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Baseline Susceptibility of European Corn Borer (Lepidoptera: Crambidae) to *Bacillus thuringiensis* Toxins

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ABSTRACT Susceptibility to Cry1Ab and Cry1Ac toxins from *Bacillus thuringiensis* was determined for 11 populations of neonate European corn borer,*Ostrinia nubilalis* (Hübner), from the United States and 1 from northern Italy. Corn borer larvae were exposed to artificial diet treated with increasing *B. thuringiensis* concentrations, and mortality and growth inhibition were evaluated after 7 d. The range of variation in *B. thuringiensis* susceptibility indicated by growth inhibition was very similar to that indicated by mortality. Although interpopulation variation in susceptibility to both proteins was observed, the magnitude of the differences was small (≤4-fold) and comparable to the variability observed among generations within a particular population (≤3-fold). Additionally, there was no indication that *B. thuringiensis* susceptibility was influenced by pheromone race, voltine ecotype, or geographic location. These results suggest that the observed susceptibility differences reflect natural variation in *B. thuringiensis* susceptibility among corn borer populations rather than variation caused by prior exposure to selection pressures. Therefore, European corn borers apparently are susceptible to *B. thuringiensis* toxins among populations across most of their geographic range.

KEY WORDS *Bacillus thuringiensis, Ostrinia nubilalis,* geographic variability, bioassay

INCREASING PUBLIC CONCERNS about environmental hazards and widespread resistance in pest populations are threatening the continued effectiveness of conventional insecticides and should increase the use of insecticidal products derived from *Bacillus thuringiensis* Berliner (Bt) in the next decade (Tabashnik 1994). This is particularly true for transgenic Bt crops, which represent an important new tool for pest management (Fischhoff 1996). However, increased use of *B. thuringiensis* in agriculture via transgenic plants and other modes of application likely will increase the intensity of selection for *B. thuringiensis* resistance in pest populations. The development of *B. thuringiensis* resistance in target pests threatens the continued effectiveness of *B. thuringiensis* technology, both as transgenic Bt plants and all other *B. thuringiensis*-based products. The possibility of resistance development highlights the need to develop and implement resistance management strategies to prevent or delay the evolution of resistance to *B. thuringiensis* (Hokkanen and Wearing 1994). These strategies are dependent on the development of effective resistance monitoring programs capable of early detection of resistance that will allow implementation of appropriate management decisions in a timely manner (Dennehey 1987). The initial steps in implementing such programs include development of appropriate bioassay techniques and establishment of baseline susceptibility data among populations across the geographic range of the target species. With this information, potential changes in susceptibility of populations in response to selection with *B. thuringiensis* can be identified (Fischhoff 1996).

The European corn borer, *Ostrinia nubilalis* (Hübner), is one of the most destructive pests of maize, *Zea mays* L., in the United States (Mason et al. 1996) and the major target pest for control with transgenic Bt corn (Fischhoff 1996). Two pheromone races of European corn borer have been identified in North America—the Z race predominates over most of the range in Europe and North America, whereas the *E* strain is found in Switzerland, Italy, and eastern North America from Massachusetts to South Carolina (Klun and Huettel 1988, Mason et al. 1996). Additionally, Showers (1993) recognized 3 European corn borer voltine ecotypes in the United States—northern univoltine, central bivoltine, and southern multivoltine. The *E* pheromone race has bi- and multivoltine ecotypes, whereas the Z race occurs as uni-, bi-, and multivoltine ecotypes (Glover et al. 1991).

Although population variability in response to *B. thuringiensis* toxins has been assessed previously in European corn borer (Siegfried et al. 1995), variation in susceptibility among geographically distinct populations, pheromone races, and voltine ecotypes has not been

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Table 1. Source description of European corn borer populations used to establish baseline susceptibility to Cry1Ab and Cry1Ac from *B. thuringiensis*

Source	Location	Life stage	Host	Date
Colorado	Washington Co.	Eggs—larvae	Field corn	Aug. 1995
Illinois	McLean Co.	Larvae	Field corn	June 1995
Indiana	Tippecanoe Co.	Larvae	Field corn	July 1995
Iowa	Polk Co.	Larvae	Field corn	July 1995
Laboratory, NE[a]	Saunders Co.	Adults	Field corn	June 1994
Laboratory, ICI[b]	Lombardia, Italy	Larvae	Field corn	June 1993
Missouri	Pemiscot Co.	Larvae	Field corn	Sept. 1995
N. Carolina	Washington Co.	Eggs—Larvae	Potatoes	June 1995
N.-Central Italy	Lombardia, Italy	Larvae	Field corn	July 1995
N. Dakota univoltine	Burleigh Co.	Larvae	Field corn	July 1995
N. Dakota bivoltine	Burleigh Co.	Larvae	Field corn	July 1995
Nebraska	Saunders Co.	Adults	Field corn	June 1995
Pennsylvania	Snyder Co.	Larvae	Sweet corn	June 1995
Tennessee	Obion Co.	Larvae	Field corn	June 1995

[a] University of Nebraska laboratory colony in culture for 14 generations.
[b] ICI laboratory colony in culture for 20 generations.

investigated. The objective of the current study was to establish a baseline of susceptibility to Cry1Ab and Cry1Ac toxins from geographically distinct populations of corn borers, emphasizing areas where previously identified pheromone races and voltine ecotypes are known to occur.

Materials and Methods

Insect Rearing. Rearing procedures for European corn borer were based on those developed at the USDA-ARS Corn Insects Research Unit, Ames, IA (Guthrie et al. 1965). Larvae were reared at 27 ± 0.7°C in a photoperiod of 24:0 (L:D) h and 80% RH on a wheat germ-based diet (Lewis and Lynch 1969). At pupation, insects were moved to mating cages where adults were maintained with 8-h scotophase at 18 ± 0.7°C and 16-h photophase at 27 ± 0.7°C with 80% RH. Cages were misted with water twice a day, and adult diet was provided to maximize egg production (Leahy and Andow 1994). Egg masses were collected and incubated within plastic petri dishes containing moistened filter paper until hatching.

Field Collections. European corn borers were collected from corn fields in 10 states across the United States (Colorado, Illinois, Indiana, Iowa, Missouri, Nebraska, North Carolina, North Dakota, Ohio, and Pennsylvania) and from northern Italy (Table 1). Collection sites were concentrated in areas populated with the bivoltine Z race, which is the dominant race throughout the Midwest (Mason et al. 1996). Additional collections were made from areas where the univoltine Z race (North Dakota) and the multivoltine E race (North Carolina) have been previously identified (Mason et al. 1996). Of the larvae collected from North Dakota in mid-July (1st field generation), those that did not pupate were considered to represent primarily univoltine individuals. These larvae were maintained in the dark at 10°C for 120 d, and then at 30°C and 16:8 (L:D) h with 1% agar solution as a moisture source to terminate diapause and allow pupation. Two laboratory colonies also were evaluated (Table 1). The laboratory colony from Nebraska was established in 1994 and was collected from the same field site where Nebraska European corn borer were collected in 1995. This colony was tested at generation F_{14}. The ICI laboratory colony was established in 1993 from European corn borer larvae collected in the Lombardia region of north-central Italy. This colony is maintained by ICI, and egg masses from generation F_{20} were sent to our laboratory. Neonates hatching from these egg masses were tested. Additionally, insects collected in 1995 from Nebraska and North Carolina were tested for 2 or 3 consecutive generations (F_1–F_3) to evaluate variation in response among generations of a single population.

Field collections of ≈250 individuals were obtained from each site, and colonies were initiated with no fewer than 50 founder parents. Field-collected larvae, neonates from field-collected egg masses, or neonates from egg masses produced by field-collected females were placed immediately on artificial diet in individual containers to minimize possible spread of disease. Larvae were reared to adults, and the adults were allowed to mate. Egg masses from mated females were collected and allowed to hatch. Neonates obtained from field-collected parents (F_1) were used in most bioassays. In a few instances where egg production was low, neonates from the F_2 or F_3 generation were used (specifically Missouri, Tennessee, and Italy).

Bioassays. All bioassays were conducted by exposing neonates (<24 h after hatching) to treated artificial diet. Bioassays involved surface treatment of single wells of artificial diet to minimize the amount of *B. thuringiensis* required. The rearing diet developed for *Heliothis virescens* (King et al. 1985) and adapted for European corn borer (Bruce Lang, Mycogen Seeds, personal communication) was used instead of corn borer diet for *B. thuringiensis* bioassays because it was relatively inexpensive, required less time for preparation, and larval growth was comparable to growth on the standard rearing diet through the first several instars (B.D.S., unpublished data).

Bioassays were performed in 128-well trays (each well 16 mm diameter, 16 mm high; CD International, Pitman, NJ). Approximately 1 ml of diet was dispensed into each well and allowed to solidify. Eight to nine

concentrations of toxin were used, and dilutions were made in 0.1% Triton-X 100 nonionic detergent to obtain uniform spreading onto the diet surface. Each well was treated with 30 μl of the appropriate solution. Control treatments consisted of diet treated with 0.1% Triton-X 100 only. Wells were allowed to air dry for 1 h, and 1 neonate was transferred into each well. Wells then were covered with vented lids (CD International), and trays were held at 27°C, 24 h scotophase, and 80% RH. Mortality and individual larval weights were recorded 7 d later. When mortality was recorded, larvae that had not grown beyond 1st instar and weighed ≤0.1 mg were considered to be dead. As a result, the criterion for mortality used in this study accounts for both severe growth inhibition and death.

Two *B. thuringiensis* toxins were tested, Cry1Ab and Cry1Ac. Purified Cry1Ab was obtained from the *B. thuringiensis kurstaki* HD1-9 strain, which produces only the Cry1Ab protein. This material was provided by Ciba Geigy (Research Triangle Park, NC). The crystal protein preparation was obtained by density gradient centrifugation and contained ≈98% crystal protein, as determined by phase contrast microscopy. Cry1Ac was obtained from the commercial *B. thuringiensis kurstaki* MVP Bioinsecticide formulation (Mycogen, San Diego, CA), which contains a modified Cry1Ac protein produced by a recombinant strain of the bacterium *Pseudomonas fluorescens*. MVP Bioinsecticide contains only the Cry1Ac toxin and does not contain spores or any other potentially toxic ingredients (Gould et al. 1995). Nevertheless, a washing procedure was used to eliminate most inert ingredients present in the formulation. This procedure involved centrifugation of 1-ml batches of commercial product at 10,000 × g for 30 s. The supernatant was discarded and the pellet was resuspended in double-distilled water to 1 ml total volume. This material was centrifuged again for 30 s, the supernatant was removed, and the pellet was resuspended to 1 ml in double-distilled water. The resulting preparation was used as a stock solution for all bioassays with Cry1Ac.

Statistical Analysis. The bioassay was repeated 2–4 times for each population, depending on availability of larvae. Concentrations were replicated 3 times for each bioassay (total of 48 larvae per concentration per bioassay). Mortality data were analyzed by probit analysis (Finney 1971) using POLO-PC (LeOra Software 1987). A likelihood ratio test was conducted to test the hypothesis that all LC_P values (lethal concentration at which a percentage mortality P is attained) were equal. If the hypothesis was rejected, pairwise comparisons were performed and significance was declared if confidence intervals did not overlap (Savin et al. 1977). The significance of differences among slopes was determined by the likelihood ratio test for parallelism (Savin et al. 1977). Larval weights were transformed to percentage growth inhibition relative to the controls, and these data were analyzed by nonlinear regression (PROC NLIN, SAS Institute 1988) fitted to a probit model (SAS Institute 1988, pp. 1160–1162). The nonlinear probit model used was

$$E(GI|X) = c^*\Phi^* (a + b^*X),$$

where E(GI|X) is the population mean percentage growth inhibition at the logarithm of the *B. thuringiensis* protein concentration X, a and b are parameters that standardize GI at X to a mean of 0 and a standard deviation of 1, the c parameter is the upper percenage growth inhibition limit and is set at 100%, and ϕ is the distribution function of a standard normal random variable. Inverse regression was used to estimate EC_P (effective concentration at which a percentage growth inhibition level of P is attained) and set confidence intervals on EC_P (Draper and Smith 1981, pp. 47–51), using an SAS program written by D. Travnicek (Department of Biometry, UNL). First, the model was fitted to the data and confidence bands determined for percentage growth inhibition at various levels of X. Then, for a particular value of P (50, 95, or 99), the estimate of EC_P was found by solving the equation $P = 100\phi(\hat{a}+bEC_P)$ for EC_P. To determine confidence limits for EC_P, the level of EC_P corresponding to the lower and upper confidence bounds (EC_L and EC_U) at a particular level of P was determined through iteration. That is, EC_L is that value of EC for which $P_U = 100\phi(\hat{a}+bEC_L)$ and EC_U is that value of EC for which $P_L = 100\phi(\hat{a}+bEC_U)$. The subscripts for P and EC are reversed because the lower value for EC is obtained from the upper confidence band for the fitted curve, whereas the upper value of EC is obtained from the lower confidence band.

Results

Cry1Ab. Mortality data for *O. nubilalis* populations exposed to purified Cry1Ab protein are presented in Table 2. LC_{50} values ranged from 2.22 ng/cm^2 (North Carolina F_3) to 7.89 ng/cm^2 (Tennessee F_2). LC_{95} values ranged from 9.59 ng/cm^2 (North Dakota univoltine F_1) to 57.67 ng/cm^2 (Illinois F_2). Significant differences ($P < 0.05$) in susceptibility were detected among some of the populations tested, and differences between the most susceptible and most tolerant populations were 4- and 6-fold at the LC_{50} and LC_{95}, respectively.

Results regarding growth inhibition of European corn borer larvae treated with Cry1Ab are presented in Table 3. EC_{50} values ranged from 0.33 ng/cm^2 (North Carolina F_3) to 1.33 ng/cm^2 (Nebraska F_1). EC_{95} values ranged from 3.21 ng/cm^2 (North Carolina F_3) to 22.19 ng/cm^2 (North Dakota univoltine F_1). The range of variation in susceptibility indicated by larval growth inhibition (Table 3) was similar to that indicated by mortality (Table 2)—4- and 7-fold for EC_{50} and EC_{95}, respectively.

Significant differences in susceptibility, reflected by mortality and growth inhibition, were noted between generations of the same population (Tables 2 and 3). These differences were 2- to 3-fold at the LC_{50} and EC_{50} and 2- to 4-fold at the LC_{95} and EC_{95}, respectively. For mortality data (Table 2), differences among generations of the same population were observed in the Nebraska F_2 in relation to F_3 and F_{14} (≈2-fold) but not to F_1. However, these differences were significant

Table 2. Probit analysis of mortality of European corn borer neonates exposed to the Cry1Ab protein from *B. thuringiensis*

Population	Generation	n	Slope ± SE[a]	LC_{50} (95% FL)[ab]	LC_{95} (95% FL)[ab]	χ^2	df
Colorado	F_1	1,283	1.98 ± 0.14bcdef	4.70 (3.38–6.24)bcdef	31.74 (20.28–66.82)bcd	16.17[c]	6
Illinois	F_2	1,144	1.41 ± 0.11a	3.90 (2.27–6.16)abcdf	57.67 (27.19–252.9)cd	22.48[c]	6
Indiana	F_1	1,281	2.15 ± 0.17bcdefg	3.94 (3.10–4.81)bcd	23.04 (16.76–37.09)bcd	9.03	6
Iowa	F_1	857	1.87 ± 0.19bc	4.97 (4.04–5.97)cde	37.57 (27.05–60.07)cd	2.81	6
Italy	F_2	1,288	1.90 ± 0.15bcd	3.16 (2.62–3.70)ab	23.12 (18.70–31.60)bc	3.56	6
Italy lab.	F_{20}	1,292	1.88 ± 0.16bc	3.43 (2.61–4.26)abc	25.48 (18.66–41.70)bcd	6.11	6
Nebraska	F_1	1,386	1.97 ± 0.15bcde	5.24 (4.51–6.03)de	35.99 (27.89–50.09)cd	4.88	7
Nebraska	F_2	1,718	2.27 ± 0.20bcdefg	7.33 (5.93–8.75)ef	38.86 (28.48–62.74)cd	7.30	6
Nebraska	F_3	433	2.38 ± 0.32defg	3.62 (1.67–5.61)abcd	17.81 (10.15–87.34)abc	27.94[c]	6
Nebraska lab.	F_{14}	1,715	2.30 ± 0.16cdefg	3.80 (2.98–4.63)abcd	19.75 (14.77–30.29)bc	10.85	6
N. Dakota (UV)	F_2	864	2.78 ± 0.30fg	2.45 (2.02–2.87)a	9.59 (7.68–12.90)a	9.24	6
N. Dakota (BV)	F_1	1,296	1.71 ± 0.16ab	6.00 (4.37–7.81)def	55.20 (34.00–125.2)d	5.52	6
N. Carolina	F_1	1,853	1.75 ± 0.12b	5.15 (4.49–5.86)d	44.8 (34.08–63.66)d	5.02	7
N. Carolina	F_2	1,292	1.90 ± 0.14bcde	3.90 (3.12–4.73)bcd	28.49 (20.96–43.43)bcd	6.44	6
N. Carolina	F_3	1,292	2.32 ± 0.14cdefg	2.22 (1.49–3.05)a	11.34 (7.40–23.73)ab	9.19	6
Missouri	F_3	1,148	2.54 ± 0.31efg	2.66 (0.67–4.10)abc	11.86 (7.30–72.47)abc	28.41[c]	6
Pennsylvania	F_1	1,287	1.89 ± 0.13bcd	3.67 (3.15–4.23)bc	27.35 (21.56–36.82)bcd	2.41	6
Tennessee	F_2	1,148	2.98 ± 0.28g	7.89 (6.04–9.84)f	28.12 (19.89–54.34)bcd	14.51[c]	6

[a] Values followed by the same letter within a column are not significantly different ($P > 0.05$). Significance of differences among slopes determined by likelihood ratio test of equality followed by pairwise comparisons using nonoverlapping fiducial limits (Savin et al. 1977).
[b] ng Cry1Ab/cm^2 of treated artificial diet surface.
[c] Chi-square significant ($P < 0.05$).

only at the LC_{50} level. The LC_{50} values of North Carolina F_1 and F_2 were greater than those of North Carolina F_3 (2-fold). For growth inhibition data (Table 3), the 3 consecutive generations tested (F_1, F_2, and F_3) were different from each other (2- to 3-fold) for both Nebraska and North Carolina populations. Despite the observed differences among generations of the same population, no differences in susceptibility were observed between the 1st generation tested (Nebraska F_1 or Italy F_2) and the corresponding laboratory colony in culture for multiple generations (Nebraska F_{14} or Italy F_{20}).

Cry1Ac. Mortality data for European corn borer challenged with Cry1Ac protein are presented in Table 4. LC_{50} values ranged from 0.20 ng/cm^2 (Ital F_2 and Italy Lab F_{20}) to 0.78 ng/cm^2 (Illinois F_2). LC_{95} values ranged from 1.15 ng/cm^2 (Iowa F_1) to 4.53 ng/cm^2 (Illinois F_2). These values represent a 4-fold variation in susceptibility to Cry1Ac at both the LC_{50} and LC_{95}.

Results regarding growth inhibition of corn borer larvae treated with Cry1Ac are presented in Table 5. EC_{50} values ranged from 0.05 ng/cm^2 (Italy Lab F_{20}) to 0.13 ng/cm^2 (Indiana F_1). EC_{95} values ranged from 0.36 ng/cm^2 (Iowa F_1) to 1.56 ng/cm^2 (North Carolina F_1). The range of variation in Cry1Ac susceptibility indicated by larval growth inhibition (Table 5) again was comparable with that indicated by larval mortality (Table 4)—2- and 4-fold for EC_{50} and EC_{95}, respectively.

Table 3. Nonlinear regression of growth inhibition fitted to a probit model for European corn borer neonates exposed to the Cry1Ab protein from *B. thuringiensis*

Population	Generation	n	Pairwise comparison[a]	EC_{50} (95% FL)[b]	EC_{95} (95% FL)[b]
Colorado	F_1	1,283	e	0.67 (0.56–0.78)	5.21 (3.36–7.56)
Illinois	F_2	1,144	d	0.60 (0.54–0.66)	14.05 (10.69–18.16)
Indiana	F_1	1,281	cd	0.54 (0.44–0.64)	7.93 (4.79–12.33)
Iowa	F_1	857	efg	0.84 (0.69–0.99)	14.01 (8.54–21.59)
Italy	F_2	1,288	abc	0.35 (0.31–0.38)	4.97 (4.00–6.11)
Italy lab.	F_{20}	1,292	bc	0.34 (0.31–0.43)	8.07 (5.35–11.83)
Nebraska	F_1	1,386	h	1.33 (0.93–1.38)	8.10 (4.90–12.19)
Nebraska	F_2	1,718	efg	0.78 (0.60–0.99)	12.86 (6.58–22.62)
Nebraska	F_3	433	bcd	0.38 (0.22–0.57)	9.02 (3.21–21.99)
Nebraska lab.	F_{14}	1,715	gh	0.99 (0.75–1.30)	6.71 (3.39–11.38)
N. Carolina	F_1	1,853	efg	0.75 (0.66–0.86)	7.95 (5.58–10.81)
N. Carolina	F_2	1,292	h	1.07 (0.90–1.27)	5.79 (3.69–8.33)
N. Carolina	F_3	1,292	a	0.33 (0.31–0.35)	3.21 (2.72–3.77)
N. Dakota (BV)	F_2	864	bcd	0.48 (0.39–0.58)	4.47 (2.65–6.98)
N. Dakota (UV)	F_1	1,296	fg	0.98 (0.73–1.29)	22.19 (10.38–42.07)
Missouri	F_2	1,148	ab	0.34 (0.29–0.39)	5.26 (3.65–7.38)
Pennsylvania	F_1	1,287	ef	0.70 (0.60–0.82)	6.58 (4.20–9.64)
Tennessee	F_2	1,148	efg	0.79 (0.73–0.85)	10.49 (8.42–12.87)

Data not fitting the probit model ($P < 0.05$) not included.
[a] An overall F-test indicated that the parameters were not equal for all populations tested. Therefore, pairwise comparisons were conducted to determine whether the parameters of the nonlinear probit model differed significantly ($P < 0.05$) for any particular population pair. Values followed by the same letter within a column indicate populations that could be fit with common parameters.
[b] ng Cry1Ab/cm^2 of treated artificial diet surface.

Table 4. Probit analysis of mortality of European corn borer neonates exposed to the Cry1Ac protein from *B. thuringiensis*

Population	Generation	n	Slope ± SE[a]	LC_{50} (95% FL)[ab]	LC_{95} (95% FL)[ab]	χ^2	df
Colorado	F_1	1,160	2.09 ± 0.13ab	0.33 (0.25–0.43)abcd	2.02 (1.42–3.34)abc	13.24	6
Illinois	F_2	1,288	2.15 ± 0.15ab	0.78 (0.67–0.89)e	4.53 (3.68–5.87)d	5.26	6
Indiana	F_1	1,150	1.19 ± 0.12a	0.40 (0.28–0.53)cd	2.90 (1.98–5.09)bcd	13.48	6
Iowa	F_1	1,292	2.23 ± 0.17ab	0.21 (0.17–0.25)a	1.15 (0.93–1.47)a	3.63	6
Italy	F_2	1,282	1.90 ± 0.11a	0.20 (0.16–0.25)a	1.46 (1.08–2.19)ab	9.20	6
Italy lab.	F_{20}	1,290	1.76 ± 0.12a	0.20 (0.14–0.26)ab	1.72 (1.21–2.78)abc	10.00	6
Nebraska (F_1)	F_1	1,288	1.82 ± 0.11a	0.29 (0.21–0.37)abc	2.30 (1.62–3.73)bcd	11.45	6
Nebraska lab.	F_{14}	1,291	2.02 ± 0.12ab	0.32 (0.24–0.42)abc	2.09 (1.47–3.51)abc	14.54[c]	6
N. Dakota (BV)	F_2	862	2.12 ± 0.17ab	0.53 (0.42–0.66)d	3.19 (2.38–4.76)cd	6.28	6
North Carolina	F_1	2,135	1.83 ± 0.09a	0.33 (0.25–0.42)bcd	2.64 (1.89–4.17)bcd	17.45[c]	6
North Carolina	F_2	1,001	2.52 ± 0.19b	0.42 (0.30–0.55)cd	1.88 (1.31–3.37)abc	15.95[c]	6
Pennsylvania	F_1	1,420	1.95 ± 0.10ab	0.42 (0.30–0.57)cd	2.97 (1.93–5.72)bc	24.58[c]	6
Tennessee	F_2	1,285	2.33 ± 0.14b	0.44 (0.36–0.52)cd	2.23 (1.73–3.12)bc	8.50	6

[a] Values followed by the same letter within a column are not significantly different ($P \geq 0.05$). Significance of differences among slopes determined by likelihood ratio test of equality followed by pairwise comparisons using nonoverlapping fiducial limits (Savin et al. 1977).
[b] ng Cry1Ac/cm^2 of treated artificial diet surface.
[c] Chi-square significant ($P < 0.05$).

Differences in susceptibility to Cry1Ac among generations within a population (Tables 4 and 5) were more subtle (<2-fold) than for Cry1Ab. In fact, significance was achieved only at the EC_{50} level, between North Carolina F_1 and F_2 (Table 5). However, only 2 generations were evaluated with Cry1Ac, which may have decreased the likelihood of observing significant differences among populations. As with Cry1Ab, no differences were noted among generations of recently collected insects (Nebraska F_1 and Italy F_2) and laboratory colonies in culture for many generations (Nebraska F_{14} and Italy F_{20}). These results suggest that although variation in susceptibility to *B. thuringiensis* toxins were observed from generation to generation, laboratory culturing of European corn borers for multiple generations does not seem to affect susceptibility to *B. thuringiensis*. Overall, susceptibility to Cry1Ac from MVP Biopesticide was about an order of magnitude greater than to purified Cry1Ab (Tables 2–5).

Significant differences were noted for probit line slopes, both among generations within a particular population and among geographically distinct populations (Tables 2 and 4). However, the range of variation was relatively small (<2-fold). The slopes of concentration-mortality lines generated for European corn borer in response to *B. thuringiensis* toxins (1.7–3.0, Tables 2 and 4) were ≈2-fold less steep than those observed for corn borer treated with Fipronil (B.D.S., unpublished data), supporting other observations that shallow slopes are characteristic of the response of most insects to microbial insecticides (Burges 1971, Stone and Sims 1993).

Discussion

Although interpopulation variation in susceptibility to Cry1Ab and Cry1Ac was observed, the magnitude of the differences was small (i.e., 2–7-fold, at the LC_{50} and EC_{50}) and comparable with the variation observed among generations within populations (i.e., (≤3-fold at the LC_{50} and EC_{50}). Additionally, susceptibility to *B. thuringiensis* was not related to pheromone race, voltine ecotype, or geographic location. Because verification of pheromone strain and ecotype

Table 5. Nonlinear regression of growth inhibition fitted to a probit model for European corn borer neonates exposed to the Cry1Ac protein from *B. thuringiensis*

Population	Generation	n	Pairwise comparison[a]	EC_{50} (95% FL)[b]	EC_{95} (95% FL)[b]
Colorado	F_1	1,160	b	0.076 (0.072–0.081)	0.44 (0.37–0.51)
Illinois	F_2	1,288	f	0.11 (0.083–0.14)	1.56 (0.78–2.83)
Indiana	F_1	1,150	g	0.13 (0.12–0.14)	0.66 (0.53–0.81)
Iowa	F_1	1,292	d	0.071 (0.064–0.077)	0.36 (0.29–0.45)
Italy lab.	F_{20}	1,290	a	0.050 (0.045–0.055)	0.44 (0.35–0.54)
Italy	F_2	1,282	a	0.052 (0.044–0.058)	0.45 (0.33–0.60)
Nebraska	F_1	1,288	c	0.079 (0.070–0.089)	0.84 (0.62–1.12)
Nebraska lab.	F_{14}	1,291	bcd	0.069 (0.066–0.073)	0.48 (0.42–0.55)
North Carolina	F_1	2,135	bc	0.067 (0.059–0.080)	0.71 (0.49–0.99)
North Carolina	F_2	1,001	e	0.11 (0.096–0.13)	0.79 (0.51–1.13)
N. Dakota (BV)	F_2	862	e	0.12 (0.097–0.15)	1.18 (0.67–1.90)
Tennessee	F_2	1,285	e	0.11 (0.10–0.11)	0.65 (0.56–0.76)

Data not fitting the probit model ($P < 0.05$) not included.
[a] An overall F-test indicated that the parameters were not equal for all populations tested. Therefore, pairwise comparisons were conducted to determine whether the parameters of the nonlinear probit model differed significantly ($P < 0.05$) for any particular population pair. Values followed by the same letter within a column indicate populations that could be fit with common parameters.
[b] ng Cry1Ab/cm^3 of treated artificial diet surface.

was not performed, it may be inappropriate to conclude that these differences in susceptibility do not exist. However, based on known distributions of these genetic variants, our results do not indicate any strong differences among strains.

In a similar study, Stone and Sims (1993) found considerable interpopulation variation in *B. thuringiensis* susceptibility among U.S. populations of the corn earwom, *Helicoverpa zea*, and tobacco budworm, *Heliothis virescens* (16- and 4-fold, respectively). These data were reexamined by Sims et al. (1996), who suggested that interpopulation variation in *B. thuringiensis* susceptibility in these species may reflect nongenetic variation or sampling error, because the populations tested represented a small sample, taken at one point in time, of considerably larger multivoltine populations. Similar levels of variability in *B. thuringiensis* susceptibility were noted previously for the European corn borer using different bioassay techniques (Siegfried et al. 1995). Rossiter et al. (1990) found significant variation in *B. thuringiensis* susceptibility among 3 populations of gypsy moth, *Lymantria dispar*. A significant proportion of that variation was among siblings within a family, whereas differences among families (caused by genetic and mean maternal effect differences among families) accounted for only 16% of the total variation.

As reported for the gypsy moth (Rossiter et al. 1990), the variation in susceptibility to *B. thuringiensis* in European corn borers observed in the current study may reflect vigor differences in growth and developmental capability, attributes that are the product of both genotype and the maternally determined nutritional status of the egg. Similar variation in LC values has been reported among repeated bioassays with *B. thuringiensis* proteins against larvae from single strains of Colorado potato beetle, *Leptinotarsa decemlineata*, and diamondback moth, *Plutella xylostella*. For a laboratory strain of the Colorado potato beetle tested with *B. thuringiensis* subsp. *tenebrionis* over an 83-wk period, LC_{50} values ranged from 20 to 255 mg (AI)/ml, a 13-fold difference in response to the *B. thuringiensis* toxin (Robertson et al. 1995). Similarly, for a diamondback moth population tested with *B. thuringiensis* subsp. *kurstakii* for 24 wk, LC_{50} values varied from 0.18 to 0.66 ($\approx$4-fold) (Robertson et al. 1995). Based on the data from the current study, it is not possible to determine whether the observed differences in susceptibility among corn borer populations are partially heritable or simply reflect environmental influences upon response of insects to the *B. thuringiensis* toxins. However, the generation-to-generation variation in response to the *B. thuringiensis* toxins suggests that at least part of the variation is caused by nonheritable factors associated with repeated bioassays. Overall, there was no indication of increased tolerance associated with prior exposure to *B. thuringiensis* selection or to long-term rearing on artificial diet. The results of this study support the assumption that the European corn borer remains susceptible to Cry1Ab and Cry1Ac from *B. thuringiensis* across most of its geographic distribution.

Intrapopulation variation in response to chemical or microbial insecticides is a common phenomenon when any bioassay is repeated (Robertson et al. 1995), and the extent of both intra- and interpopulation natural variation in susceptibility to a given pesticide should be investigated before biologically important changes can be identified with any certainty. Ideally, this should be done before the product is used commercially rather than after resistance is already widespread. Knowledge of the natural variation in response to *B. thuringiensis* among corn borer populations before widespread commercial use of Bt corn is necessary to avoid unwarranted concerns about resistance to *B. thuringiensis* in field surveys of European corn borer populations.

The diet surface treatment bioassay used in this study provided reliable and consistent results and required significantly less purified *B. thuringiensis* protein than a comparable diet incorporation assay. Testing insects with purified *B. thuringiensis* toxins is recommended over commercial formulations containing these proteins because the purified forms may elucidate shifts in susceptibility to transgenic plants more readily than a complex mixture of several proteins, spores, and formulation ingredients (Stone and Sims 1993). For example, although the MVP formulation used in this study should have been devoid of potentially toxic inert ingredients, we are not certain whether the apparently greater potency of Cry1Ac was inherent to the toxin or an artifact of the formulation. Our results are inconsistent with previous comparisons of European corn borer susceptibility to Cry1Ab and Cry1Ac (Denolf et al. 1993) that indicated higher susceptibility to Cry1Ab. Further testing with purified Cry1Ac would be required to compare toxicity of the 2 toxins adequately.

Growth inhibition data (established by weighing individual larvae) were more sensitive than mortality data (which included both extreme growth inhibition and mortality responses) for detecting the sublethal effects of the *B. thuringiensis* toxins (i.e., EC_{50} values were one order of magnitude lower than LC_{50} values). However, there was no clear advantage of using this response instead of mortality data. The range of variation in susceptibility among European corn borer populations was similar between the 2 response criteria, and because mortality data are easier and faster to collect, this response should be more appropriate for large-scale monitoring. However, scoring this type of bioassay requires some training to determine visually which larvae are severely stunted ($\leq$0.1 mg), although the chance for error in interpretation could be minimized by concurrently testing sample larvae on control diet to provide a direct size comparison (Sims et al. 1996).

Development of *B. thuringiensis* baseline susceptibility data before widespread commercial release of transgenic Bt corn represents the first step toward the development of a monitoring program designed to identify resistant European corn borer populations should they develop. These data also may provide information that will allow development of diagnostic bioassays that would be more efficient in detection of populations resistant to *B. thuringiensis*. Monitoring for potential resistance in corn borer populations would be facilitated by the establishment of such a

diagnostic bioassay, and future efforts should concentrate on development of this critical tool for effectively monitoring the resistance status of European corn borer field populations to *B. thuringiensis* as they become exposed to transgenic Bt corn.

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