragua; and the National Autonomous University of Nicaragua in León.

This study focused on monitoring insecticide resistance in five insect species: *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), *Spodoptera exigua*

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0022-0493/00/1779–1787$02.00/0

(Hübner) (Lepidoptera: Noctuidae), *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), and *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae). These insect species are key pests in export and food crops, and are subject to intense selection by insecticides during a single crop season. The damage and brief history of their evolution of resistance to pesticides in Nicaragua, or elsewhere, is discussed below.

The coffee berry borer, *H. hampei*, is a key pest of coffee, currently the most important export crop in Nicaragua, and the standard control strategy is two or more applications of endosulfan per year. Resistance of *H. hampei* to endosulfan has not been documented in Nicaragua; however, studies conducted with populations from New Caledonia revealed the presence of endosulfan resistant *H. hampei* (Brun et al. 1989, ffrench-Constant and Brun 1994). Other studies conducted by Kern et al. (1989) with *H. hampei* populations from Guatemala, Brazil, Cameroon, and Philippines failed to document resistance to this insecticide.

The diamondback moth, *P. xylostella*, is the most important insect pest of crucifers in Nicaragua and other Central American countries. Farmers rely almost exclusively on synthetic or microbial insecticides to control *P. xylostella* and, in some years, cabbage growers have applied as many as 15–20 sprays either singly or tank mixed during the crop cycle. This pattern of pesticide use against *P. xylostella* has prompted the evolution of resistance to >46 commercial formulations of insecticide worldwide, including the microbial insecticide *Bacillus thuringiensis* Berliner (Shelton et al. 1993, Sun et al. 1986, Tabashnik et al. 1990). Studies conducted in Honduras (Ovalle and Cave 1989) documented resistance to methomyl, methamidophos and cypermethrin in local populations of *P. xylostella.* Other studies documented resistance to *B. thuringiensis* subsp. *kurstaki* in populations of *P. xylostella* from Honduras, Nicaragua, and Guatemala (Perez and Shelton 1997).

The beet armyworm, *S. exigua*, is a key pest of tomato, cotton, bean, soybean, and onions in Nicaragua. As with the other insect pests mentioned above, insecticides are the standard method of control. Insecticide resistance in *S. exigua* has not been documented in Nicaragua; however, >100-fold resistance to deltamethrin was documented in an *S. exigua* population collected from cotton fields in Guatemala (Delorme et al. 1988). Although it appears that deltamethrin has not been used against *S. exigua* in Nicaragua, this pest has been exposed to pyrethroid applications directed against other pests occurring simultaneously (e.g., *Helicoverpa zea*). Studies conducted elsewhere indicate the potential of *S. exigua* to evolve resistance to pyrethroids and methomyl in California (Brewer and Trumble 1989; Brewer et al. 1990). Resistance to the crystal protein CryIC, an endotoxin produced by *B. thuringiensis* subsp. *aizawaii*, has also been documented after seven generations of selection in the laboratory (Moar et al. 1995).

The cotton bollworm, *H. zea*, is one of the most economically important insect pests of cotton in Nicaragua, although the importance of this crop has declined drastically. It is also a key pest in other crops such as tomato, soybean, bean, corn, sorghum, and sesame. In all cases, farmers rely on frequent insecticide applications for its control. The use of insecticides, especially pyrethroids and organophosphates for *H. zea* control, has been more intensive in cotton and vegetables where up to 12 applications have been applied per crop. *H. zea* resistance to methyl parathion and endrin has been documented in Nicaraguan populations (Wolfenbarger et al. 1973), and resistance to methyl parathion increased 10-fold from 1970 to 1972 (Wolfenbarger et al. 1981).

Bemisia tabaci is a key pest of melons, other cucurbit crops, cotton, tobacco, pepper, tomato, bean, and other crops in Nicaragua. The economic importance of whitefly as a pest of export crops and vegetables in Central America seems to expand continually (Monge 1993) and farmers rely exclusively on insecticide applications for its control. At least 10 different insecticide formulations, including five organophosphates, carbamates, pyrethroids, and insect growth regulators, have been used against *B. tabaci* in Nicaragua. Dittrich et al. (1990) documented *B. tabaci* resistance to common pesticides including organophosphates, carbamates, and pyrethroids in populations collected from cotton fields in Guatemala and Nicaragua. In the same study, it was determined that the Nicaraguan strain contained highly active nonspecific esterases as well as enhanced mixed-function oxidase activity, and these were important detoxification mechanisms conferring resistance to at least three insecticides. Studies conducted by Byrne and Devonshire (1993) demonstrate that *B. tabaci* populations collected in Nicaragua carry an insensitive acetylcholinesterase known to confer strong carbamate and organophosphate resistance. It is not known which biotype of *B. tabaci* prevails in Nicaragua, but there is evidence that the B-biotype of *B. tabaci* is present throughout Central America (Brown et al. 1995).

Materials and Methods

Hypothenemus hampei. Infested coffee berries, 671–7584 per site, were collected from six representative coffee growing regions: Yasica Norte and San Ramón (Department of Matagalpa), Masatepe and San Marcos (Department of Carazo), Crucero (Department of Managua), and Aranjuez (Department of Jinotega). After collection the infested berries were kept separately in wooden boxes 40 by 35 by 30 cm (L × H × D) which contained nine 30-ml glass vials screwed into the rear of the box. The glass vials were screwed into their caps but the caps had a 1.5-cm opening. Upon hatching, the adults of *H. hampei* crawled through the cap and into the vials where they were held until tested. Only the females collected in a single day were tested.

We used a bioassay procedure previously described and used by Brun et al. (1991) for endosulfan resistance monitoring in *H. hampei.* The method consists of a Plexiglas kit made of three superimposed sheets. The sheet in the middle contains 15 holes, ≈15 mm diam-

eter. A filter paper is placed between first and second Plexiglas sheets, and the third sheet serves as a cover. Two diagnostic concentrations, 0.2 and 0.4 mg [AI]/ml, of endosulfan were diluted in distilled water. A water-only solution was also prepared for the bioassays. The filter paper was soaked with 0.6 ml of one of the three concentrations (0, 0.2, or 0.4 mg [AI]/ml) by using a micropipet. After the application of the treatments, the filter paper was allowed to air-dry for a period of 1 h, after which, it was placed on top of the first Plexiglas sheet, then the 15-holes sheet was placed on top of the filter paper. Then, 10–15 female adults of *H. hampei* were placed in each hole. The insects were exposed to the endosulfan residues for a period of 6 h at 26°C, 70% RH, and a photoperiod of 12:12 (L:D) h before assessing mortality. A single bioassay consisted of two endosulfan concentrations and a control, 15 replicates per concentration, and 10–15 insects per replicate for a total of 450–675 individuals per bioassay. Except in one case, all bioassays were replicated twice. A *H. hampei* beetle was considered dead if it did not move when prodded.

Plutella xylostella. Insects were collected from cabbage (*Brassica olearacea* variety *capitata*) fields at six localities: La Concha (Department of Masaya), Tisey and La Laguna (Department of Esteli), Las Brisas and Chagüitillo (Department of Matagalpa), and Jinotega (Department of Jinotega). At least 200 larvae of *P. xylostella* were collected from each field. The larvae were brought to the laboratory at the National Agricultural University and reared separately on rapeseed (*Brassica napus*) seedlings. An insecticide-susceptible population of *P. xylsotella*, Geneva 88 (Shelton et al. 1993), was also reared at the same laboratory for comparisons. For *P. xylostella*, and the remaining species, the number of generations over which the insects were reared in the laboratory before bioassay is given in the tables.

We tested several commercial formulations of insecticides commonly used in the field against *P. xylostella.* At least six concentrations were prepared from each of the following insecticides: cypermethrin (Cymbush 25 EC, ICI, PLC, England), deltamethrin (Decis 2.5 EC, Roussel UCLAF, Paris, France), methamidophos (Metamidofos 60 SL, Servicio Agrícola Gurdián S.A., León, Nicaragua), thiocyclam (Evisect 50 SP, Sandoz, Basel, Switzerland), and chlorfluazuron (Jupiter, Ciba Geigy, Basel, Switzerland). In all cases, the insecticides were diluted in distilled water before the tests. A solution containing distilled water was also used as control. A spreader-sticker (Bond, Loveland Industries) was added to each solution at a concentration of 0.02% (vol:vol).

The susceptibility of *P. xylostella* to these insecticides was evaluated by using a bioassay technique similar to that described by Shelton et al. (1993). Leaf disks (32 mm diameter) were obtained from the cabbage variety 'Superette'. Five leaf disks were dipped individually for 10 s in each insecticide solution, air-dried for 2 h, then individually placed in 30-ml plastic cups (Solo, Solo Cup Co., Urbana, IL). Five larvae (second instar) were placed in each cup and allowed to feed for a period of 24 h for treatments with cypermethrin, deltamethrin, thiocyclam, and methamidophos, and 72 h for treatments with chlorfluazuron. Larvae surviving the chlorfluazuron treatment for 72 h were then transferred to untreated fresh leaf disks for an additional period of 48 h. During the tests, the larvae were allowed to feed at 27°C, 50% RH, and a photoperiod of 12:12 (L:D) h. A single bioassay was composed of at least six concentrations, five replicates, and 25 larvae per concentration. All bioassays were replicated at least twice. A larva was considered dead if it did not move when prodded.

Spodoptera exigua and *H. zea.* Larvae of *S. exigua* were collected from onion fields at two localities, Sébaco and Darío, both in the Department of Matagalpa. Larvae were brought to the laboratory at the National Autonomous University in León and reared on artificial diet. A colony of *S. exigua* from the University of California at Riverside was also reared and used as a standard susceptible population. Larvae of *H. zea* were collected from irrigated cornfields from two localities (Telica and Nagarote, Department of León), and from a tomato crop near the city of Nandaime (Department of Granada). Larvae of *H. zea* were brought to the laboratory at the National Autonomous Univesity in León and reared on artificial diet. A colony from the Department of Entomology of the New York State Agricultural Experiment Station in Geneva, NY, was also established in the laboratory and used as a standard susceptible population.

The topical application technique was used to evaluate the susceptibility of *S. exigua* and *H. zea* to synthetic insecticides (Anonymous 1970). Six to eight solutions of each of the following insecticides were prepared from technical material using acetone (98% technical grade): cypermethrin (92% [AI], Zeneca Ag Products, Wilmington, DE), deltamethrin (99% [AI], Roussel UCLAF, Lyon, France), chlorpyriphos (96.8% [AI], Dow, Indiannapolis, IN), and methomyl (98.9% [AI], DuPont, Wilmington, DE). Larvae of *S. exigua* and *H. zea* were exposed to deltamethrin, cypermethrin, and chlorpyriphos. Only *S. exigua* was exposed to methomyl.

The larval weight was recorded from a sample of 60 larvae before insecticide exposure. The average body weight ($\pm$SD; $n = 60$) for third-instar *S. exigua* and *H. zea* was 18.2 $\pm$ 1.6 mg and 28.1 $\pm$ 2 mg, respectively. For each bioassay, a 1-μl droplet of each insecticide solution was applied on the dorsal side of the second thoracic segment of each larva by using a microsyringe equipped with a repetitive dispenser. Each insecticide dose was applied to three groups of 10 larvae each, for a total of 30 larvae per dose. Usually, six to seven doses were used as treatments. A single group of 30 larvae was treated with acetone alone as a control. Each bioassay was repeated at least twice on two different days. After insecticide exposure the larvae were allowed to feed on artificial diet at 27°C, 50% RH, and a photoperiod of 12:12 (L:D) h for a period of 48 h, after which mortality was recorded. A larva was considered dead when it did not move when prodded. We conducted >50 bioassays with both species.

Table 1. Response of six populations of *H. hampei* to two diagnostic concentrations of endosulfan

Location	*n*	Mortality, %		
		Controls	0.2 mg (AI)/ml	0.4 mg (AI)/ml
Yasica Norte	1,350	0	98.9	99.6
Masatepe	1,350	0	97.8	100
Crucero	1,125	0	99.1	100
San Marcos (S. Dionisio)	900	0	99.7	100
San Ramón	1,350	0	99.3	99.5
Aranjuez	450	0	100	100

Bemisia tabaci. Nymphs of *B. tabaci* were collected from tomato and cucurbit fields at four localities. In two of the four localities, Nandaime (Department of Granada) and Sébaco (Department of Matagalpa), *B. tabaci* were collected from tomato fields. In El Viejo (Department of Chinandega), nymphs of *B. tabaci* were collected from a watermelon field (*Citrullus lanatus*), and in León (Department of León), nymphs were collected from a cucurbit field (*Cucurbita pepo* variety *ovifera* L.). Four populations of *B. tabaci* were brought to the Department of Plant Protection at the Panamerican School of Agriculture in Honduras and then reared on cotton seedlings (*G. hirsutum* variety 'Deltapine 20'). A susceptible population of *B. tabaci* from the Agricultural Experiment Station of Rothamstead, England, was also established in the laboratory for further comparisons.

We used a modified yellow sticky card bioassay method similar to that described by Prabhaker et al. (1992) and Sanderson and Roush (1992). In this study a single nozzle electrostatic sprayer (Electrostatic Spraying Systems, Watkinsville, GA) was used to treat the yellow sticky cards with one of several concentrations of commercial formulations of each of three insecticides, bifenthrin (Talstar, FMC, Philadelphia, PA), methamidophos (Tamaron 600) and endosulfan (Thiodan 35 EC, Hoechst, Berlin, Germany), diluted in distilled water.

Data Analyses. The concentration or dose-mortality relationship was estimated assuming a probit model by using POLO (LeOra Software 1987). Except for mortality data from the experiments with *H. hampei* (see below), the median lethal dose or concentration (LD_{50} for *S. exigua* and *H. zea*; LC_{50} for *P. xylostella* and *B. tabaci*) and the corresponding 95% fiducial limits (FL) were estimated for each insecticide and population. The responses of two populations within a given insect species were considered different if the corresponding 95% FL did not overlap. The resistance ratios (RR) were also calculated by dividing the LD_{50} or LC_{50} of a field population of a given insect species by the corresponding LD_{50} or LC_{50} of the susceptible strain. The Pearson correlation (SYSTAT 1992) was used to examine possible cases of cross-resistance or multiple-resistance between insecticides tested on a single insect species. The LD_{50}s and LC_{50}s were transformed to the $\log_{10}$ equivalent before correlation analysis. For mortality data of the experiments with *H. hampei*, the chi-square test for significant deviations between the observed and the expected mortality was used. Deviations associated with chi-square values > 3.84 ($df = 1$; $P < 0.05$) were considered significant.

Results

Hypothenemus hampei. According to Brun et al. (1991), the expected mortality at 0.4 mg [AI]/ml is 99.8% in an endosulfan susceptible population of *H. hampei.* In this study, mortality of *H. hampei* was always ≥99.5% (Table 1), and the deviations from the expected mortality were not significant ($\chi^2 < 3.84$, $P > 0.05$). Therefore, our results do not provide evidence of resistance to endosulfan in *H. hampei* from Nicaragua.

Plutella xylostella. The LC_{50}s of cypermethrin, deltamethrin, clorfluazuron, thiocyclam, and methami-

Table 2. Concentration-mortality response of *P. xylostella* from Nicaragua to commonly used insecticides

Population	*n*	Slope ± SE	LC_{50}[a]	95% FL[b]	RR[c]
Cypermethrin					
Geneva 88 G_{179}[d]	300	2.29 ± 0.38	0.63	(0.2–1.9)	1
La Concha G_6	300	1.06 ± 0.12	6.4	(3.6–10.3)	10
Sébaco 2 G_2	300	0.66 ± 0.08	24.7	(11.4–51.9)	39
Tisey G_3	445	1.64 ± 0.17	75.0	(40.0–140.0)	119
Sébaco 1 G_4	300	1.07 ± 0.11	81.3	(31.2–273.2)	129
Jinotega G_1	175	1.86 ± 0.49	146.0	(36.1–266.1)	232
La Laguna G_6	300	1.05 ± 0.10	76.0	(45.0–135.0)	121
Deltamethrin					
Geneva 88 G_{183}	750	0.67 ± 0.06	0.91*	(0.54–1.46)	1
Tisey G_6	350	1.25 ± 0.12	45.3	(17.3–110.1)	49,800
Jinotega G_3	350	0.98 ± 0.11	10	(5.5–16.0)	11,000
La Concha G_6	350	1.14 ± 0.10	37	(20.0–65.0)	40,700
Sébaco 1 G_3	350	1.22 ± 0.11	38	(25.0–56.0)	41,800
Sébaco 2 G_2	300	1.12 ± 0.12	43.9	(26.6–68.0)	48,200
La Laguna G_5	300	1.47 ± 0.16	34.4**	(24.0–46.8)	37,800
Clorfluazuron					
Geneva 88 G_{182}	300	0.68 ± 0.07	0.03	(0.01–0.11)	1
La Concha G_5	350	0.97 ± 0.1	0.4	(0.23–0.64)	13
La Laguna G_3	300	0.91 ± 0.08	1.0	(0.3–1.7)	33
Jinotega G_3	300	0.82 ± 0.08	1.1	(1.0–2.0)	37
Tisey G_7	300	0.86 ± 0.08	2.1	(0.9–5.3)	70
Sébaco 1 G_1	300	0.75 ± 0.07	3.0	(1.0–7.0)	100
Sébaco 2 G_2	300	1.01 ± 0.10	6.0	(3.0–13.0)	200
Thiocyclam					
Geneva 88 G_{182}	325	0.55 ± 0.05	0.52	(0.11–2.2)	1
La Concha G_5	300	0.82 ± 0.07	2.8	(0.4–21.0)	5
Tisey G_6	325	2.17 ± 0.20	11.0	(8.7–13.2)	21
La Laguna G_4	400	2.11 ± 0.17	18.0	(10.0–36.0)	35
Sébaco 1 G_2	350	2.15 ± 0.22	19.0	(15.0–24.0)	37
Jinotega G_2	225	1.45 ± 0.17	11.0	(7.0–17.0)	21
Methamidophos					
Geneva 88 G_{186}	350	1.70 ± 0.17	0.47	(0.32–0.66)	1
La Concha G_5	300	1.78 ± 0.21	60	(30–90)	127
Tisey G_8	350	2.04 ± 0.18	80	(60–100)	170
La Laguna G_6	300	2.47 ± 0.23	160	(110–220)	340
Sébaco 1 G_4	300	2.70 ± 0.32	220	(170–270)	468
Jinotega G_4	350	2.13 ± 0.36	110	(60–150)	234
Sébaco 2 G_3	150	3.03 ± 0.41	130	(70–230)	276

[a] LC_{50} (μg [AI]/ml); LC_{50} values marked with * are expressed in ng (AI)/ml.

[b] Fiducial limits (95%).

[c] Resistance ratios = LC_{50} of the field population/LC_{50} of Geneva 88.

[d] Generation in the laboratory at which the larvae were tested.

Table 3. Dose-mortality relationship in two populations of *S. exigua* exposed to cypermethrin, deltamethrin, chlorpyrifos, and methomyl

Populations	*n*	Slope ± SE	LD_{50}[a]	95% FL[b]	Weight[c]	RR[d]
			Cypermethrin			
Susceptible G_4	360	1.38 ± 0.12	5.8	(4.0–8.6)	18.5 ± 1.6	1
Darío G_2	420	1.21 ± 0.09	928.8	(585–1427)	18.8 ± 1.1	160
Sébaco G_2	360	1.64 ± 0.17	7,091.0	(5634–9086)	18.8 ± 1.5	1,200
			Deltamethrin			
Susceptible G_5	420	0.80 ± 0.06	2.6	(0.63–11.8)	19.1 ± 1.1	1
Sébaco G_3	360		>5,464[e]		18.3 ± 0.3	>2,100
Darío G_3	360		>5,485[e]		18.4 ± 0.5	>2,100
			Chlorpyrifos			
Susceptible G_5	360	2.34 ± 0.20	36.8	(28.5–47.2)	18.2 ± 1.3	1
Sébaco G_3	360	2.38 ± 0.34	150.2	(111.6–207.8)	18.3 ± 1.3	4
Davío G_4	360	2.54 ± 0.22	347.9	(298.0–408.4)	18.5 ± 1.5	9
			Methomyl			
Susceptible G_7	300	0.95 ± 0.09	2.0	(0.87–4.9)	18.4 ± 0.5	1
Sébaco G_5	300	1.20 ± 0.12	11.9	(7.7–17.6)	18.2 ± 0.2	6
Darío G_5	420	0.60 ± 0.48	14.8	(8.2–26.8)	18.3 ± 0.4	7

[a] LD_{50} (μg [AI]/gr of larva).
[b] Fiducial limits (95%)
[c] Average weight (± SD) of larvae in mg (n = 60).
[d] Resistance ratios = LD_{50} of the field population/LD_{50} of the susceptible population.
[e] Mortality in populations from Sébaco and Darío at an extremely high dose (100 μg[AI]/larva) was 5.0 ± 1.1 and 10.9 ± 0.9% (agverage ± SD), respectively.

dophos of the field populations were 10–232, 11,000–49,800, 13–200, 5–37, and 127–468 times higher, respectively, than the corresponding LC_{50} of Geneva 88 (Table 2). These results indicate that several *P. xylostella* populations occurring in crucifer fields in Nicaragua have evolved high levels of resistance to at least four insecticides from four different groups: two pyrethroids (cypermethrin and deltamethrin), one insect growth regulator (IGR) (chlorfluazuron), one nereistoxin based insecticide (thiocyclam) and one organophosphate (methamidophos). High levels of resistance to pyrethroid insecticides (2,132–82,475 times) have also been documented in *P. xylostella* from Florida (Yu and Nguyen 1992).

Spodoptera exigua. The LD_{50}s of cypermethrin in two field populations of *S. exigua* were 160–1,200 times higher than the corresponding LD_{50}s of the susceptible population (Table 3). In the case of deltamethrin, the LD_{50}s in the two field populations could not be estimated because larval mortality for the Sébaco and Darío populations was 5.0 ± 1.1 and 10.9 ± 0.9% (n = 360), respectively, at the highest dose used (100 μg [AI]/g of larvae). Deltamethrin was 2.2 times more active than cypermethrin on the susceptible population, indicating that the two field populations collected in onion fields have evolved high levels of resistance to at least two pyrethroid insecticides. The LD_{50}s of chlorpyriphos and methomyl in field popu-

Table 4. Dose mortality-relationship in three field populations of *H. zea* exposed to cypermethrin, deltamethrin and chlorpyrifos

Populations	*n*	Slope ± SE	LD_{50}[a]	95% FL[b]	Weight[c]	RR[d]
			Cypermethrin			
Susceptible G_2	360	1.52 ± 0.14	0.28	(0.21–0.35)	28.6 ± 0.7	1
Nandaime G_2	420	1.71 ± 0.09	2.34	(1.90–2.90)	28.2 ± 0.3	8
Telica G_1	420	1.50 ± 0.16	3.34	(2.36–4.44)	28.4 ± 0.4	12
Nagarote G_1	420	1.55 ± 0.12	4.08	(3.17–5.0)	28.2 ± 0.8	15
			Deltamethrin			
Susceptible G_2	660	0.75 ± 0.05	0.11	(0.07–0.21)	28.5 ± 0.6	1
Nandaime G_2	360	1.07 ± 0.10	1.63	(0.80–2.80)	28.1 ± 0.2	15
Telica G_1	420	1.00 ± 0.07	0.91	(0.42–2.08)	28.4 ± 0.5	8
Nagarote G_1	360	0.76 ± 0.07	1.24	(0.50–3.61)	28.2 ± 0.2	11
			Chlorpyrifos			
Susceptible G_2	360	1.23 ± 0.11	0.76	(0.2–5.1)	28.7 ± 0.7	1
Nandaime G_3	360	1.97 ± 0.18	20.4	(16.2–25.3)	28.5 ± 2.0	26
Telica G_2	420	1.15 ± 0.93	75.0	(53.1–108.9)	28.3 ± 0.4	97
Nagarote G_1	420	1.20 ± 0.96	48.5	(36.1–65.5)	28.3 ± 0.3	63

[a] LD_{50} (μg [AI]/g of larva).
[b] Fiducial limits (95%).
[c] Average weight (± SD) of larvae in mg (n = 60).
[d] Resistance ratios = LD_{50} of the field population/LD_{50} of the susceptible population.

Table 5. Concentration-mortality relationship in field populations of *B. tabaci* exposed to bifenthrin, methamidophos and endosulfan

Populations	*n*	Slope ± SE	LC_{50}[a]	95% FL[b]	RR[c]
		Bifenthrin			
Rothamstead G_4	361	1.14 ± 0.16	0.11	(0.06–0.18)	1
El Viejo G_3	369	0.98 ± 0.16	0.53	(0.22–0.97)	4
Sébaco G_3	420	1.73 ± 0.30	1.05	(0.62–1.56)	9
Nandaime G_3	302	2.46 ± 0.76	4.95	(2.27–8.84)	45
		Methamidophos			
Rothamstead G_4	426	1.06 ± 0.11	0.07	(0.05–0.12)	1
El Viejo G_2	350	0.98 ± 0.16	5.1	(2.8–9.6)	68
León G_2	446	1.04 ± 0.17	4.2	(2.8–9.6)	56
Sébaco G_3	406	2.86 ± 0.78	32.1	(16.9–46.7)	423
Nandaime G_3	406	2.70 ± 0.39	41.5	(31.5–51.5)	546
		Endosulfan			
Rothamstead G_4	399	0.64 ± 0.07	0.007	(0.003–0.016)	1
El Viejo G_3	376	1.47 ± 0.24	0.22	(0.10–0.36)	31
Sébaco G_3	416	1.89 ± 0.34	0.41	(0.21–0.63)	59
Nandaime G_3	368	0.76 ± 0.16	0.37	(0.10–0.81)	53

[a] mg (AI)/ml.
[b] Fiducial limits (95%).
[c] Resistance ratios = LC_{50} of the field population/LC_{50} of the Rothamstead population.

lations were only 4–9 and 6–7 times higher, respectively, than the corresponding LD_{50} of the susceptible population of *S. exigua.* However, because the corresponding 95% CLs do not overlap, the LD_{50}s of chlorpyriphos and methomyl were significantly different than the corresponding LD_{50}s of the susceptible population.

Helicoverpa zea. The LD_{50}s of cypermethrin, deltamethrin and chlorpyriphos in the field populations were 8–15, 8–15, and 26–97 times higher than the corresponding LD_{50} of the susceptible population of *H. zea* (Table 4).

Bemisia tabaci. For bifenthrin the LC_{50}s of *B. tabaci* collected at El Viejo, Sébaco, and Nandaime were 4–45 times higher than the LC_{50} of the susceptible population (Table 5). Of the latter three field populations, the LC_{50} of bifenthrin in the population from Nandaime was significantly higher than the LC_{50} of the populations from El Viejo and Sébaco, suggesting that the use of bifenthrin is more intensive in Nandaime than the other two localities. In the case of methamidophos, the LC_{50}s of the four field populations were ≈56–546 times higher than the corresponding LC_{50} of the susceptible population (Table 5). The populations from Sébaco and Nandaime were collected from tomato fields and showed significantly higher LC_{50}s to methamidophos than the populations from El Viejo and León, both collected from cucurbit crops, where insecticides are not as intensively used compared with tomato crops. The *B. tabaci* populations from El Viejo, Sébaco and Nandaime had 31 to ≈59 times higher LC_{50} values than the susceptible population (Table 5), indicating the presence of endosulfan-resistant populations of *B. tabaci* in Nicaragua. The responses to endosulfan in the populations from El Viejo, Sébaco, and Nandaime were similar, because the 95% FL of the corresponding LC_{50}s overlap.

Cross-resistance or multiple-resistance. We examined the correlation among LC_{50}s or LD_{50}s across insecticides tested against *P. xylostella*, *S. exigua*, *H. zea*, and *B. tabaci* (Tables 6–9). Significant correlations among LC_{50}s or LD_{50}s can be due to either simultaneous selection for resistance to each insecticide or cross-resistance in which resistance to one insecticide confers resistance to the other. For *P. xylostella*, the LC_{50}s of the selected insecticides were significantly correlated ($r > 0.82$, $P < 0.045$; $n = 6$; Table 6). Further biochemical studies will be required to determine whether or not a mechanism is present conferring cross-resistance to all five insecticides or if several mechanisms are conferring multiple resis-

Table 6. Correlation analysis of LC_{50} of insecticides tested against *P. xylostella*

	Cypermethrin	Deltamethrin	Thiocyclam	Chlorfluazuron
Deltamethrin	0.82[a] (0.045)[b]	—		
Thiocyclam	0.97 (0.001)	0.86 (0.029)	—	
Chlorfluazuron	0.94 (0.006)	0.91 (0.012)	0.95 (0.003)	—
Methamidophos	0.91 (0.013)	0.97 (0.001)	0.94 (0.006)	0.95 (0.004)

[a] Correlation coefficient (r).
[b] The correlation between LC_{50s} is significant if $P < 0.05$, or highly significant if $P < 0.01$.

Table 7. Matrix of correlation coefficients of LD_{50s} of insecticides tested against *S. exigua*

	Cypermethrin	Chlorpyrifos
Chlorpyrifos	0.80[a] (0.41)[b]	—
Methomyl	0.98 (0.11)	0.89 (0.30)

[a] Correlation coefficient (r).
[b] The correlation between LD_{50s} is not significant if $P > 0.05$.

Table 9. Matrix of correlation coefficients of LC_{50s} of insecticides tested against *B. tabaci*

	Bifenthrin	Methamidophos
Methamidophos	0.91[a] (0.094)[b]	—
Endosulfan	0.84 (0.16)	0.98 (0.02)

[a] Correlation coefficient (r).
[b] The correlation between LC_{50s} is not significant if $P > 0.05$.

tance. The presence of resistance to thiocyclam was rather surprising because this is a recently introduced insecticide in Nicaragua. Resistance to methamidophos and cypermethrin was documented in *P. xylostella* populations occurring in Honduras (Ovalle and Cave 1989). The results from that study and the results reported herein suggest that resistance to methamidophos and cypermethrin evolved sooner than resistance to thiocyclam. Similar correlations were observed among the LD_{50s} of three insecticides tested against *H. zea* (Table 8) and similar biochemical studies are required to clarify the reasons for the correlations.

For *S. exigua,* the correlation among LD_{50}s of three insecticides (cypermethrin, chlorpyriphos, and methomyl) was not significant (Table 7), suggesting that the mechanism conferring resistance to cypermethrin and probably to deltamethrin is independent of the mechanism conferring resistance to chlorpyriphos or methomyl.

For *B. tabaci*, no significant correlations were observed between the LC_{50}s of bifenthrin and methamidophos, or bifenthrin and endosulfan ($r < 0.91$, $P > 0.09$; $n = 4$), but the correlation between the LC_{50}s of methamidophos and endosulfan was significant ($r = 0.98$, $P = 0.02$; $n = 4$; Table 9). Methamidophos is an insecticide that has been widely recommended for *B. tabaci* management in different crops, and endosulfan has been only recently used against *B. tabaci* in Nicaragua. As shown with *P. xylostella,* the correlation between LC_{50}s of methamidophos and endosulfan tested against *B. tabaci* suggests that there is one or more mechanisms conferring resistance to both insecticides but further biochemical studies are needed to differentiate between cross and multiple resistance.

Discussion

In this study, five insect pest species of economic importance in Nicaragua were evaluated for insecticide resistance. Our data indicate that populations of *Hypothenemus hampei* are not resistant to endosulfan and our results are consistent with those reported by Kern et al. (1991). Although episodes of *H. hampei* resistance to endosulfan have already been documented elsewhere (Brun et al. 1989), a resistance management program for endosulfan should be implemented for this most important coffee insect pest in Nicaragua and other Central American countries. The basis for this program should be continued monitoring of susceptibility and the use of biological and cultural controls for *H. hampei* management.

Our data indicate that populations of *P. xylostella, S. exigua, H. zea,* and *B. tabaci* occurring in Nicaragua have evolved resistance to at least one insecticide. Insecticide resistance in the above three lepidopterous species and *B. tabaci* has been documented in previous studies with populations collected in Nicaragua (Delorme et al. 1988, Dittrich et al. 1990, Ernst and Dittrich 1992), but no previous evidence was found of the evolution of resistance to synthetic insecticides in local field populations of *P. xylostella.* High levels of resistance documented during the course of this study may be the result of several decades of intense use of insecticides in field and vegetable crops. The extreme case was documented in six field populations of *P. xylostella* that have evolved resistance to all five insecticides tested. Additionally, other studies with populations also collected from crucifer fields in Nicaragua indicate that *P. xylostella* has evolved resistance to commercial formulations of *B. thuringiensis* subsp. *kurstaki* (Perez and Shelton 1997). The high levels of resistance to pyrethroids reported in this study are consistent with the high levels of resistance to pyrethroids and other insecticide groups documented in *P. xylostella* from Florida (Yu and Nguyen 1992) and Honduras (Ovalle and Cave 1989). The broad-scale resistance found in *P. xylostella* has a negative effect on the livelihood of thousands of cabbage growers from Central America, where small-scale farmers, usually cultivating 0.5–1 ha of crucifers, depend on crucifer production to obtain ready cash income (Andrews et al. 1992). In fact, during the field collection trips to the cabbage growing regions of Nicaragua, five cabbage growers were plowing under the residues of cabbage crops because they could not control *P. xylostella*, regardless of multiple insecticide applications during the crop season.

From a geographical point of view, special attention must be given to the overuse of insecticides at the Sébaco Valley. In that region, field and vegetable crops

Table 8. Matrix of correlation coefficients of LD_{50s} of insecticides tested against *H. zea*

	Cypermethrin	Deltamethrin
Deltamethrin	0.95[a] (0.053)[b]	—
Chlorpyrifos	0.98 (0.014)	0.91 (0.092)

[a] Correlation coefficient (r).
[b] The correlation between LD_{50s} is not significant if $P > 0.05$.

(onions, tomato, cabbage, corn, rice, and others) are grown year round. Three insect pests, *P. xylostella*, *S. exigua*, and *B. tabaci*, collected from vegetable crops in that valley showed resistance problems with at least three of the insecticides tested.

Establishing a resistance management program in Nicaragua poses a tremendous challenge and will require an integrated struggle against several decades of primary dependence on insecticides for pest management. A coalition of forces must be brought to bear upon this problem: the farmers, the industry, technicians, the government (especially the Ministry of Agriculture), and consumers of agricultural products. As a first step, however, the resistance management program must focus on monitoring for changes in susceptibility in different insect-crop systems. During the course of this study, nine Nicaraguan technicians were trained in basic laboratory and field skills for resistance monitoring of insect pests. Those technicians will need additional resources to continue this effort, otherwise, resistance monitoring will be discontinued.

What can be done to implement a resistance management program in Nicaragua? The scientific literature offers several theoretical models and recommendations to manage resistance with multiple tactics (Tabashnik and Croft 1982; Roush and Miller 1986; Tabashnik 1986, 1989; Hoy 1995), but it will require several years of training before the local entomologists are updated with complex resistance monitoring and management strategies. A more hands-on approach should be implemented that will require the involvement of the pesticide industry and the local pesticide registration and regulatory mechanisms. First, the procedures for insecticide registration must be modified so that the new pesticide will be tested against resistant populations of the target pest before it is widely used. If no resistance to the new compound has been documented elsewhere with a similar species, a baseline susceptibility level can then be established for any compound. Those insecticides that have already been registered and widely used in the field should go through a reregistration process at least every 5 yr. If widespread resistance is found, its use against the prescribed pest species should be discontinued until reversion of resistance has been assessed. The presence of cross-resistance will make it even more complicated to establish which insecticide can be used alternatively against a given insect pest species.

The use of other components of a resistance management program must be increased, and these include cultural practices and increased use of biological control agents. Future resistance management studies must address the relationship between *B. tabaci* resistance and the biotype attacking field and vegetable crops. Additionally, continued studies are required to obtain a better understanding of the resistance mechanisms affecting two or more insecticides within a single pest species. The latter may help to fine-tune the pesticide registration regulations and design of a resistance management strategy.

Acknowledgments

We are thankful to Mario Vaughan (Pesticide Management Program at the Ministry of the Environment and Natural Resources) for his support throughout this study. We are also thankful to John Trumble and Gregory Kund (University of California at Riverside) for supplying a susceptible strain of *S. exigua* and to Luc O. Brun (ORSTOM, Montpellier, France) for providing us with a test kit to evaluate resistance to endosulfan in *H. hampei*. This work was a multi-institutional effort to develop and implement an insecticide resistance-monitoring program in Nicaragua. It was developed in cooperation with the Pesticide Management Program, a subcomponent of the Land Management and Agricultural Technology Project financed with resources from the International Development Association of the World Bank, under agreement No. 2636-NI, signed on 25 August 1993, between the Government of Nicaragua and the World Bank.

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Received for publication 20 October 1999; accepted 29 August 2000.