Resistance of Diamondback Moth (Lepidoptera: Plutellidae) to *Bacillus thuringiensis* Subspecies in the Field

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ABSTRACT Eleven populations of diamondback moth, *Plutella xylostella* (L.), were collected in 1990 from *Brassica* plants in six states of the United States and in Indonesia and tested for their responses to two formulations of *Bacillus thuringiensis* subsp. *kurstaki* (Javelin WG and Dipel 2X), permethrin, and methomyl. Populations from Florida that had been treated extensively over several years with these insecticides displayed significantly higher LC_{50}s. In 1992, field tests in geographically separate areas in Florida and laboratory assays of populations from those fields indicated control failures and resistance to products containing *B. thuringiensis* subsp. *kurstaki* and low levels of resistance to a product containing *B. thuringiensis* subsp. *aizawai* (XenTari). These *B. thuringiensis* subspp. differ in the number of toxins produced, but whether resistance to them is a result of cross-resistance or independent selection was not determined. We documented significant differences between the response of resistant and susceptible populations to two products containing *B. thuringiensis* subsp. *kurstaki*, thus suggesting that the products actually differed in the number or amounts of toxins. In laboratory bioassays of three products containing *B. thuringiensis* subsp. *aizawai* and two products containing *B. thuringiensis* subsp. *kurstaki*, the variation in response (as determined by resistance ratios) varied by 321- to 461-fold for *B. thuringiensis* subsp. *kurstaki* and by 3- to 4.1-fold for *B. thuringiensis* subsp. *aizawai*. These studies indicate increasing resistance problems caused by intensive use of any *B. thuringiensis* product. We conclude that if *B. thuringiensis* is to remain a durable insecticide in parts of the world where resistance does not already occur, other tactics such as biological control, host-free periods, plant resistance, and cultural controls must be incorporated into the management programs.

KEY WORDS *Bacillus thuringiensis*, resistance, diamondback moth

DIAMONDBACK MOTH, *Plutella xylostella* (L.), a cosmopolitan defoliator of brassica crops, is the most destructive insect of crucifers worldwide; the annual cost for managing this species is estimated to be U.S. $1 billion (Talekar & Shelton 1993). In tropical regions, the diamondback moth has developed resistance to a large number of synthetic insecticides (Mayata et al. 1986) because of the continuous production of crucifers and the heavy use of insecticides. In 1981, Georghiou reported that diamondback moth was resistant to 36 insecticides in 14 tropical countries. In North America, diamondback moth is a sporadic pest of cruciferous crops (Harcourt 1954, Baker et al. 1982). Until recently, synthetic pesticides have adequately controlled populations (Shelton et al. 1993). However, control failures in several states (including Florida, Georgia, North Carolina, Texas, Wisconsin, and New York) prompted the creation of a resistance monitoring project in 1988–1989. When 41 populations from 19 states were tested for resistance to methomyl, permethrin, and methamidophos (Shelton et al. 1993), many populations displayed resistance to one or more of the insecticides.

Resistance to conventional insecticides and concern about their environmental effects has led to increased interest in use of *Bacillus thuringiensis* for insect control. However, laboratory selection of a population of Indianmeal moth, *Plodia interpunctella* (Hübner), collected from grain silos in which *B. thuringiensis* subsp. *kurstaki* had been used extensively resulted in rapid and high levels of resistance (McGaughey 1985). Although resistance to *B. thuringiensis* subsp. *kurstaki* has occurred in laboratory populations of several species, Georghiou (1990) reported that no serious control failures in the field have been documented despite the use of *B. thuringiensis* for >20 yr.

In 1990, Tabashnik et al. reported results from a survey of responses of diamondback moth collected from commercial fields of watercress, cab-

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bage, and broccoli in Hawaii. In laboratory bioassays, diamondback moth collected from watercress fields that had been heavily treated with *B. thuringiensis* subsp. *kurstaki* had LC_{50}s and LC_{95}s that were 25 and 33 times, respectively, greater than those of two susceptible laboratory colonies reared for 13 or 60 generations on cabbage. Nine generations of selection against *B. thuringiensis* subsp. *kurstaki* in the laboratory resulted in significantly higher resistance levels although five additional applications in the field did not (Tabashnik et al. 1991).

Documentation of field resistance to any insecticide is difficult but necessary because laboratory assays may not reflect the performance of insecticides under field conditions (Dennehy 1987). Tabashnik et al. (1990) noted that the watercress grower suspected resistance to *B. thuringiensis* subsp. *kurstaki* but had no data to indicate a decline in effectiveness over time. Kirsch & Schmutterer (1988) reported failure of *B. thuringiensis* subsp. *kurstaki* to control diamondback moth in the Philippines, but did not provide data that could eliminate factors other than resistance (e.g., unfavorable environmental conditions, poor coverage). Such information is needed to document resistance. Because of the increasing reliance on *B. thuringiensis* as an alternative to traditional synthetic insecticides, studies to verify resistance to this material must be done. This is especially relevant because biotechnology companies have committed substantial resources to the development and modification of existing *B. thuringiensis* products, and *B. thuringiensis*-transgenic plants will be made available soon. Studies on resistance are necessary to exclude as many epigenetic factors as possible from laboratory experiments, and laboratory data should be related to field performance.

Insecticidal activity of *B. thuringiensis* results from ingestion of crystal proteins, and different *B. thuringiensis* subspecies produce crystal proteins with different insecticidal spectra (Hofte & Whiteley 1989). Before 1991, commercial *B. thuringiensis* products for control of Lepidoptera contained only the subspecies *kurstaki,* which produces the crystal proteins CryIA(a), CryIA(b), CryIA(c), CryIIA, and CryIIB. Beginning in 1990, an experimental use permit was granted for XenTari (Abbott Laboratories, North Chicago, IL) to be used in several states including Florida. This product contains *B. thuringiensis* subsp. *aizawai,* which produces CryIA(a), CryIA(b), CryIC, CryID, and CryIIB.

Beginning in 1990, we conducted studies to compare the responses of 11 populations of diamondback moth to two formulations of *B. thuringiensis* subsp. *kurstaki,* a pyrethroid, and a carbamate. Some populations were selected from or near locations where high levels of resistance to selected synthetic insecticides have been documented (Shelton et al. 1993). Our collections were made over a large geographic area, and all insects originated from *Brassica.* As much as possible, we related responses in the laboratory to reports about use patterns and control problems in the areas of collection. We continued this study in 1992 in Florida and included field and laboratory trials to compare responses to products that contained either *B. thuringiensis* subsp. *kurstaki* or *B. thuringiensis* subsp. *aizawai.*

Materials and Methods

Insects. In 1990, nine populations of diamondback moth were established from commercial cabbage fields in six states (California, Florida, Georgia, New York, North Carolina, and Texas), one from a commercial cabbage field in Indonesia, and one from wild *Brassica campestris* L. in a nature preserve in Pensaquitos Canyon near San Diego, CA. From 50 to 400 larvae were collected at each site and then were transferred to the New York State Agricultural Experiment Station, Geneva, NY, where they were reared on rape (*Brassica napus* L.) seedlings (Shelton et al. 1991).

In 1992, we established a total of five populations from commercial fields in Florida as described above. Two populations (Zellwood and Parrish) were collected from commercial cabbage fields; two populations, Loxahatchee (a) and Loxahatchee (b), were collected from commercial fields of kohlrabi; and one population (Belle Glade) was collected from a commercial field of Chinese cabbage. A susceptible population (Geneva 1988) was used as our standard for comparison. This population was collected in 1988 from a cabbage research field near Geneva, NY, and reared continuously in our laboratory on rape seedlings.

Insecticides. For the 11 populations collected in 1990, bioassays were performed for two formulations of *B. thuringiensis* subsp. *kurstaki* (Dipel 2X, 6.4% AI, Abbott Laboratories, North Chicago, IL; Javelin WG, 6.4% AI, Sandoz, Des Plaines, IL). Dipel 2X and Javelin WG contained 32,000 International Units (IU) per mg. We also tested the pyrethroid permethrin (Ambush 2E; ICI Americas, Goldboro, NC) and the carbamate methomyl (Lannate 1.8L; E. I. du Pont de Nemours, Wilmington, DE). All materials were diluted in distilled water. For the five populations collected in 1992 and our standard susceptible population, bioassays were done for Dipel 2X, Javelin WG, and a commercial formulation of *B. thuringiensis* subsp. *aizawai* (XenTari, 3% AI, Abbott Laboratories, North Chicago, IL). XenTari contained 15,000 IU per mg.

In another series of separate tests we investigated the responses of the Loxahatchee (a) and Geneva 1988 populations against products that contained either *B. thuringiensis* subsp. *kurstaki*

or *B. thuringiensis* subsp. *aizawai*. The *B. thuringiensis* subsp. *aizawai* products, NB200 (Entotech, Davis, CA), Agree (CIBA-GEIGY, Greensboro, NC), and XenTari, were compared with Javelin WG and Biobit HP (E. I. du Pont de Nemours, Wilmington, DE).

Leaf-Dip Bioassay. We used a method similar to that described by Tabashnik et al. (1987). Leaves from the outer layers of cabbage heads (not including wrapper leaves) were cut into disks (7.5 cm diameter). Each disk was dipped into the test solution for 10 s, held vertically to allow excess solution to drip off, and placed on a rack to dry. After 2 h drying time, the disks were placed in petri dishes and five late second instars (weighing 0.4 mg) were added. Dishes were placed in a rearing room at 28°C. Larval mortality was assessed after 48 h for permethrin and methomyl and after 96 h for the *B. thuringiensis* formulations. (Our previous studies with Dipel 2X, Javelin WG, XenTari, methomyl, and permethrin indicated that mortality reached a plateau at those times.) Larvae were considered dead if they did not move when prodded.

Concentrations (mg [AI]/ml), based on a dilution series using a "field" rate of 280 liters of water per hectare, tested in 1990 were as follows: permethrin: 5.62, 1.78, 0.56, 0.18, 0.056, and 0.018; methomyl: 100, 17.78, 3.16, 0.56, 0.1, and 0.018. For Dipel 2X and Javelin WG, concentrations (mg [AI]/liter) were 1,280, 128, 12.8, 1.28, and 0.128. Experiments with each concentration and the untreated control (distilled water and sticker spreader) were replicated five times. Overall control mortality was 4.8%, with a range of 0–16%. In 1992, a preliminary test was done with each compound on a small group of individuals in each population so that an appropriate range of concentrations (10–90% mortality range) could be selected for a full-scale test. Thus, depending on the population, concentrations for Dipel 2X and Javelin WG varied from 0.006 to 64. For XenTari, concentrations varied from 0.003 to 1. Experiments with each concentration and the untreated control were replicated 10 times on different days, except for the Belle Glade population, which was replicated five times. Overall control mortality was 5.9%, with a range of 2–10%. In 1992 the same batch of each insecticide was used for laboratory assays and the field tests described below. In both years five drops of Bond sticker spreader (Loveland Industries, Loveland, CO) per 100 ml of water (0.1% vol/vol) were added to each test solution. In 1990, all populations, except Hilton, NY, were tested in the first three generations. The Hilton population was tested in the ninth laboratory generation. In 1992, all populations were tested in the second generation.

Statistical Analyses. All data were first plotted, then analyzed by logit regression with the model $y_i = \alpha + \beta \log(c_i)$ where y is the logit of response, α is the intercept, β is the slope of the regression, and c_i is the i^{th} concentration. A population's response to a particular insecticide was considered significantly different from another population's response if their LC_{50} confidence intervals at the 90 or 95% level did not overlap.

In several analyses of the *B. thuringiensis* data in 1990, logit regression did not fit the data because only two or three response levels (almost no response at lower concentrations and almost 100% response at higher concentrations) were present. When the model did not fit, the estimated probabilities of response were calculated by fitting logit models with categorical concentration-response (i.e., $y_i = \alpha + \beta_k$ where $k = 2$ if there were only two response levels, one for low concentrations and one for high concentrations), or $k = 3$ if there were three response levels. Logit regression models or categorical concentration logit models were estimated with the computer program GLIM (Baker & Nelder 1978).

In 1992, all data were analyzed with logit models using the POLO program (Russell et al. 1977). Because of a more appropriate selection of concentrations, categorical concentration logit models were not used. Resistance ratios (RRs), the ratio of the LC_{50} of a given population to that of the susceptible population (Geneva 1988), were calculated.

Field Tests. In 1992, field tests were done at four sites in the main cabbage-growing areas of central Florida. Field tests were conducted at the same sites where larvae were collected for the 1992 laboratory assays (i.e., in the two fields in Loxahatchee and the single fields in Zellwood and Parrish). No tests were performed at the Belle Glade field because of low populations. In each field we used a randomized complete-block design with four blocks. We tested Javelin WG, Dipel 2X, and XenTari at the recommended field rates of 1.12, 1.12, and 0.56 kg formulated material per ha, respectively. Plot size varied between fields but was at least 5 m long and 6 m wide with untreated borders on all sides. A single treatment was applied to each plot between 13 and 14 April. Treatments were applied with a CO_2 backpack sprayer at 2.8 kg/cm^2, 467 liters/ha, and four hollow-cone nozzles per row (two over the top and two drop nozzles). Nu-film 17 was added at 1/4% vol/vol. One week after treatments a single evaluation was done by selecting three plants per plot and counting and classifying the stadium of each larva and enumerating the pupae of diamondback moth. In 1991, we conducted a trial in Geneva, NY, at the Vegetable Research Farm of the New York State Agricultural Experiment Station. The test was similar except that we artificially infested the cabbage plants. Three plants per plot were inoculated with 50 eggs from the Geneva 1988 culture on 16 July, a single treatment was applied on 25 July,

Table 1. Susceptibility of diamondback moth populations to Dipel 2X and Javelin WG

Insecticide	Population[a]	Slope ± SE	LC_{50} (95% CL) mg(AI)/liter
Dipel 2X	Belle Glade, FL	2.14 ± 2.12	0.12 (0.03– 0.42)
	Rio Grande, TX	1.92 ± 1.69	0.10 (0.03– 0.36)
	Fletcher, NC	0.91 ± 0.23	0.23 (0.08– 0.64)
	Hilton, NY	0.94 ± 0.10	0.51 (0.32– 0.80)
	Zellwood, FL	0.66 ± 0.16	4.15 (0.94– 18.4)
	Sanford, FL	0.86 ± 0.08	6.27 (3.72– 10.5)
	Sarasota, FL	0.44 ± 0.13	6.68 (1.02– 43.5)
	Albion, NY	1.04 ± 0.22	21.4 (7.71– 59.6)
	Bogor, Indonesia	[b]	0.13[c]
	Tifton, GA	[b]	0.13[c]
	Penasquitos, CA	[b]	2.00[c]
Javelin WG	Fletcher, NC	1.21 ± 0.28	0.21 (0.11– 0.42)
	Hilton, NY	0.88 ± 0.10	0.47 (0.28– 0.79)
	Sanford, FL	0.54 ± 0.08	4.84 (1.88– 12.5)
	Sarasota, FL	0.53 ± 0.09	5.48 (1.94– 15.5)
	Zellwood, FL	0.66 ± 0.18	11.94 (2.17– 65.6)
	Albion, NY	0.77 ± 0.22	27.54 (5.08–149)
	Bogor, Indonesia	[b]	0.13[c]
	Tifton, GA	[b]	0.13[c]
	Belle Glade, FL	[b]	0.13[c]
	Penasquitos, CA	[b]	0.13[c]
	Rio Grande, TX	[b]	0.13[c]

[a] Sample size for each experiment was 150. Mortality was assessed 96 h after treatment.
[b] Slope for a logit regression could not be estimated because the lowest concentration caused about 100% mortality.
[c] 95% CL for the LC_{50} could not be estimated because of extremely poor fit of the probit regression model.

and the inoculated plants were evaluated on 31 July.

For analysis we only included the third and fourth instars and pupae because earlier instars would probably not have been subjected to the original spray or its residue. When necessary, data were transformed (log), then analyzed by analysis of variance. When treatments were significant, their means were separated ($P = 0.05$) with Tukey's procedure (SAS Institute 1988).

Results and Discussion

1990 *B. thuringiensis*. Within the 11 geographically isolated populations there were significant differences in response to *B. thuringiensis* subsp. *kurstaki*. Patterns of response to Javelin WG and Dipel 2X were the same for all locations and we identified two distinct patterns (Table 1). Populations from Belle Glade, FL; Rio Grande, TX; Fletcher, NC; Hilton, NY; Bogor, Indonesia; Tifton, GA; and Penasquitos, CA, were significantly more susceptible to Javelin and Dipel 2X than diamondback moth larvae from three locations in Florida (Sanford, Sarasota, and Zellwood) and Albion, NY.

Past usage patterns of *B. thuringiensis* in the areas in which we collected populations provide insights about the development of these differences. Reports from crop consultants and growers indicate that Dipel 2X and Javelin WG have been used as a tank mix with a pyrethroid, organophosphate, or carbamate in the Zellwood, FL, area since 1987 (Kevin Short, Sarasota, FL, personal communication). These mixes have often been applied weekly or even more frequently. For the first 3 yr, the mixture was applied by air (an ineffective method, especially because diamondback moth feeds on the undersides of the leaves); in 1989 the mixture was applied by ground sprayer. A consultant (Kevin Short) said that he was not surprised by our data because the ground sprays were not working well. Part of the reason for control failures in the Zellwood area is that there is little if any crucifer-free growing period. This results in more generations being produced, higher populations, and more selection pressure for resistance.

The Sarasota and Sanford areas have shorter crucifer growing seasons than Zellwood and have traditionally had lower diamondback moth populations. In addition, before 1990, *B. thuringiensis* was not used as extensively or for as long in these areas (Kevin Short, personal communication). Despite these factors, LC_{50} values were not significantly different than in the Zellwood population.

The Albion, NY, population, which had the highest LC_{50}, originated from transplants that were shipped from a greenhouse in Bradenton, FL. The transplant grower in Florida had sprayed his greenhouse approximately three times per week; Javelin WG or Dipel 2X was usually included in each spray. In a single set of transplants, plants may have been sprayed 15 times. When a sequence of cabbage transplants are grown in the same greenhouse (as often occurs), the diamondback moth population may be subjected to about 50 sprays during a 3-mo pe-

riod. Because of the concentration of transplants and sprays in greenhouses, greenhouses may play a major role in the development and distribution of *B. thuringiensis* resistance.

The population from Hilton, NY, was significantly more susceptible to *B. thuringiensis* than the Zellwood, Sarasota, Sanford, and Albion populations. This greater susceptibility could be the result of testing this population in the ninth generation rather than in the first three, or may be a result of the cabbage in Hilton being transplanted with plants that originated near Tifton, GA, where *B. thuringiensis* had not been used extensively for control of diamondback moth (Richard Chalfant, personal communication). In addition, this field was treated only once with *B. thuringiensis* products during the cropping period before our collections (A.M.S., personal observation).

The cabbage field in Rio Grande, TX, had been treated in a manner similar to that documented by Magaro & Edelson (1990) (i.e., with pyrethroids, carbamates, and organophosphates). Unconfirmed reports from that area indicate high levels of resistance to these compounds, resulting in several growers now using *B. thuringiensis* products or mass releases of *Cotesia plutellae* (Kurdjumov) for control of diamondback moth.

The population from Indonesia was very susceptible to *B. thuringiensis* products. A previous report by Sastrodihardjo (1986) also indicated that diamondback moth from Indonesia was resistant to many synthetic insecticides, but not to *B. thuringiensis*.

Over all 11 populations, we found that much of the susceptibility to *B. thuringiensis* could be explained by past patterns of use. In their survey, Tabashnik et al. (1990) also noted in Hawaii that diamondback moth from fields that were heavily treated with *B. thuringiensis* products had elevated LC_{50}s; however, such values only occurred in watercress fields. Whether this was caused by an increased use of *B. thuringiensis* in watercress fields or by the influence of host origin merits further investigation. In our study, elevated LC_{50}s appear to be linked with use patterns, apart from any influence of host. Differences between LC_{50}s estimated from our study and those reported by Tabashnik et al. (1990) may reflect our use of a longer time (96 h compared with 48 h) before mortality was assessed after treatment. We expected our results after 96 h to give lower LC_{50}s; for all populations that we tested, this was correct. The highest value that we observed, 27.5, was about twice as low as the highest value reported by Tabashnik et al. (1990), but direct comparisons between LC_{50}s in the two studies are not possible because of differences in observation times.

A difficulty of our 1990 study is that LC_{50}s for *B. thuringiensis* could not be estimated accurately for all 11 populations. Our use of one population (Zellwood) to choose the concentrations for all subsequent tests resulted in some concentrations that were too high for certain other populations. Although we thought that it was important to test the populations as soon as possible after they came from the field, it would have been advisable to stagger the individuals within a generation so that an initial test to find the appropriate dose range could have been done on the early individuals. Despite this limitation in methods, use of the categorical logit model permitted the analyses of the resultant data.

1990 Methomyl and Permethrin. Significant differences in response to methomyl and permethrin were apparent in several populations (Table 2). The highest LC_{50}s for methomyl were associated with plants grown in Florida (i.e., Sarasota, Sanford, Zellwood) and Albion, NY. With the exception of Hilton, NY, the five highest LC_{50}s for permethrin also occurred in these Florida populations. Four of the five populations that had the highest LC_{50}s for methomyl also had the highest values for permethrin. This may or may not be the result of cross or multiple resistance in diamondback moth (Sun 1992). More interesting, however, is the fact that of these five populations four were the same as those that had the highest LC_{50}s for *B. thuringiensis*. Because the mechanism for resistance to *B. thuringiensis* in diamondback moth is thought to be caused by lack of binding of the crystal proteins to the brush-border membrane, either because of greatly reduced binding affinity or the complete absence of the receptor molecule (Ferre et al. 1991), it is unlikely that cross-resistance or multiple resistance developed between *B. thuringiensis* and permethrin or methomyl. It is most likely that resistance first developed to permethrin and methomyl. When they failed, products containing *B. thuringiensis* became more widely used, and resistance to them developed.

1992 Collections. Patterns of response to Javelin WG and Dipel 2X were generally similar, with the highest LC_{50}s for populations from the two Loxahatchee and Zellwood fields and lower values for the Parrish and Belle Glade populations (Table 3). Unlike the 1990 data, much higher LC_{50}s were obtained for Javelin WG than for Dipel 2X. For Javelin WG, the Loxahatchee (a) population was significantly more resistant than all but the Loxahatchee (b) population and had an RR value of 1,641. Both the Parrish and Belle Glade populations were significantly more susceptible than either of the Loxahatchee populations. For Dipel 2X, both Loxahatchee populations and the Zellwood population were significantly less susceptible than the Belle Glade population. The Loxahatchee (a) population was significantly less susceptible than the Parrish and Belle Glade populations. Comparing the Dipel 2X and Javelin WG data, we noted that the

Table 2. Susceptibility of diamondback moth populations to methomyl and permethrin

Insecticide	Population[a]	Slope ± SE	LC_{50} (95% CL) mg(AI)/mg
Methomyl	Penasquitos, CA	0.47 ± 0.29	0.06 (0.003– 1.34)
	Bogor, Indonesia	0.50 ± 0.21	0.14 (0.01 – 1.93)
	Hilton, NY	1.03 ± 0.21	0.37 (0.14 – 0.99)
	Rio Grande, TX	0.97 ± 0.17	1.18 (0.50 – 2.80)
	Fletcher, NC	0.90 ± 0.19	1.92 (0.63 – 5.87)
	Tifton, GA	0.81 ± 0.16	2.46 (0.81 – 7.47)
	Zellwood, FL	0.93 ± 0.26	7.38 (1.91 – 28.5)
	Sanford, FL	1.62 ± 0.17	13.5 (9.9 – 18.5)
	Sarasota, FL	0.86 ± 0.32	47.8 (7.0 –311)
	Albion, NY	0.86 ± 0.31	52.5 (8.09 –340)
	Belle Glade, FL	[b]	[b]
Permethrin	Penasquitos, CA	1.47 ± 0.13	0.007 (0.005– 0.009)
	Bogor, Indonesia	0.93 ± 0.19	0.04 (0.02 – 0.09)
	Rio Grande, TX	1.30 ± 0.33	0.21 (0.087– 0.53)
	Tifton, GA	2.43 ± 0.37	0.35 (0.25 – 0.48)
	Fletcher, NC	1.93 ± 0.33	0.43 (0.28 – 0.68)
	Zellwood, FL	1.30 ± 0.22	0.45 (0.24 – 0.82)
	Sanford, FL	1.71 ± 0.35	0.47 (0.26 – 0.85)
	Hilton, NY	1.78 ± 0.72	0.65 (0.22 – 1.98)
	Sarasota, FL	2.43 ± 0.35	0.67 (0.50 – 0.90)
	Albion, NY	1.55 ± 0.67	1.66 (0.51 – 5.44)
	Belle Glade, FL	[b]	[b]

[a] Sample size for each experiment was 175. Mortality was assessed 48 h after treatment.
[b] Not tested.

LC_{50}s for Javelin WG tended to be lower than those for Dipel 2X in the more susceptible populations (Parrish, Belle Glade, and Geneva 1988). For the more resistant Loxahatchee populations, however, Javelin had higher LC_{50}s. A likely explanation is that, although the products claim to contain the same toxins derived from *B. thuringiensis* subsp. *kurstaki*, one product may not contain all the toxins or may contain a different amount of a particular toxin. The presence of a higher concentration of one toxin in Javelin may cause lower LC_{50}s in the more susceptible populations, but a lack of (or lower concentration of) another toxin in Javelin may account for higher LC_{50}s in the resistant populations. Previous studies by Ferre et al. (1991) indicated that different crystal proteins of *B. thuringiensis* act at different target sites of diamondback moth. Loss of a gene that codes for the CryIA(b) toxin in the HD-1 strain of *B. thuringiensis* subsp. *kurstaki* has been documented (Wilcox et al. 1986), and such loss may diminish toxicity (Moar et al. 1990). Whether this is the case in our tests is not clear.

Table 3. Laboratory assays of *Bacillus thuringiensis* products against diamondback moth populations

Insecticide	Population[a]	Slope ± SE	LC_{50} (90% CL) mg(AI)/liter	RR[b]
Javelin WG	Geneva 1988	2.58 ± 0.34	0.02 (0.01– 0.03)	1
	Belle Glade	0.9 ± 0.2	0.14 (0.01– 0.52)	7
	Parrish	0.54 ± 0.1	0.65 (0.35– 1.13)	32
	Zellwood	0.45 ± 0.08	2.73 (0.81–16.58)	136
	Loxahatchee (b)	0.45 ± 0.08	12.48 (4.64–69.82)	624
	Loxahatchee (a)	1.29 ± 0.29	32.83 (22.27–53.95)	1641
Dipel 2X	Geneva 1988	1.84 ± 0.87	0.32 (0.20– 0.49)	1
	Belle Glade	0.89 ± 0.12	0.43 (0.25– 0.69)	1.3
	Parrish	1.45 ± 0.23	2.51 (1.71– 3.50)	7.8
	Zellwood	1.11 ± 0.15	4.74 (2.69– 9.47)	15
	Loxahatchee (b)	1.05 ± 0.18	4.15 (1.54– 8.77)	13
	Loxahatchee (a)	1.19 ± 0.32	7.04 (3.99–12.42)	22
XenTari WDG	Geneva 1988	1.56 ± 0.16	0.06 (0.04– 0.10)	1
	Belle Glade	1.81 ± 0.36	0.14 (0.06– 0.28)	2.3
	Parrish	1.15 ± 0.14	0.33 (0.22– 0.58)	5.5
	Zellwood	1.57 ± 0.41	0.29 (0.17– 0.41)	4.8
	Loxahatchee (b)	1.48 ± 0.19	0.17 (0.10– 0.28)	2.8
	Loxahatchee (a)	1.61 ± 0.29	0.50 (0.33– 0.92)	8.3

[a] Sample size for each population was 261 to 434, except for Belle Glade, which was 106 and 128 for Dipel 2X and Javelin WG, respectively. Mortality was assessed 96 h after treatment.
[b] Resistance ratio (RR) is calculated by dividing the LC_{50} obtained for a particular population by the LC_{50} for the susceptible laboratory colony (Geneva 1988).

Table 4. Field tests of *Bacillus thuringiensis* products against five populations of diamondback moth

Insecticide	kg/ha	Mean ± SEM of DBM[a] per 3 plants				
		Zellwood	Loxahatchee (a)	Loxahatchee (b)	Parrish	Geneva
XenTari WDG	0.6	30.2 ± 7.3b	21.5 ± 12.3b	29.5 ± 13.4b	26.7 ± 4.8a	[b]
Dipel 2X	1.1	58.0 ± 19.3a	45.2 ± 19.7ab	72.3 ± 8.9a	30.5 ± 18.2a	2.5 ± 1.9b
Javelin WG	1.1	54.7 ± 11.0a	58.7 ± 8.5a	61.2 ± 16.8ab	45.0 ± 21.9a	3.0 ± 2.1b
Untreated		74.5 ± 14.4a	46.7 ± 17.0ab	67.6 ± 16.4ab	42.5 ± 6.9a	21.2 ± 11.7a

Means in each column followed by the same letter are not significantly different ($P = 0.05$) by Tukey's test (SAS Institute 1988).
[a] Third and fourth instars and pupae.
[b] Not tested.

For XenTari, significant differences between LC_{50}s were apparent between the Loxahatchee populations and between the Loxahatchee (a), Belle Glade, and Geneva 1988 populations. Variation in response, as noted by RR values between populations, was much lower than for Dipel 2X or Javelin. Whether variation in responses was a result of more intensive field use or other factors is not clear, but these data provide evidence that geographically isolated populations display significant differences in their responses to XenTari after only 2 yr of use.

1992 Field Tests. In all four field trials in Florida, XenTari provided better control than Javelin or Dipel 2X, although differences were not always significant (Table 4). Only in the Parrish location were no significant differences detected between the untreated control and at least one of the *B. thuringiensis* subsp. *kurstaki* products. In Parrish, we observed no significant differences between XenTari and the other treatments and the untreated control. This may be the result of >2 cm of rain falling on the days before and during evaluation. Rainfall is a major mortality factor for diamondback moth (Harcourt 1954). In Florida, Javelin WG and Dipel 2X, respectively, provided at best only 27% (Zellwood) and 29% (Parrish) control compared with the untreated control, whereas XenTari provided control to 60% (Zellwood). Even this level of control may not be considered adequate for growers, but under the population pressure of this test, growers would have sprayed more than once per week. In Geneva, where plants were inoculated with the susceptible Geneva 1988 population, control by Javelin WG and Dipel 2X was 89 and 86%, respectively.

A comparison of all the laboratory and field data suggests that control can be achieved in the field when the LC_{50} for *B. thuringiensis* is <0.5 mg (AI)/liter (Tables 1, 3, and 4). This appears to be the case for all *B. thuringiensis* products we tested and may serve as a diagnostic concentration. Use of resistance ratios rather than a particular LC_{50} may not provide the same insight into field efficacy. For example, in the case of the Loxahatchee (a) population for Javelin WG and Dipel 2X, the difference in the RRs is 74.6-fold compared with only a 4.7-fold difference in LC_{50}s. The extremely high RR of Javelin WG (1,617) may simply be the result of its greater efficiency against a susceptible population and probably does not provide insight into expected field control.

***B. thuringiensis* subsp. *aizawai* and subsp. *kurstaki*.** Significant differences in the response of resistant and susceptible populations to *B. thuringiensis* subsp. *aizawai* were observed with two of the three products, but RRs varied only between 3.0 and 4.1 (Table 5). Without data on the performance of these materials in the field, it remains unclear whether RRs of this magnitude would influence control. However, with the two products that contain *B. thuringiensis* subsp. *kurstaki*, RRs were 321–462, suggesting potential problems with these products because our tests with Javelin WG and Dipel (see above) clearly demonstrate control failures with RRs of this magnitude.

These data on the magnitude and extent of resistance to *B. thuringiensis* complement our present knowledge of insecticide resistance of diamondback moth over a wide area of North America (Shelton et al. 1993). The study reported here demonstrated high levels of resistance to *B. thuringiensis* subsp. *kurstaki* in areas of the continental United States where diamondback moth pressure and use of products containing *B. thuringiensis* subsp. *kurstaki* have been intense. Based on our data on resistance to permethrin and methomyl and the earlier use of these products, we infer that once these products became ineffective, growers switched to *B. thuringiensis* subsp. *kurstaki*, and selection pressure and resistance occurred. Although there are areas in which resistance did not appear in either year (e.g., Belle Glade), we are concerned about the simultaneous development of high RRs to *B. thuringiensis* subsp. *kurstaki* in several geographically isolated populations in Florida. Whether the same mechanisms of resistance developed in each case is currently being investigated. The significant differences in several populations in their response to *B. thuringiensis* subsp. *aizawai* appear to be the first documented case of potential field resistance. These differences may be a result of a presently low level of cross-resistance to the unique toxins contained in *B. thuringien-*

Table 5. Comparison of dose-mortality regression data of *Bacillus thuringiensis* products against resistant and susceptible diamondback moth populations

Product[a]	Slope ± SE	LC_{50} (95% CL) (ppm)	RR[b]
Bacillus thuringiensis subsp. *aizawai*			
XenTari			
Resistant	1.32 ± 0.19	3.9 (1.0– 10.8)	3.0
Susceptible	1.66 ± 0.26	1.3 (0.8– 1.9)	
Agree			
Resistant	1.69 ± 0.26	20.5 (14.1– 28.3)	3.5
Susceptible	1.91 ± 0.28	7.2 (2.3– 13.5)	
NB200 FC			
Resistant	1.57 ± 0.23	4.5 (2.7– 6.8)	4.1
Susceptible	1.52 ± 0.23	1.1 (0.7– 1.6)	
Bacillus thuringiensis subsp. *kurstaki*			
Biobit HP			
Resistant	1.25 ± 0.26	161.7 (20.7–383.7)	461.6
Susceptible	1.83 ± 0.22	0.7 (0.5– 1.0)	
Javelin WG			
Resistant	0.89 ± 0.13	96.2 (54.8–163.5)	320.7
Susceptible	2.25 ± 0.30	0.3 (0.2– 0.5)	

[a] Sample size for each population was 212 to 322. Mortality was assessed 96 h after treatment. Resistant and susceptible populations were Loxahatchee (a) and Geneva 1988, respectively.

[b] Resistance ratio (RR) is calculated by dividing the LC_{50} obtained for a particular population by the LC_{50} for the susceptible laboratory colony (Geneva 1988).

sis subsp. *aizawai* (most likely CryIC), or possibly to independent selection by the toxins contained in XenTari because it has been used since 1990 under an experimental use permit. Whatever the reason, we should be concerned because where selection by one insecticide is intense, diamondback moth resistance historically occurs within 2–5 yr (Talekar & Shelton 1993).

Our data on the differences in activity of two *B. thuringiensis* subsp. *kurstaki* products are puzzling. For a specific population, Javelin WG and Dipel 2X had similar LC_{50}s in 1990 but very different values in 1992 depending on the susceptibility of the population to *B. thuringiensis* subsp. *kurstaki*. These products may have changed between 1990 and 1992 and contained different or different amounts of toxins. This possibility should be of concern to industry as well as to those who work with *B. thuringiensis* products and may necessitate the identification of batch numbers of products for all published reports.

In our previous article (Shelton et al. 1993) and in articles by Tabashnik et al. (1990, 1991), a plea was made for judicious use of *B. thuringiensis* because it appeared to be one of the few remaining insecticides without widespread resistance. Our study demonstrated that resistance to *B. thuringiensis* subsp. *kurstaki* is more widespread in the United States than previously documented and resistance to *B. thuringiensis* subsp. *aizawai* is developing. Where resistance has occurred, it appears to be directly related to past patterns of *B. thuringiensis* use. Whether or not we can manage resistance to *B. thuringiensis* is a pressing question. Tabashnik et al. (1991) reported that susceptibility to *B. thuringiensis* is not quickly restored when treatments are discontinued and, thus, rotations of *B. thuringiensis* with other insecticides with different modes of action may not be a viable strategy. Past experiences with diamondback moth have reinforced the belief that single-component strategies will fail (Talekar & Shelton 1993). Use of other strategies such as biological control, host-free periods, cultural controls, novel insecticides, and plant resistance is the only means for limiting the use of *B. thuringiensis* toxins and the development of resistance to them.

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