

## Resistance of *Plutella xylostella* (Lepidoptera: Plutellidae) to *Bacillus thuringiensis* Berliner in Central America

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**ABSTRACT** Eighteen field populations of *Plutella xylostella* (L.) from Central America were tested for susceptibility to *Bacillus thuringiensis* subsp. *kurstaki* Berliner, and 7 of the 18 field populations were tested for susceptibility to *B. thuringiensis* subsp. *aizawai*. Tests with *B. thuringiensis* subsp. *kurstaki* were done with a leaf-dip bioassay and a single concentration (20.5 mg [AI]/liter) incorporated in diet. Only leaf-dip bioassays were used in tests with *B. thuringiensis* subsp. *aizawai*. The  $LC_{50}$ s of *B. thuringiensis* subsp. *kurstaki* in the field populations from Guatemala, Honduras, and Costa Rica ranged from 4.3- to 18.3-, 9.3- to 77.2-, and 13.3- to 19.5-fold higher, respectively, than the  $LC_{50}$  of a susceptible population from New York (Geneva 88).  $LC_{50}$ s of *B. thuringiensis* subsp. *aizawai* in the 7 field populations were 1.9- to 3.3-fold higher than the  $LC_{50}$  of Geneva 88. Mortality in the single concentration test varied significantly among populations within countries, and 14 field populations displayed significantly lower mortality than Geneva 88. Results from both bioassay techniques suggest that populations of *P. xylostella* from Central America have evolved resistance to *B. thuringiensis* subsp. *kurstaki*. Monitoring populations of *P. xylostella* for baseline and changing levels of susceptibility to *B. thuringiensis* subspecies and the implementation of pesticide resistance management strategies are urgently needed in Central America.

**KEY WORDS** *Plutella xylostella*, *Bacillus thuringiensis*, resistance monitoring

THE DIAMONDBACK MOTH, *Plutella xylostella* (L.), has evolved resistance to *Bacillus thuringiensis* subsp. *kurstaki* Berliner in Asia (Syed 1992) and the United States (Tabashnik et al. 1990, Shelton et al. 1993). This insect is a key pest of crucifers in Central America, and farmers have relied almost exclusively on synthetic insecticides to control this insect (Andrews et al. 1992). Based on available information, *P. xylostella* apparently first became resistant to synthetic insecticides. Growers then switched to intensive use of *B. thuringiensis* subsp. *kurstaki*, to which *P. xylostella* next evolved resistance. For example, in Hawaii, resistance to synthetic insecticides in field populations of *P. xylostella* (Tabashnik et al. 1987) preceded resistance to *B. thuringiensis* subsp. *kurstaki* (Tabashnik et al. 1990). In Florida, high levels of resistance to synthetic insecticides were documented in a single field population of *P. xylostella*, although no resistance to *B. thuringiensis* subsp. *kurstaki* was observed (Yu and Nguyen 1992). One year later, however, Shelton et al. (1993) reported high levels of resistance to *B. thuringiensis* subsp. *kurstaki* in several field populations of *P. xylostella* from Florida.

In Central America, field populations of *P. xylostella* from Honduras have evolved resistance to synthetic insecticides commonly used for control of this insect in crucifers (Ovalle and Cave 1989).

However, data from field experiments with several formulations of *B. thuringiensis* subsp. *kurstaki*, suggest that *P. xylostella* from Honduras (Ramos 1992), and Nicaragua (Miranda 1992) were effectively controlled in the field with this bioinsecticide. The susceptibility of *P. xylostella* to *B. thuringiensis* subsp. *kurstaki* may change in Central America as growers begin to shift their patterns of insecticide use. A recent report from Guatemala indicates that synthetic insecticides were more commonly used in broccoli fields than *B. thuringiensis* formulations (Dix and Carroll 1995). The Guatemalan broccoli industry is encouraging farmers to rely more on *B. thuringiensis* formulations to control *P. xylostella* to reduce the risks of product rejection by the United States Food and Drug Administration when broccoli shipments from Guatemala enter the United States. In Honduras, the Integrated Pest Management program for crucifers at the Panamerican School of Agriculture has recommended that growers rely more on *B. thuringiensis* formulations in an effort to develop environmentally compatible pest management practices (Andrews et al. 1992). In Costa Rica, farmers have relied more on synthetic insecticides than on *B. thuringiensis* formulations (Monge 1991), but this too may change especially for exported crucifers.

Monitoring pesticide resistance is the cornerstone of resistance management strategies. Cur-

Table 1. Sites of *P. xylostella* collections in Central America

Country	Site	Location	Crop*
Honduras	San Juan del Rancho	Department of Francisco Morazan	c
	Tatumbula	Department of Francisco Morazan	c
	Aguacate (2)	Department of Francisco Morazan	c
	Linaca	Department of Francisco Morazan	b
	Lepaterique (2)	Department of Francisco Morazan	c and b
	Zamorano	Department of Francisco Morazan	b
	Signatepeque	Department of Comayagua	c
Guatemala	Tecpan	Department of Chimaltenango	c
	Patzicia	Department of Chimaltenango	b
	Chinaulta	Department of Guatemala	b
Nicaragua	Sebaco	Department of Matagalpa	c
	Esteli	Department of Esteli	c
	Miraflores	Department of Esteli	c
	Jinotega	Department of Jinotega	b
Costa Rica	Tierra Blanca	Province of Cartago	c
	Valle del Guarco	Province of Cartago	c

c, Cabbage; b, broccoli.

rently, available techniques for monitoring *P. xylostella* resistance to *B. thuringiensis* are based on leaf-dip bioassays that estimate the concentration-mortality relationship (Tabashnik et al. 1990, Shelton et al. 1993). Median lethal concentrations (LC<sub>50</sub>s) are then calculated assuming the probit model. However, use of monitoring methods that correlate bioassay results and field performance of a given insecticide are important. Shelton et al. (1993) determined that field populations of *P. xylostella* displaying LC<sub>50</sub>s of *B. thuringiensis* subsp. *kurstaki* >0.5 mg (AI)/liter of water showed significantly lower mortality in field experiments and proposed this as a discriminating dose for control failures. In another study (C.J.P. and A.M.S., unpublished data), where a probit model was used to predict field efficacy based on the LC<sub>50</sub> of different field strains of *P. xylostella*, we determined that LC<sub>50</sub>s of *B. thuringiensis* subsp. *kurstaki* >0.6 mg (AI)/liter of water would display mortalities <60% when exposed to field applications of the above bioinsecticide. Therefore, we propose to use the LC<sub>50</sub> of 0.6 mg (AI) as a reference to determine the likelihood of field control failure when monitoring resistance to *B. thuringiensis* subsp. *kurstaki* using leaf-dip bioassays.

The objectives of this study were to evaluate the current status of susceptibility to *B. thuringiensis* subsp. *kurstaki* and *B. thuringiensis* subsp. *aizawai* in *P. xylostella* from Central America and to test a bioassay technique based on a single concentration of *B. thuringiensis* subsp. *kurstaki* incorporated in diet. Based on recent studies, mortalities of several field populations of *P. xylostella* at 20.5 mg (AI) of *B. thuringiensis* subsp. *kurstaki*/liter of diet were highly correlated with mortality from field applications at the recommended field rate of a commercial formulation of *B. thuringiensis* subsp. *kurstaki* (C.J.P. and A.M.S., unpublished data).

#### Materials and Methods

**Insects.** Field populations of *P. xylostella* were collected from Guatemala, Honduras, Nicaragua,

and Costa Rica from January to June 1995. Collection sites, geographical locations, and crops are specified in Table 1. At least 300 individuals (larvae or pupae) of *P. xylostella* were collected from commercial fields of crucifers, brought to the laboratory, and reared on rapeseed seedlings (*Brassica napus*) (Shelton et al. 1991). In all cases, the recovery of *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae), a larval parasitoid of *P. xylostella* (Cordero and Cave 1992), ranged from 13 to 20% of individuals collected in the fields. A susceptible laboratory colony from the New York State Agricultural Experiment Station, Geneva, NY, was used for comparisons (Shelton et al. 1993). Hereafter, the susceptible strain will be referred to as Geneva 88. Previous studies indicated that Geneva 88, even after being reared for >100 generations, had a level of susceptibility not significantly different than F<sub>2</sub> populations taken from fields in which resistance to *B. thuringiensis* had not developed.

**Insecticides.** The bioassays were conducted with commercial formulations of Javelin (*B. thuringiensis* subsp. *kurstaki*; WG (wetttable granules) 6.4% [AI], Sandoz, Des Plaines, IL; Lot No. 76732), and Xentari (*B. thuringiensis* subsp. *aizawai*; WG 10.3% [AI], Abbott, North Chicago, IL; Lot No. 11528-04-01). These 2 insecticides vary in their composition of  $\delta$ -endotoxins. *B. thuringiensis* subsp. *kurstaki* produces crystal proteins Cry IA(a), Cry IA(b), Cry IA(c), Cry IIA, and CryIIB, whereas *B. thuringiensis* subsp. *aizawai* produces Cry IA(a), CryIA(b), CryIC, CryID, and CryIIB (Koziel et al. 1993).

**Bioassays.** We used a leaf-dip bioassay similar to that described by Shelton et al. (1993). Leaf disks (3.2 cm diameter) were cut from cabbage leaves ('Izalco') and dipped in solutions prepared with Javelin or Xentari ranging from 0.0064 to 64 and from 0.103 to 32.5 mg(AI)/liter of water, respectively. We used a 3.16-fold serial dilution factor with both insecticides and the range of concentrations was similar to that reported by Shelton

**Table 2.** Concentration-mortality relationships of *P. xylostella* populations from Central America in leaf-dip bioassays with *B. thuringiensis* subsp. *kurstaki*

Population	Generation	n	Slope ± SE	LC <sub>50</sub> mg (AI)/liter (95% CI) <sup>a</sup>	RR <sup>b</sup>
Guatemala					
Geneva 88	F <sub>139</sub>	176	1.40 ± 0.23	0.04 (0.02–0.07)	1
Tecpan	F <sub>2</sub>	195	1.30 ± 0.22	0.19 (0.1 –0.32)	4.8
Patzicia	F <sub>2</sub>	190	1.50 ± 0.19	0.17 (0.1 –0.25)	4.3
Chinautla	F <sub>2</sub>	176	1.96 ± 0.70	0.73 (0.16–3.3)	18.3
Honduras					
Geneva 88	F <sub>128</sub>	196	2.15 ± 0.32	0.06 (0.04–0.10)	1
Rancho	F <sub>2</sub>	176	3.20 ± 0.53	0.56 (0.3 –0.9)	9.3
Tatumbula	F <sub>1</sub>	226	1.23 ± 0.15	0.70 (0.4 –1.1)	11.7
Lepaterique 2	F <sub>1</sub>	204	1.60 ± 0.20	0.72 (0.5 –1.1)	12.0
Aguacate 1	F <sub>1</sub>	204	2.40 ± 0.36	0.73 (0.4 –1.4)	12.2
Lepaterique 1	F <sub>1</sub>	224	2.10 ± 0.30	0.97 (0.6 –1.4)	16.2
Linaca	F <sub>1</sub>	225	1.90 ± 0.25	1.14 (0.8 –1.7)	19.0
Aguacate 2	F <sub>1</sub>	190	2.10 ± 0.30	1.20 (0.7 –2.2)	20.0
Zamorano	F <sub>2</sub>	222	1.15 ± 0.14	2.28 (1.4 –3.7)	38.0
Siguatepeque	F <sub>2</sub>	225	1.60 ± 0.30	4.63 (1.6 –9.1)	77.2
Costa Rica					
Geneva 88	F <sub>140</sub>	199	1.64 ± 0.27	0.04 (0.01–0.08)	1
Tierra Blanca	F <sub>1</sub>	197	2.10 ± 0.34	0.53 (0.35–0.78)	13.3
Valle Guarco	F <sub>3</sub>	197	1.41 ± 0.19	0.78 (0.5 –1.2)	19.5

<sup>a</sup> 95% CL (LeOra Software 1987).

<sup>b</sup> Resistance ratio = LC<sub>50</sub> of field population/LC<sub>50</sub> of Geneva 88 tested with field populations of corresponding country.

et al. (1993). A solution without insecticide was used as control. A spreader-sticker (Bond, Loveland Industries, Loveland, CO) was added to all dilutions at a rate of 0.2% vol:vol. Each leaf disk was dipped for 10 s into the test solution and allowed to air-dry for a period of 2 h, after which the disks were individually placed into 30-ml plastic cups. Five 2nd instars (0.2–0.4 mg) were placed in each cup and 5 replicates were used for a total of 25 larvae per concentration. Larvae from all populations tested were reared on rapeseed seedlings before the bioassay. Six to 7 concentrations, including the control, were used with individual populations. Larvae were allowed to feed on the leaf disks for 48 h at 27°C, 50–70% RH, and a photoperiod of 12:12 (L:D) h, after which mortality was assessed. Larvae were considered dead if they did not move when prodded. Mortality in the controls was always <4%. Field populations of *P. xylostella* from Costa Rica were not tested with *B. thuringiensis* subsp. *aizawai*.

A single concentration of *B. thuringiensis* subsp. *kurstaki* incorporated in artificial diet was also tested. The diet used in this study was described by Shelton et al. (1991), although 2 modifications were made. First, no formaldehyde was added; 2nd, cabbage juice (65 mg cabbage leaves per 500 ml of water) was used to replace 10% of the total water specified in the original diet. The cabbage juice was preheated to 80°C in a microwave oven before adding it to the diet blender. Javelin was added to the diet at a rate of 20.5 mg(AI)/liter when still liquid (65–70°C). Before the *B. thuringiensis* subspecies was incorporated into the diet, it was diluted in 10 ml distilled water for 30 min. Ten cups with treated diet, each with 10 larvae, were used as treatment replicates; and 3 cups with untreated diet, also with 10 larvae each, were used as controls to give a total of 104–130 insects per population. Mortality in the controls was always <7%.

**Interviews with Farmers.** Fourteen crucifer growers from Honduras, 4 from Nicaragua, and

**Table 3. Concentration-mortality relationship of *P. xylostella* populations from Central America in leaf-dip bioassays with *B. thuringiensis* subsp. *aizawai***

Population	Generation	n	Slope $\pm$ SE	LC <sub>50</sub> mg (AI)/liter (95% CL) <sup>a</sup>	RR <sup>b</sup>
Guatemala					
Geneva 88	F <sub>139</sub>	185	1.46 $\pm$ 0.14	3.0 (1.5- 6.8)	1
Tecpan	F <sub>2</sub>	216	1.14 $\pm$ 0.14	8.3 (3.4-25.6)	2.8
Patzicia	F <sub>2</sub>	207	1.86 $\pm$ 0.37	8.7 (3.7-14.8)	2.9
Chinautla	F <sub>2</sub>	203	1.20 $\pm$ 0.12	5.6 (1.1-62.8)	1.9
Honduras					
Geneva 88	F <sub>129</sub>	172	1.94 $\pm$ 0.33	4.0 (2.8- 6.3)	1
Aguacate 1	F <sub>1</sub>	178	1.54 $\pm$ 0.25	12.9 (6.3-42.5)	3.2
Aguacate 2	F <sub>2</sub>	174	1.04 $\pm$ 0.17	12.3 (4.2-15.2)	3.0
Nicaragua					
Geneva 88	F <sub>138</sub>	185	1.46 $\pm$ 0.14	3.0 (1.5- 6.8)	1
Sebaeo	F <sub>2</sub>	208	2.16 $\pm$ 0.30	10.0 (7.2-13.9)	3.3
Esteli	F <sub>2</sub>	181	1.10 $\pm$ 0.14	5.3 (3.1- 9.0)	1.8

<sup>a</sup> 95% CL (LeOra Software 1987).

<sup>b</sup> Resistance ratio = LC<sub>50</sub> of field population/LC<sub>50</sub> of Geneva 88 tested with field populations of corresponding country.

broccoli industry personnel from Guatemala were interviewed to assess the current use of insecticides against *P. xylostella*. Questions asked of farmers or industry personnel included the type of formulation used, frequency of application, application rates per hectare, insect sampling method before or after pesticide applications, and crop area. Only farmers that had planted crucifers at the time of the interview were approached (January to June 1995). Farmers that allowed collections of *P. xylostella* in their fields were asked to show the insecticide containers to verify the insecticide labels.

**Statistical Analyses.** Data from leaf-dip bioassays were analyzed assuming the probit model (LeOra Software 1987). Median lethal concentrations were estimated for each population. The response of 2 populations was considered significantly different if the 95% CL of the corresponding LC<sub>50</sub>s did not overlap. Resistance ratios (RR) were calculated by dividing the LC<sub>50</sub> of a *P. xylostella* field population with the LC<sub>50</sub> of Geneva 88.

Data recorded from the tests with the single concentration of *B. thuringiensis* subsp. *kurstaki* were transformed to the arcsine of the square root of proportion of dead larvae before analysis of variance (SYSTAT 1992). Mortality in the treatments was corrected with the mortality in the controls using Abbott's (1925) formula. When analysis of variance indicated that the population effect was significant ( $P < 0.05$ ), differences in average mortalities between populations were separated by the

Tukey honestly significant difference (HSD) procedure ( $P = 0.05$ ; SYSTAT 1992). Pearson correlation (SYSTAT 1992) was used to determine the degree of association between LC<sub>50</sub>s (log<sub>10</sub>) and mortality (arcsine transformed square-root of the proportion of dead larvae) in the single concentration bioassay.

## Results and Discussion

**Leaf-dip Bioassays with *B. thuringiensis* Subspecies.** In 3 independent bioassays in which the susceptible laboratory population (Geneva 88) was tested with *B. thuringiensis* subsp. *kurstaki*, the average LC<sub>50</sub> ranged from 0.04 to 0.06 mg (AI)/liter of water (Table 2). Field populations of *P. xylostella* from Guatemala, Honduras, and Costa Rica displayed LC<sub>50</sub>s of *B. thuringiensis* subsp. *kurstaki* 4.3- to 18.3-, 9.3- to 77.2-, and 13.3- to 19.5-fold higher, respectively, when compared with Geneva 88. One field population from Guatemala, 8 from Honduras, and 1 from Costa Rica had LC<sub>50</sub>s of *B. thuringiensis* subsp. *kurstaki* beyond the proposed threshold LC<sub>50</sub> of 0.6 mg (AI)/liter of water. We hypothesize that those populations with LC<sub>50</sub>s >0.6 mg (AI) that also showed significantly lower mortality in the single concentration tests may be causing control failures in the field. Field populations from Guatemala, Honduras, and Nicaragua displayed LC<sub>50</sub>s of *B. thuringiensis* subsp. *aizawai* 1.8- to 3.3-fold higher than the LC<sub>50</sub> of Geneva 88 (Table 3). Although the LC<sub>50</sub>s of Xentari in 1 population from Honduras (Aguacate 1) and 1 popu-

**Table 4.** Response of *P. xylostella* from Central America to a discriminating concentration of *B. thuringiensis* subsp. *kurstaki* incorporated in diet

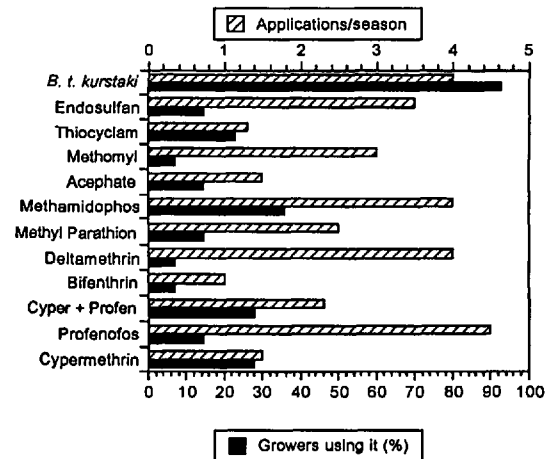
Populations	Javelin incorporated in diet (20.5 mg [AI]/liter of diet)		
	Generation	n larvae	Mortality (% ± SEM)
Guatemala ( $F = 16.8$ ; $df = 3, 36$ ; $P < 0.0001$ )			
Geneva 88	F <sub>139</sub>	129	97.4 ± 0.02a
Tecpan	F <sub>2</sub>	130	89.5 ± 0.7ab
Patzicia	F <sub>2</sub>	129	83.3 ± 1.8b
Chinautla	F <sub>2</sub>	131	64.7 ± 0.4c
Honduras ( $F = 6.14$ ; $df = 9, 90$ ; $P < 0.0001$ )			
Geneva 88	F <sub>128</sub>	129	97.5 ± 0.01a
Lepaterique 1	F <sub>1</sub>	134	85.8 ± 0.6ab
Linaca	F <sub>1</sub>	128	73.5 ± 2.3bc
Tatumbula	F <sub>1</sub>	136	71.6 ± 0.8bc
Aguacate 2	F <sub>1</sub>	128	71.5 ± 2.2bc
Rancho	F <sub>1</sub>	131	70.0 ± 0.4bc
Lepaterique 2	F <sub>1</sub>	133	69.8 ± 0.9bc
Aguacate 1	F <sub>1</sub>	123	69.5 ± 1.2bc
Signatepeque	F <sub>1</sub>	123	63.4 ± 1.0c
Zamorano	F <sub>2</sub>	119	59.1 ± 2.2c
Nicaragua ( $F = 16.5$ ; $df = 4, 45$ ; $P < 0.0001$ )			
Geneva 88	F <sub>137</sub>	127	96.8 ± 0.1a
Jinotega	F <sub>2</sub>	126	85.7 ± 0.8ab
Esteli	F <sub>2</sub>	115	49.5 ± 1.0d
Miraflores	F <sub>2</sub>	129	76.7 ± 2.0bc
Sebaco	F <sub>2</sub>	131	55.0 ± 2.1cd
Costa Rica ( $F = 5.9$ ; $df = 2, 27$ ; $P = 0.008$ )			
Geneva 88	F <sub>140</sub>	131	97.5 ± 0.1a
Tierra Blanca	F <sub>1</sub>	130	93.4 ± 1.0a
Valle Guarco	F <sub>2</sub>	130	88.3 ± 0.5b

Average mortalities (percentages) within country followed by the same letter are not significantly different ( $P = 0.05$ ) by the Tukey HSD procedure (SYSTAT 1992).

lation from Nicaragua (Sebaco) were significantly higher than the LC<sub>50</sub> of Geneva 88, the somewhat reduced susceptibility may not be sufficient to declare meaningful levels of resistance.

**Single Concentration Tests with *B. thuringiensis* subsp. *kurstaki*.** Field populations from all 4 countries were exposed to a single concentration of Javelin incorporated in diet. When compared with mortality of Geneva 88, 1 population from Guatemala, 8 from Honduras, 3 from Nicaragua, and 1 from Costa Rica showed significantly lower mortalities ( $F > 5.9$ ,  $P < 0.008$ ; Table 4). For all populations tested with both bioassay methods, LC<sub>50</sub>s of *B. thuringiensis* subsp. *kurstaki* and percentage of mortality were significantly correlated ( $r = -0.88$ ,  $P < 0.0001$ ,  $n = 17$ ). For populations from Honduras, the correlation between LC<sub>50</sub>s and percentage of mortality was even higher and also highly significant ( $r = -0.95$ ,  $P < 0.0001$ ,  $n = 10$ ). Because LC<sub>50</sub>s in the leaf-dip bioassays and mortality in the single concentration tests were significantly correlated, we conclude that either method can be used to distinguish between *B. thuringiensis* subsp. *kurstaki*-susceptible and resistant *P. xylostella*.

**Pesticides Used Against *P. xylostella*.** Based on our interviews, 13 of the 14 farmers interviewed



**Fig. 1.** Pesticides used against *P. xylostella* in Honduras during the crop season of 1994–1995.

(>90%) in Honduras used *B. thuringiensis* subsp. *kurstaki*, alone or in mixtures with other synthetic insecticides, for control of *P. xylostella* (Fig. 1). Profenofos and *B. thuringiensis* subsp. *kurstaki* were the most frequently used pesticides during the crop season. Also in Honduras, the number of pesticide applications against *P. xylostella* ranged from 4 to 16 (mean = 7.2) per season. None of the farmers interviewed in Guatemala, Honduras, or Nicaragua used economic thresholds, insect sampling or calibration procedures for sprayers. Because farmers did not calibrate sprayers, the rate applied per hectare could not be estimated. The use of *B. thuringiensis* subsp. *kurstaki* has been, and continues to be, strongly recommended by the broccoli industry of Guatemala during the last 4 yr (Erich Sundfeld, ALCOSA, Guatemala City, Guatemala, personal communication). Although farmers in Nicaragua claimed that they did not use *B. thuringiensis* formulations during the above crop season, they stated that they had used it in the past. However, the 4 farmers interviewed in Nicaragua had switched from *B. thuringiensis* and other synthetic insecticides to insect growth regulators because of apparent loss of efficacy against *P. xylostella*.

Resistance is a genetically based decrease in susceptibility of a population to an insecticide (Tabashnik 1994), and the LC<sub>50</sub> is one of the most widely measured and reported parameters in studies of *P. xylostella* resistance to *B. thuringiensis* (Tabashnik et al. 1990, Shelton et al. 1993, Chilcutt and Tabashnik 1995). The results obtained with the 2 bioassay techniques used in our study indicate that the susceptibility of *P. xylostella* from Central America to *B. thuringiensis* subsp. *kurstaki* was significantly lower compared with a standard susceptible population. The relative magnitude of the LC<sub>50</sub>s displayed by several populations from Honduras were similar to resistance ratios displayed by resistant populations of *P. xylostella* from Hawaii

(Tabashnik et al. 1990). The concentration–mortality data from 7 populations collected in the region suggest that *P. xylostella* were susceptible to *B. thuringiensis* subsp. *aizawai*. This result is not surprising because commercial formulations of *B. thuringiensis* subsp. *aizawai* had not been used in the field until the 1st half of 1995 (Erich Sundfeld, personal communication).

Based on available information, the significantly reduced susceptibility of *P. xylostella* to *B. thuringiensis* subsp. *kurstaki* in Central America may have been the result of an increased use of this microbial insecticide at least during the past 6 yr. In Honduras, the increased use of *B. thuringiensis* may have evolved because of resistance to synthetic insecticides (Ovalle and Cave 1989). In 1980, none of the Honduran farmers surveyed used *B. thuringiensis* for pest management in crucifers, but 34% of farmers surveyed used it by 1989 (Andrews et al. 1992). Based on our interviews, the use of *B. thuringiensis* subsp. *kurstaki* in Honduras is apparently more intensive than indicated in previous reports. In 1990, the United States Food and Drug Administration detected 120 pesticide residue violations in nontraditional export produce from Guatemala (Barrett 1995), and broccoli accounted for the majority of nontraditional export crops that year (Murray and Hoppin 1992). These events may have persuaded the frozen broccoli industry to implement a biological control program beginning in 1993, which combined applications of *B. thuringiensis* and releases of parasitoids of *P. xylostella* (Biever et al. 1994).

Resistance studies conducted in Hawaii showed that *P. xylostella* displayed significant intra-island variation in susceptibility among populations separated <10 km (Tabashnik et al. 1987). Our data may provide additional insight to explain the geographical variation in susceptibility to *B. thuringiensis* in *P. xylostella*. The insects collected in all 4 countries came from crucifer fields within 150 km, and in some cases, significant differences in mortality were observed in populations from Nicaragua collected from cabbage fields <40 km apart.

In Central America, crucifer growers continue to rely on insecticide applications to control *P. xylostella* in crucifers. Integrated pest management tactics based on biological control, cultural practices, and insect sampling methods must be implemented to reduce dependence on both synthetic and biological pesticides. Current development of transgenic plants (Metz et al. 1995) may lead to the release of crucifer crops expressing *B. thuringiensis* toxins, but evolution of resistance to these toxins may limit the use of these crops in Central America. Continued monitoring of insecticide resistance is crucial to development of insecticide resistance management tactics in this region. Finally, farmers should be trained in the rational use of pesticides for pest management.

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