Susceptibility of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) Populations in Mexico to Commercial Formulations of *Bacillus thuringiensis*

OVIDIO DÍAZ-GOMEZ,[1] J. C. RODRÍGUEZ,[2] A. M. SHELTON,[3] A. LAGUNES-T.,[2] AND R. BUJANOS-M.[4]

J. Econ. Entomol. 93(3): 963–970 (2000)

ABSTRACT Populations of diamondback moth, *Plutella xylostella* (L.), sampled from commercial fields of crucifers in three states of Mexico, were tested for susceptibility to commercial formulations of *Bacillus thuringiensis* subsp. *kurstaki* (Berliner) (Dipel 2X), *B. thuringiensis* subsp. *aizawai* (XenTari), delta endotoxin Cry 1C (MC), and CryIA(c) (MVP), and a mixture of *B. thuringiensis* subsp. *kurstaki* and subsp. *aizawai* (Agree). Leaf-dip bioassays confirmed variation in susceptibility of up to 13-fold for MVP, 12-fold for Dipel 2X, sevenfold for XenTari, fivefold for Agree, and less than fivefold for MC. Comparisons with previously published data indicate that at least the 12-fold variation in Dipel 2X would result in significant differences in control in the field. Based on the LC_{99} values observed for the products, we propose discriminating concentrations for each product. To ensure continued performance in the field we suggest that a resistance monitoring program be implemented to detect any changes in susceptibility to *B. thuringiensis* products and specific toxins and that their use be restricted to one generation per crop and that they be rotated with other groups of insecticides. Furthermore, we suggest enforcement of a crucifer host-free period and the development and implementation of cultural and biological control strategies to reduce overall population pressure so that fewer insecticidal treatments will be needed.

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

MEXICO IS A major producer of broccoli and related crucifers used for processing and export to the United States. Most production is located in 'El Bajio' region where >30,000 ha of broccoli are produced. The most abundant lepidopteran pest of cruciferous plants in Mexico is the diamondback moth, *Plutella xylostella* (L.). It greatly reduces the yield and quality of the crop and accounts for a large percentage of insecticide use in crucifer production (Laborde 1992, Bujanos et al. 1995). This, coupled with its demonstrated ability to develop resistance to many insecticides (Talekar 1986; Georghiou and Lagunes-Tejeda 1991; Tabashnik et al. 1991; Shelton et al. 1993a. 1993b), has caused ongoing problems for production during the past 25 yr. In Mexico before 1988, management of diamondback moth depended largely on the use of organosynthetic insecticides. However, because of declining levels of effectiveness to the standard insecticide products, *Bacillus thuringiensis* Berliner subsp. *kurstaki* has been heavily used by the farmers since 1989 in "El Bajio" and, since 1991, in San Luis Potosi. Various *B. thuringiensis* products containing one or more endotoxins (Hofte and Whitely 1989) are now widely used and there is concern that resistance to one or more of the toxins may occur in *P. xylostella* as it has in other regions (Shelton et al. 1993a, Tabashnik 1994, Perez and Shelton 1997) if efforts to manage resistance are not implemented in the near future.

In Mexico, the risk of developing *B. thuringiensis* resistance in *P. xylostella* was reported and discussed by Ibarra (1993) and Díaz (1992). These studies strongly suggest the existence of genes for resistance to *B. thuringiensis* in field populations. The consequences of *B. thuringiensis* resistance could be severe and the need for design and implementation of tactics and strategies to delay *B. thuringiensis* resistance is extremely urgent. As a first step, initial surveys of insect susceptibility (Roush and Miller 1986) to *B. thuringiensis* are necessary to establish a baseline for monitoring possible changes in population sensitivity to *B. thuringiensis* products and their specific toxins. This information can then be used for implementing appropriate resistance management and product use strategies to ensure their efficacious long-term use.

Herein we report results of a study documenting the susceptibility in *P. xylostella* populations from three states in Mexico to toxins and subspecies of *B. thuringiensis*.

[1] Universidad Autonoma de San Luis Potosi, Facultad de Agronomia. Alvaro Obregon 64. C.P. 78000. San Luis Potosi, S.L.P., Mexico.
[2] Instituto de Fitosanidad, Colegio de Postgraduados Km. 35.5 carretera Mexico-Texcoco, Montecillo, Texcoco, Estado de Mexico 56230, Mexico.
[3] Department of Entomology, Cornell University/NYSAES, Geneva, NY 14456.
[4] Campo Experimental Bajio. Apdo. Postal 112, CP 38000, Celaya, Gto. Mexico.

Materials and Methods

Field Populations. Six field populations of *P. xylostella* were bioassayed to compare their susceptibility to *B. thuringiensis* products. From 50 to 200 larvae and pupae were collected from three states of Mexico during 1997: San Luis Potosi (SLP), Aguascalientes (Aguas), and Guanajuato. In the state of Guanajuato, we collected in six locations: Salamanca, Rodríguez, Celaya, Leon, Irapuato, and Cortazar. Salamanca and Rodríguez insects were bioassayed independently; however, the insects collected in Celaya, Leon, Irapuato, and Cortazar were pooled to obtained the "Guanajuato" population. SLP and Aguas populations were derived from a small region of commercial crucifers that had little exposure to *B. thuringiensis* products. The other populations, Salamanca, Rodríguez, and Guanajuato, were collected in the major crucifer-growing region of the state of Guanajuato and they are representative of extensive *B. thuringiensis* use on crucifers in Mexico. A laboratory population, Geneva 88 from Cornell University, was used as a susceptible reference. It was collected in a field in Geneva, NY, and maintained in culture without exposure to insecticides for 205 generations. This population has been used as a standard in several studies of *P. xylostella* susceptibility to *B. thuringiensis* products and toxins (Shelton et al. 1993a, Tang et al. 1996, Perez and Shelton 1997).

We followed the rearing procedure suggested by Shelton et al. (1991). Adults were kept in cylinder cages and fed 10% sugar-water. Eggs were collected on foil sheets and sections of the egg sheets containing an estimated number of eggs were distributed on *Brassica oleracea* L. subsp. *botrytis* ('Snowball') plants in the greenhouse. Egg density was adjusted to plant size to ensure that overcrowding did not occur and reduce the fitness of the larvae. Late second instars were used for bioassay.

Insecticides. The bioassays were conducted with commercial formulations of Dipel 2 (*Bacillus thuringiensis* subsp. *kurstaki* [Berliner]; wettable powder [WP] 6.4% [AI], Abbott, North Chicago, IL), XenTari (*B. thuringiensis* subsp. *aizawai;* wettable granule [WG] 10.3% [AI], Abbott), Agree (*B. thuringiensis* subsp. *kurstaki* and subsp. *aizawai;* WP 3.8% [AI], Novartis, Greensboro, NC), MC (Cry 1C delta endotoxin, derived from *B. thuringiensis* subsp. *aizawai* aqueous flowable suspension [AF] 15% [AI], Mycogen, San Diego, CA) and MVP (Cry 1A(c) delta endotoxin derived from *B. thuringiensis* subsp. *kurstaki*, AF 10% [AI], Mycogen).

Bioassays. The mortality data for log dose-probit responses were determined following the leaf dip bioassay method used by Tang et al. (1996). Leaf disks (3.2-cm-diamter) obtained from cabbage leaves. Each disk was dipped for 10 s in an insecticide solution and then allowed to air dry at room temperature. A single disk was then placed into a 20-ml plastic cup and five second instars were added. Mortality data were recorded 72 h after treatment. Larvae were considered dead if they did not move when prodded. We used a 3.16-fold serial dilution factor with all insecticides. A solution without insecticide was used as a control. Six to seven concentrations, including the control, were used with individual populations. The bioassays included two to four replications with each replication performed on a different day. Control mortality never exceeded 6% and data were corrected based on Abbott's (1925) formula.

Table 1. Susceptibility of *P. xylostella* populations to Cry 1A(c) delta endotoxin of *B. thuringiensis* (MVP)

Population	Generation	n	LC_{50} mg (AI)/liter (95% CL)	Slope ± SE	RR
Geneva 88	F_{205}	343	0.95 (0.29–1.76)	1.41 ± 0.21	1.0
Salamanca	F_2	556	6.61 (4.67–8.87)	2.03 ± 0.19	7.0
Rodríguez	F_2	541	7.38 (5.45–10.07)	1.57 ± 0.11	7.7
SLP	F_2	546	7.67 (4.68–10.75)	2.29 ± 0.30	8.0
Aguas	F_2	543	12.64 (9.98–15.53)	2.04 ± 0.21	13.3

Guanajuato population not available for testing. RR, resistance ratios, calculated by dividing the LC_{50} of a *P. xylostella* field population by the LC_{50} of Geneva 88.

Statistical Analyses. Data from leaf-dip bioassays were analyzed assuming the probit model (POLO-PC; LeOra Software 1987). Median lethal concentrations (LC_{50}) and LC_{90} values and their 95% CL values were estimated for each population. The responses of two populations were considered significantly different if their 95% CL values did not overlap. Resistance ratios were calculated by dividing the LC_{50} of a *P. xylostella* field population by the LC_{50} of Geneva 88. Using the LC_{99} values for each insecticide we also propose discriminating concentration that can be used to classify populations as resistant or susceptible (Roush and Miller 1986).

Results

Significant differences in susceptibility of *P. xylostella* were found among populations and for all insecticides. Our data confirmed variation of up to 13-fold for MVP, 12-fold for Dipel 2X, sevenfold for XenTari, fivefold for Agree and less than fivefold for MC.

Cry 1A(c) Delta Endotoxin (MVP). The most susceptible population was the Geneva 88 (LC_{50} = 0.95 mg [AI]/l) and it was significantly different from all other populations based on the nonoverlap of the 95% CL values (Table 1). The four field populations of *P. xylostella* displayed LC_{50} values from 7.0 to 13.3 times more than Geneva 88. The highest LC_{50} was 12.64 mg (AI)/liter, and it occurred in the Aguas population, which had an average of only 43% (uncorrected) mortality at the highest concentration tested on the Geneva population (10 mg [AI]/liter).

Based on the susceptibility responses to Cry 1A(c) delta endotoxin observed, we established two general groups. The first, represented by the Geneva 88, reflects baseline susceptibility of *P. xylostella* to Cry 1A(c) delta endotoxin. This population may be expected to have little survivorship at treatments of >7.0 mg (AI)/liter (Fig. 1). The second group, represented by the field populations, had high proportions of sur-

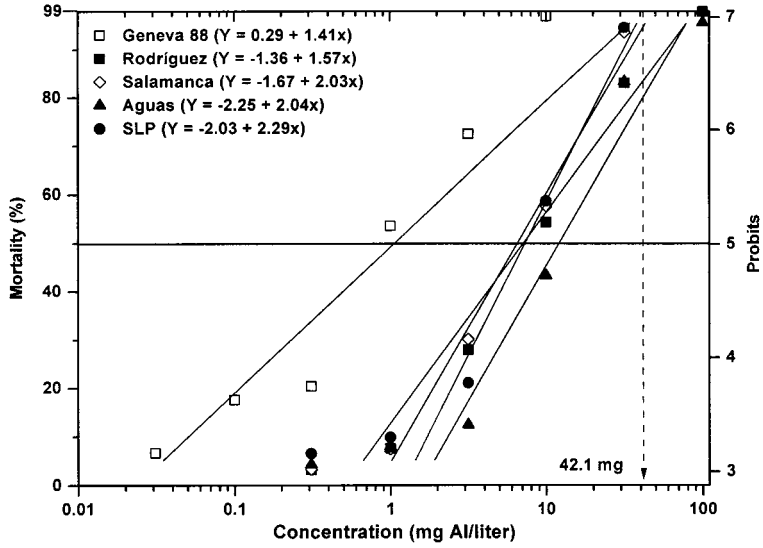

Fig. 1. Concentration mortality lines for Cry 1A(c) delta endotoxin of *B. thuringiensis* against *P. xylostella* populations from Mexico. The concentration identified represents the LC_{99} of Geneva 88.

vivors at 7.0 mg (AI)/liter treatments, but relatively low survivorship at concentrations between 20 and 31 mg (AI)/liter. We suggest that a provisional discriminating concentration of 42.1 mg (AI)/liter (LC_{99} of our susceptible population) may be used to classify populations for susceptibility and to predict the potential usefulness of Cry 1A(c) in field treatments.

***Bacillus thuringiensis* subsp. *kurstaki* (Dipel 2X).** The most susceptible populations were Geneva 88 and Guanajuato, which had LC_{50} values of 0.12 and 0.13 mg (AI)/liter, respectively (Table 2). Of the five field populations evaluated, four had LC_{50}s <1.0 mg (AI)/liter, and one population had a value >1.0 mg (AI)/liter (Table 2). LC_{90} values for these five populations were >1.5 mg (AI)/liter and there was relatively more survivorship at a concentration between 2.0 and 4.0 mg (AI)/liter than Geneva 88 (LC_{90} of Geneva 88 was 1.0 [95% CL, 0.6–1.7] mg [AI]/liter). Therefore, we suggest using a concentration of 5.6 mg (AI)/liter (LC_{99} of our susceptible standard) as a provisional discriminating concentration for surveying susceptibility to *B. thuringiensis* subsp. *kurstaki* (Fig. 2). The population least susceptible to *B. thuringiensis* subsp *kurstaki* was Rodríguez, which had a LC_{50} of 1.44 mg (AI)/liter. Three field populations of *P. xylostella* yielded LC_{50} values of *B. thuringiensis* subsp. *kurstaki* from 5.3 to 12 times more than Geneva 88.

Table 2. Susceptibility of *P. xylostella* populations to *B. thuringiensis* subspecies *kurstaki* (Dipel 2X)

Population	Generation	*n*	LC_{50} mg (AI)/liter (95% CL)	Slope ± SE	RR
Geneva 88	F_{204}	377	0.12 (0.09–0.17)	1.41 ± 0.14	1.0
Guanajuato	F_1	296	0.13 (0.05–0.23)	1.21 ± 0.22	1.0
Aguas	F_2	351	0.33 (0.20–0.55)	1.31 ± 0.11	2.8
SLP	F_1	368	0.64 (0.47–0.85)	1.57 ± 0.16	5.3
Salamanca	F_1	299	0.97 (0.69–1.41)	1.18 ± 0.12	8.1
Rodríguez	F_1	315	1.44 (1.06–2.04)	1.34 ± 0.14	12.0

RR, resistance ratios, calculated by dividing the LC_{50} of a *P. xylostella* field population by the LC_{50} of Geneva 88.

***Bacillus thuringiensis* subsp. *aizawai* (XenTari).** Geneva 88, SLP, and Rodríguez populations were the most susceptible and not significantly different from each other in their LC_{50} values, but were significantly less susceptible than Guanajuato (Table 3). LC_{50} values for six populations bioassayed ranged from 0.23 mg (AI)/liter for the population from Geneva 88 to 1.66 mg (AI)/liter for the population from Guanajuato.

Based on the Geneva 88 population as our susceptible standard with an LC_{50} of 0.23 mg (AI)/liter, and a LC_{90} of 2.42 mg (AI)/liter (95% CL, 1.25–7.66), little survivorship can be expected at a concentration of 16.2 mg (AI)/liter (LC_{99} of our susceptible population), which could be a provisional discriminating concentration for surveys of susceptibility of *P. xylostella* (Fig. 3).

Mixture of *B. thuringiensis* subsp. *kurstaki* and subsp. *aizawai* (Agree). Responses revealed some significant differences in susceptibility among the populations Geneva 88, SLP, Aguas, and Guanajuato. They were all significantly more susceptible than Rodríguez (Table 4). The highest LC_{50}, 1.1 mg ([AI]/liter), occurred in the Rodríguez population, which had an average of 79% (uncorrected) mortality at the highest concentration evaluated on the Geneva population (3.8 mg (AI)/liter) and this concentration killed all the Geneva 88 insects tested. Field populations of *P. xylostella* displayed LC_{50}s to 5.0 times higher when compared with Geneva 88.

Based on the observed responses to a mixture of toxins Geneva 88 represents the baseline susceptibility of *P. xylostella* to this complex set of toxins of *B.*

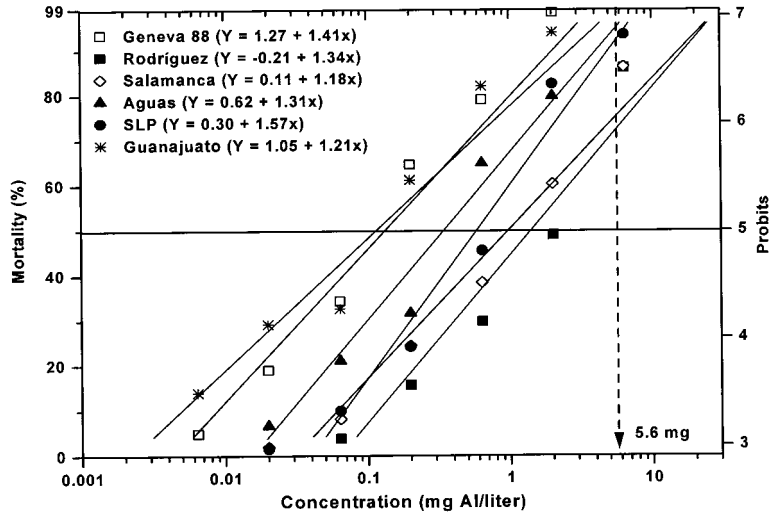

Fig. 2. Concentration mortality lines for *B. thuringiensis* subsp. *kurstaki* against *P. xylostella* populations from Mexico. The concentration identified represents the LC_{99} of Geneva 88.

thuringiensis. This population may be expected to have little survivorship at treatments of 1.2 mg (AI)/liter, its CL_{90}. Rodríguez and Salamanca populations had relatively low survivorship at concentrations between 2.0 and 4.0 mg (AI)/liter, so we suggest 5.2 mg (AI)/liter (LC_{99} of our susceptible population) as a provisional discriminating concentration (Fig. 4).

Cry 1C delta endotoxin (MC). The differences in susceptibility between our susceptible standard, Geneva 88, and field populations were less than fivefold (Table 5). The most susceptible populations were Geneva 88 and Aguas with LC_{50} values of 0.23 and 0.36 mg (AI)/liter, respectively, and, although they were not significantly different from each other, they were significantly more susceptible than Salamanca and SLP. The two former populations can be considered to represent baseline susceptibility of *P. xylostella* to Cry 1C delta endotoxin. Relative to LC_{90} values, the field populations had values >2.5 mg (AI)/liter and these populations would have relatively more survivorship at a concentration between 2.0 and 4.0 mg (AI)/liter than Geneva 88 (LC_{90} of Geneva 88 was 0.9 [95% CL, 0.71–1.21] mg [AI]/liter). Thus, a concentration of 2.8 mg (AI)/liter (LC_{99} of our susceptible population) could be used as a provisional discriminating concentration for surveying susceptibility to Cry 1C delta endotoxin (Fig. 5).

Table 3. Susceptibility of *P. xylostella* populations to *B. thuringiensis* subspecies *aizawai* (Xen Tari)

Population	Generation	*n*	LC_{50} mg (AI)/liter (95% CL)	Slope ± SE	RR
Geneva 88	F_{204}	365	0.23 (0.12–0.39)	1.26 ± 0.14	1.0
Rodríguez	F_1	638	0.49 (0.38–0.64)	1.08 ± 0.07	2.1
SLP	F_1	363	0.53 (0.34–0.77)	1.26 ± 0.15	2.3
Salamanca	F_1	532	0.93 (0.63–1.40)	1.10 ± 0.08	4.0
Aguas	F_2	538	1.25 (0.66–1.98)	1.72 ± 0.18	5.4
Guanajuato	F_1	336	1.66 (0.95–2.70)	1.76 ± 0.23	7.2

RR, resistance ratios, calculated by dividing the LC_{50} of a *P. xylostella* field population by the LC_{50} of Geneva 88.

Discussion

Although the Geneva 88 population was the most susceptible population in all the trails, previous reports (Shelton et al. 1993b, Perez and Shelton 1997) indicate it is a realistic standard and representative of a pristine population and, in some cases, is even less susceptible than recently collected field populations that have not been exposed to *B. thuringiensis.* Thus, any elevated lethal concentration values we observed in the Mexican populations are the result of either natural variation or the development of resistance. Because we do not have accurate spray records for each area over several years, it is impossible to accurately relate the variation in susceptibility to the variation in the pattern of use, but there are some indications of such a relationship (see below). Our data do indicate significant regional variation in susceptibility of *P. xylostella* to *B. thuringiensis* toxins up to 13-fold. Our current studies do not indicate whether such variation did result in actual control failures in the field but a previous report indicated that an resistance ratios value of 7.8 for Dipel 2X in laboratory assays such as these resulted in significant differences in control in the field (e.g., the Parris population, Shelton et al. 1993a). In Mexico, *B. thuringiensis* derivatives have been used in crucifers since the end of the 1980s (Laborde 1992). Tabashnik et al. (1991) and Shelton et al. (1993a) expressed concern that intensive use of *B. thuringiensis* against *P. xylostella* might lead to selection of resistance and eventual control failures.

In our study the largest variation among populations in susceptibility was to the Cry 1A(c) delta endotoxin and *B. thuringiensis* subspecies *kurstaki.* The increase in tolerance level probably reflects an increase in

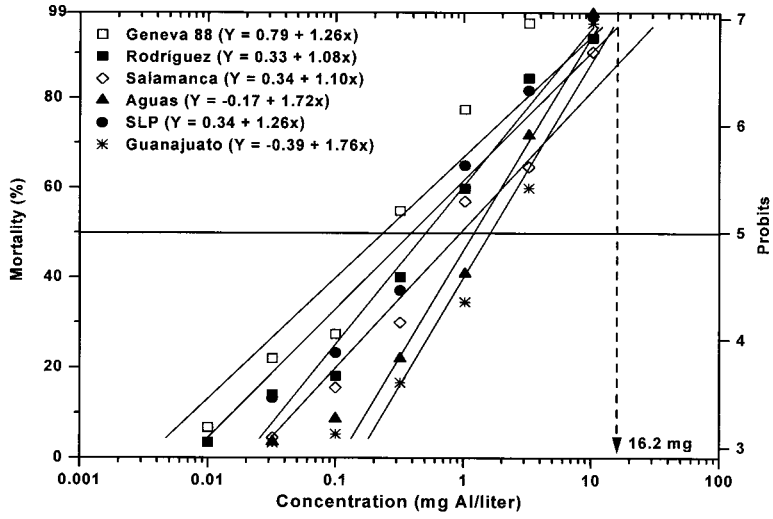

Fig. 3. Concentration mortality lines for *B. thuringiensis* subsp. *aizawai* against *P. xylostella* populations from Mexico. The concentration identified represents the LC_{99} of Geneva 88.

frequency of tolerant individuals in the populations because, when probit regressions for *B. thuringiensis* toxins and subspecies in the field populations were compared, slopes were generally higher with Cry 1A(c) than with the other products. Relatively high resistance ratios at the confidence limits$_{50}$, coupled with higher slopes for Cry 1A(c) in SLP, Aguas, and Salamanca populations, indicate that populations in these regions are becoming less heterogeneous and more tolerant to this toxin. As a monitoring indicator, the evolution of higher slope values should be regarded as the forerunner of a major increase in tolerance because of the increase in frequency of resistant genotypes. Subsequent increase in slope values, followed by a decrease, would then indicate the progression of tolerance to higher intensities but it must be tested using quantitative genetic techniques (Chilcutt and Tabashnik 1995).

The states of San Luis Potosi and Aguascalientes, where *P. xylostella* populations had the highest tolerance levels to Cry 1A(c), do not reflect a pattern of MVP use because this product has not been used in those states. We believe that the tolerance was developed from selection with the commercial formulation used (Dipel 2X) because it contains the crystal proteins Cry 1A(a), Cry 1A(b), Cry 1A(c), Cry IIA, and Cry IIB. These toxins have common receptors in many lepidopteran species (Fiuza et al. 1996). This result is similar to previous reports that indicate that *P. xylostella* can be resistant to a delta endotoxin, Cry 1A(b), but not to Dipel (McGaughey and Johnson 1994, Denis et al. 1997). However, Aguas was highly susceptible to Cry 1C delta endotoxin and the mixture of *B. thuringiensis* subsp. *kurstaki* and subsp. *aizawai.* Therefore, it can be presumed that the exposure to Dipel in the field has not induced tolerance to Cry 1C nor to the complex set of toxins of *B. thuringiensis* contained in the mixture tested (Ferré et al. 1991).

In contrast to responses to Cry 1A(c) delta endotoxin, higher levels of tolerance to *B. thuringiensis* subsp. *kurstaki* in the Rodríguez and Salamanca populations could be explained by past use patterns of this subspecies of *B. thuringiensis.* Díaz et al. (1994) documented levels of tolerance to *B. thuringiensis* subsp. *kurstaki* in field populations of *P. xylostella* collected in SLP and Irapuato, Gto., and they were 2.5 and 2.2 times higher, respectively, than a susceptible population. However, 8 yr later the levels are 5.3 higher for the SLP population and from 8.1 to 12.0 times higher for

Table 4. Susceptibility of *P. xylostella* populations to mixture of *B. thuringiensis* subspecies *kurstaki* and subspecies *aizawai* (Agree)

Population	Generation	*n*	LC_{50}mg (AI)/liter (95% CL)	Slope ± SE	RR
Geneva 88	F_{206}	543	0.22 (0.17–0.27)	1.69 ± 0.14	1.0
Guanajuato	F_1	311	0.25 (0.14–0.44)	1.71 ± 0.15	1.1
SLP	F_1	363	0.39 (0.30–0.50)	1.49 ± 0.13	1.8
Aguas	F_3	367	0.42 (0.32–0.56)	1.38 ± 0.12	1.9
Salamanca	F_2	356	0.69 (0.30–1.17)	1.45 ± 0.18	3.1
Rodríguez	F_1	413	1.10 (0.78–1.48)	1.65 ± 0.21	5.0

RR, resistance ratios, calculated by dividing the LC_{50} of a *P. xylostella* field population by the LC_{50} of Geneva 88.

Table 5. Susceptibility of *P. xylostella* populations to Cry 1C delta endotoxin of *B. thuringiensis* (MC)

Population	Generation	*n*	CL_{50} mg (AI)/liter (95% CL)	Slope ± SE	RR
Geneva 88	F_{205}	550	0.23 (0.18–0.27)	2.14 ± 0.19	1.0
Aguas	F_2	355	0.36 (0.23–0.56)	1.50 ± 0.13	1.6
Rodríguez	F_2	538	0.50 (0.34–0.72)	1.66 ± 0.11	2.2
Salamanca	F_2	559	0.85 (0.62–1.10)	1.51 ± 0.15	3.7
SPL	F_2	540	1.06 (0.84–1.30)	2.06 ± 0.20	4.6

Guanajuato population not available for testing. RR, resistance ratios, calculated by dividing the LC_{50} of a *P. xylostella* field population by the LC_{50} of Geneva 88.

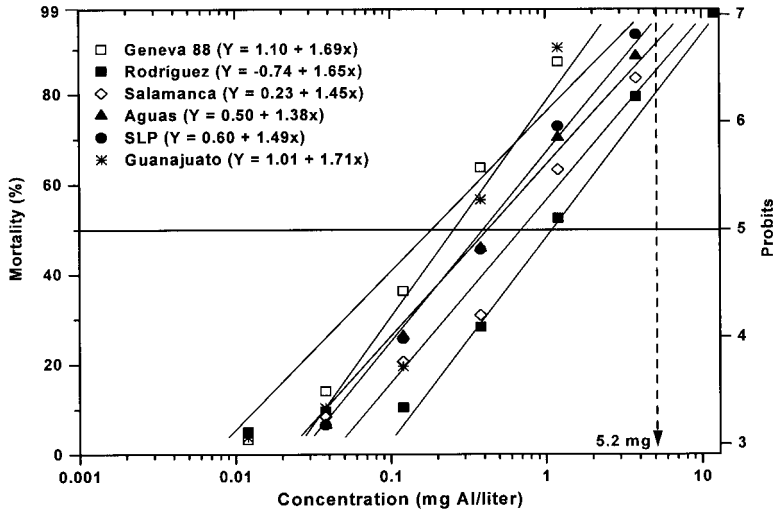

Fig. 4. Concentration mortality lines for mixture of *B. thuringiensis* subsp. *kurstaki* and subsp. *aizawai* against *P. xylostella* populations from Mexico. The concentration identified represents the LC_{99} of Geneva 88.

Salamanca and Irapuato populations, respectively. Taking this into account and based on available information, the reduced susceptibility of *P. xylostella* to *B. thuringiensis* subsp. *kurstaki* in these states may reflect higher selective pressure because of greater frequency of *B. thuringiensis* subsp. *kurstaki* applications, because the growers indicated that commercial formulations of this subspecies were one of the first and most widely used products for >10 yr in the state of Guanajuato. Growers continue to use them in the field to control *P. xylostella* but additional studies to correlate larval tolerance with *B. thuringiensis* efficacy are needed to determine whether present tolerance levels result in field control failures.

The susceptibility to *B. thuringiensis* subsp. *aizawai* was intermediate between the low values for tolerance to the mixture of subsp. *kurstaki* and *aizawai* and the higher values for *B. thuringiensis* subsp. *kurstaki* toxins. For the mixture of *B. thuringiensis* subsp. *kurstaki* and subsp. *aizawai* and Cry 1C delta endotoxin the variation in response was much lower than for the other products tested. These data probably reflect genetic variation in some geographically isolated populations because they displayed significant differences in their responses to those *B. thuringiensis* derivatives (Shelton et al. 1993a). Those products have been used less frequently in the state of Guanajuato since the mid-1990s. Thus, the selective pressure has been lower than with the other *B. thuringiensis* derivatives, but might be expected to attain higher levels of tolerance if exposure to Cry 1C continued.

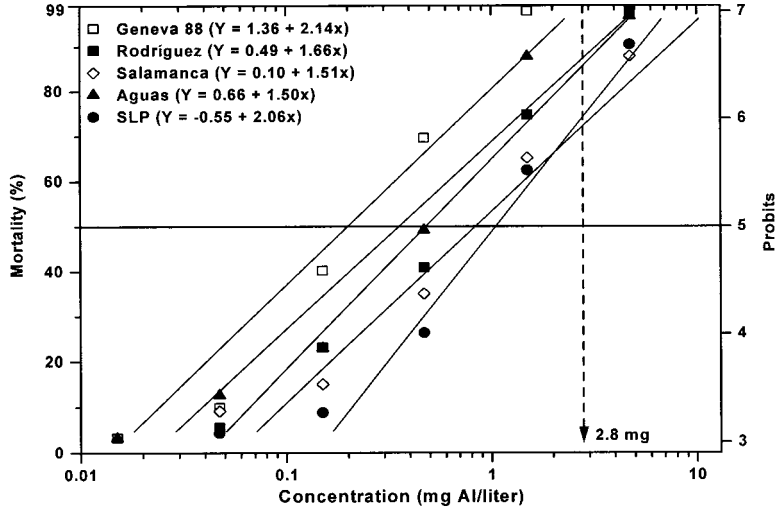

Fig. 5. Concentration mortality lines for Cry 1C delta endotoxin of *B. thuringiensis* against *P. xylostella* populations from Mexico. The concentration identified represents the LC_{99} of Geneva 88.

We suggest that crucifer growers in the three states of Mexico (Aguascalientes, San Luis Potosi, and Guanajuato) use our data on the current susceptibility levels to commercial formulations of *B. thuringiensis* available in the market as baseline data for a resistance management program. Our data may be especially timely for Guanajuato, which is the most prolific crucifer growing region of Mexico. Previous studies indicated that with *B. thuringiensis* subsp. *kurstaki* LC_{50} values between 0.5 (Shelton et al. 1993) and 0.6 mg(AI)/liter (Perez and Shelton 1997) would result in field control problems and the current study indicates that this range is a reasonable one (Table 2). Additionally, this current study provides information on other subspecies and toxins and proposes LC_{99} values that can be used as discriminating concentrations to classify populations for susceptibility and predict the potential usefulness of subspecies and toxins of *B. thuringiensis* in field treatments. Thus, these current data will not only establish an adequate database for regionwide monitoring for several products but will also aid in quick diagnosis of any shifts in tolerance. Such a process also will help growers decide whether failures of *B. thuringiensis* toxins and subspecies are because of the development of resistance or faulty application methodology or timing, poor coverage or unfavorable environmental conditions.

Although other studies have documented higher levels of field resistance (e.g., Shelton et al. 1993a, Perez and Shelton 1996), we believe that the relatively low resistance levels we found for *B. thuringiensis* can be problematic at present and may become more so in the future. However, perhaps resistance has not been as intense because growers are also using other types of management strategies that may be effective. Nevertheless, to reduce the risks of product rejection by the United States Food and Drug Administration, the growers are relying more upon *B. thuringiensis*, therefore the selection pressure during the crucifer season is increasing.

In summary, we documented the susceptibility of *P. xylostella* populations collected in three states of Mexico to all five commercial formulations of *B. thuringiensis* using our data as a baseline. It is important to take measures to prevent the further buildup of resistance. We suggest that a resistance monitoring program be implemented to detect any changes in susceptibility to *B. thuringiensis* products and specific toxins and that their use be restricted to one generation per season and that it be rotated with other groups of insecticides; crucifer host-free periods be enforced, and other cultural and biological control strategies (Talekar and Shelton 1993, Bujanos et al. 1995) be developed and implemented to reduce overall population pressure so that fewer pesticidal treatments will be needed.

Acknowledgments

We thank Juliet Tang, Nina Barcenas, Raquel Alatorre, and David Mota for their excellent technical advices. We are very grateful to Leticia Zapata-Martinez for her valuable help with this work. We also acknowledge James M. Lawrence for his review of the manuscript. The research was partially supported by CONACyT (Project 3223-PB 2 d.96). This research was part of a dissertation submitted to the Colegio de Postgraduados by Ovidio Díaz-Gomez in partial fulfillment of the requirements for a Doctor degree.

References Cited

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Bujanos, M. R., A. Marin J., F. Galvan C., and K. F. Byerly M. 1995. Plagas de los cultivos de cruciferas en el Bajio, Mexico. INIFAP-APFVG.A.C. Celaya, Gto., Mexico.

Chilcutt, M. R., and B. E. Tabashnik. 1995. Evolution of pesticide resistance and slope of the concentration-mortality line: are they related? J. Econ. Entomol 88: 11–20.

Denis, J. W., M. Iqbal, F. Granero, and J. Ferré. 1997. A change in a single midgut receptor in the diamondback moth (*Plutella xylostella*) is only in part responsible for field resistance to *Bacillus thuringiensis* subsp. *kurstaki* and *B. thuringiensis* subsp. aizawai. Appl. Environ. Microbiol. 63: 1814–1819.

Díaz, G. O. 1992. Susceptibilidad de la palomilla dorso de diamante *Plutella xylostella* (Lepidoptera: Yponomeutidae) a insecticidas organosinteticos y microbiales. Tesis de Maestria en Ciencias, Colegio de Postgraduados, Chapingo, Mexico.

Díaz, G. O., A. Lagunes T., H. Sanchez A., and R. Alatorre R. 1994. Susceptibility of *Plutella xylostella* (L.) to microbial insecticides. Southwest. Entomol. 19: 403–408.

Ferré J., M. D. Real, J. Van Rie, S. Jansens, and M. Peferoen. 1991. Resistance to the *Bacillus thuringiensis* bioinsecticide in a field population of *Plutella xylostella* is due to a change in a midgut membrane receptor. Proc. Natl. Acad. Sci. U.S.A. 88: 5119–5123.

Fiuza, L. M., C. Nielsen-Leroux, E. Goze, R. Frutos, and J. F. Charles. 1996. Binding of *Bacillus thuringiensis* CryI toxins to the midgut brush border membrane vesicles of *Chilo suppressalis* (Lepidoptera: Pyralidae): evidence of shared binding sites. Appl. Environ. Microbiol. 62: 1544–1549.

Georghiou, G. P., and A. Lagunes-Tejeda. 1991. The occurrence of resistance to pesticides in arthropods. An index of cases reported through 1989. Publication AGPP/Misc/91–1. FAO (United Nations), Rome.

Hofte, H., and H. R. Whitely. 1989. Insecticides crystal proteins of *Bacillus thuringiensis.* Microbiol. Rev. 53: 242–255.

Ibarra, J. 1993. Desarrollo de resistencia hacia *Bacillus thuringiensis*, pp. 231–232. *In* Memorias del XXVII Congreso Nacional de Entomologia. Entomological Society of Mexico, Cholula, Pue. Mexico.

Laborde, C.J.A. 1992. Palomilla dorso de diamante en el Bajio, control mediante un programa integral regional, pp. 245–248. *In* R. S. Ananya [ed.], Manejo Fitosanitario de las Hortalizas en Mexico. CP-SARH, Chapingo, Mexico.

LeOra Software. 1987. POLO-PC. A user's manual for Probit Or Logit analysis. LeOra Software, Berkeley, CA.

McGaughey, W. H., and D. E. Johnson. 1994. Influence of crystal protein composition of *Bacillus thuringiensis* strains on cross-resistance in Indianmeal moths. J. Econ. Entomol. 87: 535–540.

Perez, C. P., and A. M. Shelton. 1997. Resistance of *Plutella xylostella* to *Bacillus thuringiensis* Berliner in Central America. J. Econ. Entomol. 90: 87–93.

Roush, R. T., and G. L. Miller. 1986. Considerations for design of insecticide resistance monitoring programs. J. Econ. Entomol. 79: 293–298.

Shelton, A. M., R. J. Cooley, M. K. Kroening, W. T. Wilsey, and S. D. Eigenbrode. 1991. Comparative analysis of two rearing procedures for diamondback moth (Lepidoptera: Plutellidae). J. Entomol. Sci. 26: 17–26.

Shelton, A. M., J. L. Robertson, J. D. Tang, C. Perez, S. D. Eigenbrode, H. K. Preisler, W. T. Wilsey, and R. J. Cooley. 1993a. Resistance of diamondback moth (Lepidoptera: Plutellidae) to *Bacillus thuringiensis* subspecies in the field. J. Econ. Entomol. 86: 697–705.

Shelton, A. M., J. A. Wyman, N. L. Cushing, K. Apfelbeck, T. J. Dennehy, S.E.R. Mahr, and S. D. Eigenbrode. 1993b. Insecticide resistance of diamondback moth (Lepidoptera: Plutellidae) in North America. J. Econ. Entomol. 86: 11–19.

Tabashnik, B. E. 1994. Evolution of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). Annu. Rev. Entomol. 39: 47–79.

Tabashnik, B. E., N. Finson, and M. W. Johnson. 1991. Managing resistance to *Bacillus thuringiensis:* lessons from the diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 84: 49–55.

Talekar, N. S. 1986. Diamondback moth management, pp. 241–438. *In* Proceedings of the First International Workshop. Asian Vegetable Research and Development Center, Shanhua, Taiwan.

Talekar, N. S., and A. M. Shelton. 1993. Biology, ecology, and management of the diamondback moth. Annu. Rev. Entomol. 38: 275–301.

Tang, J. D., A. M. Shelton, J. Van Rie, S. De Roeck, W. J. Moar, R. T. Roush, and M. Peferoen. 1996. Toxicity of *Bacillus thuringiensis* spore and crystal protein to resistant diamondback moth (*Plutella xylostella*). Appl. Environ. Microbiol. 62: 564–569.

Received for publication 17 March 1999; accepted 13 March 2000.