Managing Diamondback Moth (Lepidoptera: Plutellidae) Resistance to Foliar Applications of *Bacillus thuringiensis*: Testing Strategies in Field Cages

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J. Econ. Entomol. 90(6): 1462–1470 (1997)

ABSTRACT Three resistance management strategies for field-sprayed commercial formulations of *Bacillus thuringiensis* Berliner subspecies were tested in field cages during the dry and rainy seasons of 1995 in Honduras. A local field population of *Plutella xylostella* (L.) with a 21-fold resistance to *B. thuringiensis* subsp. *kurstaki* (Javelin), but no resistance to *B. thuringiensis* subsp. *aizawai* (Xentari), was selected for 5–6 generations with 16 field applications of a high (1.12 kg/ha) or low (0.3 kg/ha) dose of Javelin, a high or low dose of Javelin in the presence or absence of a refuge (25%), and Xentari (1.12 kg/ha). Resistance to Javelin increased ≈1.9–4.4 times, but was significant only with the 1.12 kg/ha rate of Javelin irrespective of the presence or absence of a refuge. Field selection with Javelin at 0.3 kg/ha or Xentari did not cause a significant increase in resistance to *B. thuringiensis* subsp. *kurstaki*, nor did *P. xylostella* selected with Xentari evolve resistance to *B. thuringiensis* subsp. *aizawai*. During the same period, the LC50 of Javelin in *P. xylostella* left unselected did not decrease. Although the rate of resistance increase was lower for lower doses of Javelin, a smaller proportion of marketable cabbage was produced in comparison with higher doses of Javelin or Xentari. Our data suggest that the deliberate inclusion of a refuge may reduce the proportion of marketable produce, and may affect use of this resistance management strategy in both sprayed *B. thuringiensis* and transgenic crops expressing *B. thuringiensis* toxins.

KEY WORDS *Plutella xylostella*, *Bacillus thuringiensis*, resistance management, foliar applications

THE DIAMONDBACK MOTH, *Plutella xylostella* (L.), has evolved resistance to virtually all commercial insecticides (Denholm and Rowland 1992), including *Bacillus thuringiensis* Berliner (Tabashnik 1994). Despite the evolution of resistance to insecticides, growers of crucifers worldwide continue to rely on pesticide applications for control of *P. xylostella* in the field (Talekar and Shelton 1993). The evolution of resistance to *B. thuringiensis* in field populations of *P. xylostella* (Tabashnik et al. 1990, Shelton et al. 1993) poses a threat to the prolonged efficacy of this bacteriological pesticide in the field (McGaughey 1994). The challenge of this research is to design effective insecticide resistance and pest management strategies that will preserve the efficacy of *B. thuringiensis* toxins against *P. xylostella*.

Resistance to insecticides has been defined both in terms of gene frequency and field control. For example, as a general rule in simulation models, resistance is said to have evolved when the frequency of resistant alleles in the population exceeds 50% (e.g, Rosenheim and Tabashnik 1990). However, the point at which a pest population no longer can be adequately controlled is probably more appropriate than any genetic criterion because resistance is rarely detected until control failures occur in the field (Rosenheim and Tabashnik 1990). Brent (1986) argued that initial detection of resistance usually requires that individuals carrying the resistant alleles comprise >5% of the population. In this report, we describe a field population of *P. xylostella* that had detectable levels of resistance to *B. thuringiensis* subsp. *kurstaki*, and we use this population to test some resistance management strategies.

Resistance management programs, or the set of pest management practices that delay the evolution of resistance, are best implemented before the pest becomes resistant (Hoy 1992). However, resistance management strategies have to be tested under the assumption that a proportion of the individuals within the population are already resistant, because most of the strategies proposed for resistance management (Roush 1989) cannot be tested effectively, either theoretically or empirically, when resistance is not present in the population. Effective resistance management strategies must consider the interaction among biological, ecological, genetic, and operational factors (Georghiou 1983, Rosenheim and

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0022-0493/97/1462–1470$02.00/0

Tabashnik 1990, Denholm and Rowland 1992), and the likelihood of adoption of the proposed resistance management strategies by farmers (Kennedy and Whalon 1995).

For the specific case of *B. thuringiensis* toxins, several resistance management strategies have been described by various authors (Tabashnik et al. 1991, McGaughey and Whalon 1992, Forrester 1994, Gould 1994, Roush 1994, Alstad and Andow 1995). Potential resistance management strategies that could be used in the *P. xylostella–B. thuringiensis* system were described in more detail by Tabashnik (1994). These include the use of mixtures of toxins, synergists, mosaic application, rotations of toxins, ultrahigh doses, and refuges (unselected fractions of an insect population).

Most of the proposed resistance management strategies for *B. thuringiensis* toxins and synthetic insecticides are based on the results of laboratory investigations (Tabashnik et al. 1991) and predictions from theoretical deterministic models (Tabashnik 1986, Rosenheim and Tabashnik 1990, Roush 1994, Alstad and Andow 1995). However, proposed resistance management strategies must be tested in the field. Because *P. xylostella* is the only insect that has evolved resistance to *B. thuringiensis* toxins in the field (Tabashnik et al. 1990, Shelton et al. 1993), it is an ideal candidate for experiments to evaluate the strategies discussed above. Results from laboratory investigations with *B. thuringiensis* indicated that *P. xylostella* resistance increased significantly after 5–9 generations of selection (Tabashnik et al. 1991), suggesting that *P. xylostella* with detectable levels of resistance could be used in field experiments to evaluate the effect of different strategies on the rate of resistance increase.

The objective of our study was to compare the effect of 3 resistance management strategies for field-sprayed commercial formulations of *B. thuringiensis* (low versus high application rate; presence or absence of refuge; and rotation of *B. thuringiensis* strains) on the rate of resistance increase in a field population of *P. xylostella* that had already evolved detectable levels of resistance. The high-dose approach to manage resistance relies on the principle that resistance can be delayed because all (or nearly all) of the resistant heterozygotes, the most common carriers of resistance, are killed; it also depends on the assumption that resistance is rare (Tabashnik and Croft 1982, Roush 1994, Tabashnik 1994). In this study, we compared the effect of a recommended field application rate of Javelin (*B. thuringiensis* subsp. *kurstaki*) at 1.12 kg/ha with a low dose of Javelin (0.3 kg/ha) that was slightly higher than a field dose (0.26 kg/ha) known to kill 90% of 2nd instars of susceptible *P. xylostella* (Perez et al. 1995). Thus, the field rate of 1.12 kg/ha of Javelin, considered here as the high dose, was ≈4 times higher than the low dose. We also evaluated the effect of the 3 resistance management strategies on yield and quality of cabbage produced during 2 continuous cropping seasons.

Materials and Methods

Insecticides. Field applications and bioassays were performed with commercial formulations of Javelin (*B. thuringiensis* subsp. *kurstaki;* wettable granules [WG] 6.4% [AI], Sandoz, Des Plaines, IL; Lot No. 7671312), and Xentari (*B. thuringiensis* subsp. *aizawai;* WG 10.3% [AI], Abbott, North Chicago, IL; Lot No. 11528-04-01). *B. thuringiensis* subsp. *kurstaki* carries the genes that produce the insecticidal crystal proteins Cry IA(a), Cry IA(b), Cry IA(c), Cry IIA, and CryIIB, whereas *B. thuringiensis* subsp. *aizawai* carries the genes that produce Cry IA(a), CryIA(b), CryIC, CryID, and CryIIB (Koziel et al. 1993).

Field Trials. We planted 2 field experiments, each consisting of 18 plots of the cabbage variety 'Izalco'. Plots contained 4 rows of cabbage 3.6 m long, with a total of 34–36 plants per plot. Plant spacing was 0.9 m between rows and 0.35 m between plants. These 2 experiments were planted at the Panamerican School of Agriculture (Zamorano) in Honduras during the dry and rainy seasons of 1995. Each plot was treated with 1 of the following 6 treatments: (1) Javelin 1.12 kg/ha, (2) Javelin 1.12 kg/ha with an untreated row equivalent to 25% refugia, (3) Javelin 0.3 kg/ha, (4) Javelin 0.3 kg/ha with 25% refugia, (5) Xentari 1.12 kg/ha, and (6) water only control. All treatments were replicated 3 times and were arranged in a randomized complete block design. Each plot was individually covered with a polypropylene screen cage (32 by 32 mesh; Lumite, Gainesville, GA) immediately after transplanting. The cages were 3.6 by 3.6 by 1.8 m (length, width, height) and had a 30-cm flap around the bottom that was buried in the soil. Such screen type was sufficiently fine to keep larvae and adult *P. xylostella* from escaping, yet excluded *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae), the most common parasitoid of *P. xylostella* in Honduras (Cordero and Cave 1992). The use of the screen cages allowed us to exclude insect migration and parasitism (admittedly potentially important factors but ones which would be site- specific) and focus only on mating frequencies and their interaction with climatic factors.

Dry Season. For the dry-season experiment, cabbage was transplanted on 17 January and harvested on 11 April. During the 84-d crop period, the average daily temperature was 22.4 ± 2.2°C and total precipitation was 95.6 mm, of which 65 mm were recorded on a single day. Water and fertilizer were supplied through a drip-irrigation system in both experiments. Larvae (5 d old) of a *P. xylostella* population, previously collected from a broccoli field at Zamorano and then reared in the laboratory on seedlings of rape, *Brassica napus* L., were inoculated at a density of 20 larvae per cabbage plant 57 d after transplanting, when the crop was at the precupping stage (Andaloro et al. 1983). Two days after plant inoculation with larvae, the treatments were applied with a CO_2-assisted sprayer equipped

with a single TXSV-4 hydraulic nozzle (R&D Sprayers, Opelusas, LA) and calibrated to deliver 200 liters of mixture per hectare at a pressure of 2.8 kg/cm^2. Thereafter, applications were performed at 67, 70, 74, and 78 d after transplanting for a total of 5 applications during a period of 19 d, during which 2 generations of *P. xylostella* were treated. Distilled water was used for all applications. A spreader-sticker (Bond, Loveland, Greeley, CO) was added to all applications at 0.2% (vol:vol). Insect sampling during the dry season was done at 62, 67, 70, 74, 78, and 80 d after transplanting. Counts of *P. xylostella* were done on 3 plants per plot of the 2 middle rows. Insect developmental stages were divided into 3 separate categories: 1st and 2nd instars, 3rd and 4th instars, and pupae. Cumulative *P. xylostella*-days (CPxDs) were calculated for each of the 3 developmental categories recorded at all sampling dates by using a formula similar to that described by Beers et al. (1990). This formula was adapted to express CPxDs as follows: $\text{CPxD} = [0.5(Px_p + Px_c)]^* d_{c-p}$, where Px_p is the average number of *P. xylostella* (larvae or pupae) found on 3 plants per plot at the previous sampling date, Px_c is the current average number of *P. xylostella* on 3 plants, and d_{c-p} is the number of days between sampling dates. At harvest, fresh weight (g) and a quality index of cabbage heads based on a scale of 1–5 (1, no damage; 5, highly damaged) were recorded from all plants within plots. Simultaneously, all surviving larvae or pupae (>150 per treatment) of *P. xylostella* were collected, regardless of replicate, and reared on rape seedlings in the laboratory in cages separated by treatments. These insects were maintained in the laboratory for 2 generations without selection and were used for the 2nd planting (see below).

Rainy Season. During the rainy season, cabbage was transplanted on 19 May and harvested on 8 August. Average daily temperature and total precipitation during the 81-d crop period were 24.8 ± 1.0°C and 308.6 mm, respectively. Larvae (5 d old) collected from the different treatments of the experiment planted during the dry season, were inoculated at a rate of 20 larvae per plant on plots of corresponding treatments at 22 d after transplanting when plants had 8–10 leaves. The 1st application of these treatments was performed at 24 d after transplanting and then repeated on each of 10 sampling dates (27, 31, 38, 44, 49, 53, 58, 63, 67, 70, 74, and 77 d after transplanting). Overall, 3–4 generations of *P. xylostella* were selected with 11 applications of *B. thuringiensis* during a period of 55 d following larval inoculation. All plants within plots were harvested, and head weight and quality were recorded as above. Cumulative *P. xylostella*-days (CPxDs) also were calculated, and surviving larvae and pupae on each plot (>75 per treatment) were collected and brought to the laboratory for further bioassays.

Bioassays. Leaf-dip bioassays were done before plants for the dry-season experiment were inoculated with *P. xylostella*. Similar bioassays were done on insects collected at the end of the rainy season experiment. We used a bioassay procedure similar to that described by Shelton et al. (1993). Leaf disks (32 mm diameter) of the cabbage variety Izalco were dipped in solutions containing 3.16-fold serial concentrations that ranged from 0.02 to 64 mg (AI)/liter of Javelin, and from 0.1 to 103 mg(AI)/liter of Xentari. At least 7 concentrations were used per population. After dipping, the leaf disks were air-dried for 2 h, after which five 2nd-instar *P. xylostella* from the different field treatments were added. We used 5 replicates (total of 25 larvae per concentration). Equal numbers of larvae also were used in control solutions without insecticide. Larvae were allowed to feed for a period of 48 h at 27 ± 1°C, 50–70% RH, and a photoperiod of 12:12 (L:D) h before mortality was assessed. A larva was considered dead if did not move when prodded.

Bioassays done before the dry-season experiment were done with Javelin and Xentari on a single field population of *P. xylostella* (Zamorano at generation 1). The bioassays after field selection were done with Javelin on *P. xylostella* collected from the 6 treatments. Only the subpopulations collected from the control plots and the plots treated with Xentari were tested against Xentari after field selection. A susceptible population of *P. xylostella*, Geneva 88 (Shelton et al. 1993), also was tested in laboratory bioassays with both bacteriological pesticides for comparison.

Data Analyses. Data from the leaf-dip bioassays were analyzed assuming the probit model by using POLO (Russell et al. 1977, LeOra Software 1987). Lethal concentrations, 95% CL, and resistance ratios (LC_{50} of the field population over the LC_{50} of Geneva 88) were estimated. The susceptibility to *B. thuringiensis* of 2 populations was considered different if the corresponding 95% CL did not overlap at LC_{50}.

We used analysis of variance (ANOVA) (PROC GLM; SAS Institute 1989) to test for treatment effects on CPxDs, cabbage head weight, and percentage marketable cabbage. Data on CPxDs was transformed to $\log_{10}(x)$, where x is the value of CPxDs for a given plot, to stabilize the variances. The arcsine transformation was used on the percentage of marketable cabbage. Data on proportion of marketable cabbage includes only cabbage heads that met our criteria for quality indexes 1 (undamaged cabbage heads) and 2 (little damage). We present ANOVA done on the pooled data of all developmental stages of *P. xylostella* because the 3 developmental categories were highly correlated ($r > 0.95$; $P < 0.0001$; Pearson correlation, PROC CORR, SAS Institute 1989). The ANOVA done on cabbage head weight and percentage marketable cabbage included all plants from each plot to simulate a realistic harvesting procedure performed by a farmer who is using refuges of the same crop as a resistance management strategy. When treatment effects were significant, means were separated by

Table 1. Response of a susceptible and a field strain of *P. xylostella* to commercial formulations of *B. thuringiensis* subsp. *kurstaki* and *B. thuringiensis* subsp. *aizawai* in leaf-dip bioassays

Populations	*n*	Slope ± SE	LC_{50}[a]	95% CL	RR[b]
Javelin (*B. thuringiensis* subsp. *kurstaki*)					
Geneva 88 F_{133}	189	0.98 ± 0.15	0.034	(0.017–0.06)	1
Zamorano F_1[c]	179	1.60 ± 0.21	0.70	(0.48–1.02)	20.6
Xentari (*B. thuringiensis* subsp. *aizawai*)					
Geneva 88 F_{133}	178	1.90 ± 0.23	4.0	(2.80–6.30)	1
Zamorano F_1[c]	179	1.60 ± 0.21	4.4	(3.10–6.40)	1.1

[a] Milligrams (AI)/liter.
[b] Resistance ratios = LC_{50} of field population/LC_{50} of Geneva 88.
[c] Response of field strain before release into field cages.

nonorthogonal contrasts; the Bonferroni correction ($P = 0.1/c$), where c is the number of contrasts, was used to separate treatment means while controlling the experiment-wise error rate (Neter et al. 1990). Correlation analysis also was done to determine the degree of association between CPxDs, cabbage head weight, and percentage marketable cabbage by using data from both cropping seasons. Efficacy of *B. thuringiensis* formulations were expressed as percentage reduction in CPxDs in plots treated with *B. thuringiensis* subspp. relative to CPxDs in control plots.

Results and Discussion

Effect of Selection on Increased Resistance. The initial LC_{50}s of Javelin and Xentari of the *P. xylostella* population from Zamorano used in this study were 20.6 and 1.1 times higher, respectively, than the LC_{50}s of Geneva 88 (Table 1). After field selection with 16 applications on 5–6 generations (Table 2), the LC_{50}s of *B. thuringiensis* subsp. *kurstaki* increased ≈1.9 (in insects selected with Xentari) to 4.4 times (in insects selected with a high rate of Javelin with refuge), compared with the initial LC_{50} of the Zamorano population (0.7 mg [AI]/liter; Table 1). The LC_{50} of unselected *P. xylostella* (Zamorano) from the control plots (0.9 mg [AI]/liter) did not vary significantly from the initial LC_{50} (0.7 mg [AI]/liter) of the Zamorano population. Compared with Geneva 88, all treatments with Javelin increased the LC_{50}s of this insecticide from 20.6 to ≈85 times (Table 2). However, only the LC_{50}s of the sub-populations selected with the high rate of Javelin (1.12 kg/ha), irrespective of the presence of refugia, were significantly higher than the initial LC_{50} of 0.7 mg (AI)/liter. The LC_{50}s of the subpopulations selected with the low rate of Javelin (0.3 kg/ha), with or without refugia, and Xentari (1.12 kg/ha) did not increase significantly when compared with both the initial and final LC_{50}s of unselected *P. xylostella* from Zamorano. Similarly, the LC_{50}s of *B. thuringiensis* subsp. *aizawai* of both the unselected and the subpopulation selected with Xentari did not increase significantly, compared with the LC_{50} of Xentari in the Zamorano population before field selection (4.4 mg [AI]/liter).

Our results indicate that field applications of *B. thuringiensis* subsp. *kurstaki* will significantly increase resistance when populations of *P. xylostella* with detectable levels of resistance are further selected with this insecticide. In addition, applications of high rates of *B. thuringiensis* subsp. *kurstaki* will result in higher rates of resistance increase than low applications rates or applications with *B. thuringiensis* subsp. *aizawai*.

Table 2. Response of a field strain of *P. xylostella* to *B. thuringiensis* subsp. *kurstaki* and *B. thuringiensis* subsp. *aizawai* in leaf-dip bioassays after exposure to selected treatments of Javelin and Xentari in field cages

Populations	*n*	Slope ± SE	LC_{50}[a]	95% CL	RR[b]
Javelin (*B. thuringiensis* subsp. *kurstaki*)					
Geneva 88 F_{112}[c]	196	1.64 ± 0.32	0.037	(0.01–0.07)	1
Zamorano (control)[d]	222	1.42 ± 0.22	0.90	(0.48–1.45)	24.3
Zamorano (Javelin 0.3 kg/ha)[d]	201	2.26 ± 0.50	2.20	(0.80–3.80)	59.5
Zamorano (Javelin 0.3 + refuge)[d]	202	1.74 ± 0.24	1.50	(0.60–3.80)	40.5
Zamorano (Javelin 1.12 kg/ha)[d]	198	1.75 ± 0.25	3.10	(2.12–4.61)	83.8
Zamorano (Javelin 1.12 + refuge)[d]	199	1.54 ± 0.21	3.14	(2.16–4.70)	84.9
Zamorano (Xentari 1.12 kg/ha)[d]	227	1.70 ± 0.21	1.30	(0.90–1.90)	35.1
Xentari (*B. thuringiensis* subsp. *aizawai*)					
Zamorano (Control)[d]	198	1.15 ± 0.22	7.60	(4.14–12.98)	1.9
Zamorano (Xentari 1.12 kg/ha)[d]	194	1.84 ± 0.26	8.60	(5.90–12.60)	2.2

[a] Milligrams (AI)/liter.
[b] Resistance ratios = LC_{50} of the field population/LC_{50} of Geneva 88.
[c] The susceptible strain (Geneva 88) was not released into the field cages.
[d] Field strain of *P. Xylostella* from Zamorano exposed to selected treatment of commercial formulations of Javelin and Xentari.

The high-rate approach to managing resistance to insecticides assumes that the rate used is sufficiently high to kill most or all resistant heterozygotes and that the resistance allele is rare (Tabashnik and Croft 1982). In principle, a rate that was so high that it also killed resistant homozygotes might manage resistance even where the frequency of resistance is high. However, 2.24 kg/ha of Javelin (twice the recommended field application rate) caused only ≈80% mortality in *P. xylostella* populations with LC_{50}s of 0.6 mg (AI) of Javelin per liter in leaf-dip bioassays, a level at which we would expect control failures (Perez and Shelton 1997). In another study, we documented that some populations of *P. xylostella* from Central America displayed LC_{50}s of *B. thuringiensis* subsp. *kurstaki* >0.7 mg (AI)/liter (similar to the LC_{50} of the population we initially released into the field cages (Table 1); a survey of crucifer growers in Honduras documented that they sprayed commercial formulations of *B. thuringiensis* subsp. *kurstaki* at a frequency of 4–5 applications during the dry season of 1994–1995 extending from late November through early May (Perez and Shelton 1997). That a high proportion of *P. xylostella* can survive twice the field rate, combined with the results of this study, suggest that a high-rate approach to managing resistance to field sprays of *B. thuringiensis* would be impractical in the field once resistance is detected (Tabashnik and Croft 1982). Despite the frequent application of recommended field rates of Javelin and Xentari against a *P. xylostella* with relatively low levels of initial resistance to *B. thuringiensis* subsp. *kurstaki*, we were unable to obtain field efficacies >74%.

The use of refuges is considered the most promising resistance management strategy (Tabashnik 1994). Our data indicate that a refuge equivalent to 25% of the area planted may not be an effective resistance management strategy once resistance is already detectable, especially when high rates are used. A refuge is intended to dilute resistance; however, if the refuge is already highly contaminated with resistance alleles, it cannot be very effective in dilution. In this study, we did not address the importance of movement of larvae from treated to untreated plants within experimental plots, nor the position of the refuge relative to treated plants, yet these are factors that require attention in future research efforts.

The use of rotations of *B. thuringiensis* products also may be problematic. Both of the currently available suspecies of *B. thuringiensis* contain Cry IA toxins, whereas *B. thuiringiensis* subsp. *aizawai* also contains Cry IC. Following the evolution of resistance to *B. thuringiensis* subsp. *kurstaki* in *P. xylostella* from Florida, many farmers switched to *B. thuringiensis* subsp. *aizawai* (Shelton et al. 1993), with the hope of control by the Cry IC toxin. Our results suggest that selection with 16 applications of *B. thuringiensis* subsp. *aizawai* against 5–6 generations of *P. xylostella* did not cause a significant increase in resistance to either product. However, the use of *B. thuringiensis* subsp. *aizawai* against *B. thuringiensis* subsp. *kurstaki*-resistant *P. xylostella* may maintain the resistance to individual toxins produced by both *B. thuringiensis* subspecies (Tang et al. 1995a). Overall, the efficacy of Xentari against *P. xylostella* in the field ranged from 63.1 to 71.4%, and was not significantly different in overall reduction of infestations of *P. xylostella* caused by Javelin, irrespective of the rate used. However, we expect continued intensive use of *B. thuringiensis* subsp. *kurstaki* would further increase resistance beyond what we documented in our experiments.

Use of *B. thuringiensis* subsp. *aizawai* did not result in significantly lower infestations of *P. xylostella* in the field cages compared with the recommended field rate of Javelin (1.12 kg/ha) used in this study. A likely explanation for these results is the difference in potency of Javelin and Xentari against the field population released in the field. The LC_{50} of Xentari for the Zamorano population was ≈6.3 times higher than the LC_{50} of Javelin for the same population (Table 1). In other studies, commercial formulations of *B. thuringiensis* subsp. *aizawai* have shown ≈3-fold cross-resistance with *B. thuringiensis* subsp. *kurstaki*-resistant *P. xylostella* (Shelton et al. 1993, Tabashnik et al. 1993). Thus, minimal cross-resistance may be preventing Xentari from reaching a higher level of efficacy when applied with a conventional application technique. Tabashnik et al. (1993) and Tang et al. (1995b) reported that the response of *P. xylostella* to Cry IC, a toxin unique to Xentari, was not affected by resistance to *B. thuringiensis* subsp. *kurstaki*, but Cry IA toxins, produced by both *B. thuringiensis* subspecies, were affected by cross-resistance.

Effects of *P. xylostella* Infestations on Cabbage Yield and Quality. During the dry-season experiment, counts of *P. xylostella* on cabbage plants were done 6 times over a 19-d period, and 11 samples were recorded during a 51-d period in the rainy-season experiment. The results of CPxDs, cabbage weight, and percentage of marketable cabbage are displayed in Table 3; ANOVA and nonorthogonal contrasts are presented in Table 4. In both experiments, the overall infestation of *P. xylostella* (CPxDs) varied significantly across treatments ($F > 8.0$; df = 5, 10; $P < 0.003$), with significantly lower infestations in plots treated with either subspecies of *B. thuringiensis* ($P \leq 0.0001$). However, the results of nonorthogonal contrasts indicate that most of the variation in CPxDs resulted from higher infestations of *P. xylostella* in the control plots because no significant differences in CPxDs were observed across treatments with *B. thuringiensis* ($F < 2.8$; df = 1, 10; $P > 0.128$; Tables 3–4). In the dry season, we recorded an average of ≈88 individuals per plant per day in the control plots (Table 3), whereas, 25.3–37.5 individuals per plant per day were sampled from the plots treated with *B. thuringiensis*. During the rainy season, the average infestation of *P. xylostella* in the untreated plots was 34.2 individuals per plant per day, whereas the

Table 3. Cumulative *P. xylostella*-days (mean CPxDs ± SE), field efficacy of *B. thuringiensis* subspecies (%), weight and quality of cabbage heads (mean ± SE) produced during 2 seasons

Treatment	CPxDs[a]	Efficacy, %[b]	Wt, g[c]	Marketable cabbage[d]
		Dry season		
Control	1,679 ± 281.0	—	771 ± 43	4.1 ± 1.0
Javelin 1.12 kg/ha	506 ± 48.2	70.0	1,277 ± 65	60.1 ± 4.1
Javelin 1.12 + refuge[e]	575 ± 47.0	65.7	1,119 ± 49	37.4 ± 1.9
Javelin 0.3 kg/ha	714 ± 133.0	57.4	993 ± 42	10.3 ± 0.6
Javelin 0.3 + refuge	641 ± 62.5	62.0	1,120 ± 72	10.9 ± 1.2
Xentari 1.12 kg/ha	481 ± 58.1	71.4	1,123 ± 57	56.5 ± 0.6
		Rainy Season		
Control	1,747 ± 199	—	1,000 ± 71	2.4 ± 1.0
Javelin 1.12 kg/ha	524 ± 41	70.1	1,544 ± 85	78.8 ± 5.8
Javelin 1.12 + refuge	557 ± 120	68.2	1,066 ± 65	65.8 ± 5.8
Javelin 0.3 + kg/ha	461 ± 76	73.6	1,201 ± 85	89.4 ± 2.3
Javelin 0.3 + refuge	679 ± 33	61.1	1,274 ± 87	45.4 ± 2.0
Xentari 1.12 kg/ha	645 ± 134	63.1	1,537 ± 70	79.6 ± 3.8

[a] Cumulative *P. xylostella*-days plant (all instars and pupae included) over a sampling period of 23 and 51 d for the dry and rainy seasons, respectively.
[b] Average efficacy of treatments relative to control: [(CPXDs in the treatment—CPxDs in the control)/CPxDs in the control] × 100.
[c] Mean weight of cabbage heads (n = 102–108 per treatment).
[d] Average % ± SEM.
[e] Indicates presence of 25% refuge (1 row of cabbage untreated).

average infestation in the treated plots ranged from 9.0 to 13.3 individuals per plant per day. The overall reduction in *P. xylostella* infestation induced by foliar sprays of *B. thuringiensis* ranged from 57.4 to 71.4% and 61.1 to 73.6% in the dry and rainy seasons, respectively.

In the dry-season experiment, CPxDs were significantly correlated with cabbage head weight and quality ($P < 0.018$; Table 5), whereas in the rainy-season trial, CPxDs were significantly correlated with cabbage quality only ($P = 0.0001$). ANOVA confirmed that cabbage head weight varied significantly across treatments during the dry-season experiment ($F = 10.7$; df = 5, 10; $P = 0.001$) but did not vary significantly across treatments during the rainy-season trial ($F = 2.9$; df = 5, 10; $P = 0.069$). Based on nonorthogonal contrasts, the lower yield observed in the control plots during the dry-season trial accounted for most of the variation in cabbage head weight; no significant differences were observed in cabbage head weight across treatments with *B. thuringiensis* ($P > 0.121$).

Cabbage quality was more sensitive than cabbage weight to the infestations of *P. xylostella* across treatments. Compared with the dry-season trial, a higher proportion of marketable cabbage was produced in plots treated with *B. thuringiensis* during the rainy-season experiment. This difference may

Table 4. Comparison of treatments by nonorthogonal contrasts

Contrasts[a]	CPxDs[b]		Cabbage wt[c]		Marketable cabbage	
	F	P[d]	F	P[d]	F	P[d]
			Dry season			
1	47.5	<0.0001	15.9	0.003	9.7	0.011
2	1.4	0.273	2.9	0.121	13.6	0.004
3	<1	0.99	<1	0.818	6.1	0.008
4	2.8	0.128	<1	0.474	12.7	0.005
5	<1	0.493	<1	0.535	<1	0.594
ANOVA[e]	10.2	0.001	10.7	0.001	6.1	0.008
			Rainy season			
1	36.7	0.0001	—	—	36.9	0.0001
2	<1	0.775	—	—	<1	0.820
3	1.6	0.241	—	—	6.2	0.033
4	<1	0.626	—	—	<1	0.496
5	<1	0.474	—	—	<1	0.617
ANOVA[e]	8.0	0.003	2.9	0.069	9.1	0.002

[a] 1, control versus all other treatments; 2, effect of dose of Javelin regardless of refuge (0.3 versus 1.12 kg/ha; 3, effect of refuge regardless of the dose of Javelin; 4, Xentari 1.12 kg/ha versus Javelin 0.3 kg/ha regardless of refuge; 5, Xentari 1.12 kg/ha versus Javelin 1.12 kg/ha regardless of refuge.
[b] Cumulative *P. xylostella*-days per plant; all instars included.
[c] Average weight (grams) of cabbage heads.
[d] If $P > 0.01$, treatments were not significantly different.
[e] df = 5, 10; if $P > 0.05$, treatment effects were not significantly different (SAS Institute 1989).

Table 5. Correlation between cumulative *P. xylostella*-days (CPxDs), and weight and quality of cabbage heads

	Dry season		Rainy season	
	r^a	P^b	r^a	P^b
Cabbage wt	−0.62	0.007	−0.28	0.26
Quality	0.55	0.018	0.86	0.0001

[a] Correlation coefficients.
[b] Correlation coefficients with $P > 0.05$ are not significantly different (SAS Institute 1989).

be explained by lower overall infestation of *P. xylostella,* which may have been the result of different weather patterns during the 2 seasons, especially rainfall. Rainfall is an important mortality factor for *P. xylostella* (Talekar and Shelton 1993) and may have contributed to lower infestations throughout the crop season.

The proportion of marketable cabbage varied significantly across treatments during both experiments ($F < 0.008$; df = 5, 10; $P < 0.008$; Table 4), with significantly lower quality produced in the control plots ($P < 0.011$). In the dry-season experiment, Javelin at 1.12 kg/ha and Xentari at 1.12 kg/ha produced significantly higher quality of cabbage than did Javelin at 0.3 kg/ha ($P = 0.004$ and $P = 0.005$), regardless of the presence of refuge. The plots treated with Javelin at 1.12 kg/ha, including those with 25% refuge, and Xentari produced similar proportion of marketable cabbage ($P > 0.594$). Although it is apparent that the presence of refuge did not affect the proportion of marketable cabbage produced in the treatments with Javelin at 0.3 kg/ha, the combination of the treatments with Javelin where a 25% refuge was present produced a much lower proportion of marketable cabbage than plots treated with Javelin without refuge ($P = 0.008$). Such difference was not observed during the rainy-season experiment ($P = 0.033$).

Despite the absence of significant statistical difference during the rainy-season experiment, our data suggests that the deliberate inclusion of a refuge may reduce the proportion of marketable produce and may affect adoption of this resistance management strategy in both sprayed *B. thuringiensis* and transgenic crops expressing *B. thuringiensis* toxins (Kennedy and Whalon 1995). Refuges are necessary to manage resistance but may require some pest management action to be cost-effective, otherwise the infestations of the key pest may cause a significant reduction of benefits.

Although the initial infestations of *P. xylostella* before selection with *B. thuringiensis* (20 larvae per plant) appear to be high, they were not unrealistic for tropical areas. During field experiments with cabbage conducted by Dickson et al. (1990) at the same site of our field experiments, average larval counts per plant in 3 commercial cultivars during the dry- and rainy-seasons were >95 and 10, respectively. In a study of the geographical distribution of susceptibility to *B. thuringiensis* in Central America (Perez and Shelton 1997), we visited crucifer fields in Honduras, Nicaragua and Guatemala; in some cases, we observed 30–90 larvae and pupae in individual plants.

Crop losses caused by the evolution of resistance are very likely if *B. thuringiensis* is the only *P. xylostella* management tool (Knight and Norton 1989). Because we were unable to obtain >60% marketable cabbage during the dry season, farmers who rely on applications of *B. thuringiensis* subsp. *kurstaki* for control of *P. xylostella* may experience crop losses from evolution of resistance to *B. thuringiensis* subsp. *kurstaki.* Despite the absence of resistance to *B. thuringiensis* subsp. *aizawai* in the population that was released in the field cages, crop quality was not significantly improved when *B. thuringiensis* subsp. *aizawai* was applied frequently.

The release of resistant insect genotypes into experimental fields and insufficient isolation between treatments are concerns that make tests of resistance management strategies difficult in the field (Tabashnik 1994). The field-cage method that we used helped cope with those 2 concerns and may be used in further investigations on resistance management strategies, including crucifers expressing *B. thuringiensis* toxins (Metz et al. 1995). One possible drawback of the field-cage method used in this study is that it does not reflect mortality of *P. xylsotella* induced by parasitism and predation, but these are more site-specific factors.

The evolution of insecticide resistance in *P. xylostella* has become a major constraint for crucifer production worldwide (Talekar and Shelton 1993). Resistance management cannot depend solely on pesticide applications. Because resistance to *B. thuringiensis* has evolved in several geographically isolated populations of *P. xylostella,* and resistant *P. xylostella* may reproduce in transgenic crucifers expressing *B. thuringiensis* toxins (Metz et al. 1995), any proposed deployment of transgenic crucifers should be preceded by monitoring for resistance to insecticidal crystal proteins expressed by these crops.

Finally, our results emphasize that pest management tactics for *P. xylostella* should be based on biological control, cultural control, resistant cultivars, and the use of other biorational pesticides. Currently, crucifer growers in the region where this study was undertaken appear to have limited viable options at the moment for managing *P. xylostella.* Neither *B. thuringiensis* subsp. *aizawai* nor low rates of *B. thuringiensis* subsp. *kurstaki* appear to provide adequate control, yet the high rates of *B. thuringiensis* subsp. *kurstaki* needed for adequate control seem to accelerate further resistance.

Acknowledgments

We are grateful to Alfredo Montes (Panamerican School of Agriculture in Honduras) for his support and recommendations for the field experiments. This research was supported in part by the *Bacillus thuringiensis* Manage-

ment Working Group, the University Development Linkages Program of the Cornell International Institute for Food and Agricultural Development of Cornell University, and the World Bank Graduate Scholarship Program. This work was part of a dissertation submitted to the Graduate School of Cornell University by C.J.P. in partial fulfillment of the requirements for a Ph.D. degree.

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Received for publication 12 September 1996; accepted 22 May 1997.