

Comparison of Leaf-Dip and Diet Bioassays for Monitoring *Bacillus thuringiensis* Resistance in Field Populations of Diamondback Moth (Lepidoptera: Plutellidae)

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ABSTRACT Seven *Plutella xylostella* (L.) populations were compared for resistance to *Bacillus thuringiensis* subsp. *kurstaki* Berliner in leaf-dip and diet-incorporated bioassays. The persistence of the microbial insecticide incorporated in diet was studied at 2 storage temperatures (26.7 and 5°C). The effect of larval age on toxicity of a discriminating concentration of Javelin incorporated in diet (20.5 mg [AI]/liter) was also studied. In the leaf-dip bioassay, LC₅₀s of 6 field populations were 2.6- to ≈829-fold higher than the LC₅₀ of Geneva 88, the susceptible population, whereas LC₅₀s in the diet-incorporated bioassay were 1.7- to ≈42-fold higher in the field populations compared with the LC₅₀ of Geneva 88. Despite the difference in resistance ratios between bioassay methods, LC₅₀s were significantly correlated. Both methods were effective in distinguishing between susceptible and resistant populations. With 1 exception, slopes of the regressions in individual populations were not significantly different. We concluded that both bioassay techniques were suitable for monitoring evolution of resistance to *B. thuringiensis* in field populations of *P. xylostella*. Compared with diet prepared the day of the tests, the toxicity of *B. thuringiensis* did not vary significantly in diet stored for 10 d at 26.7°C, and the LC₅₀s of Javelin did not vary significantly in diet stored for 14 d at 5°C. Mortality of 2nd and 3rd instars was not significantly different when exposed to a discriminating concentration of Javelin incorporated in diet, but mortality was significantly lower in 4th instars. The diet-incorporated *B. thuringiensis* assay can be used as a resistance test kit for resistance studies.

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

DIAMONDBACK MOTH, *Plutella xylostella* (L.), is a key pest of crucifers worldwide and has evolved resistance to synthetic organic insecticides (Liu et al. 1981, Sun et al. 1986) and *Bacillus thuringiensis* Berliner (Shelton et al. 1993a, Tabashnik et al. 1990). Researchers have used several bioassay techniques to assess insecticide resistance of this insect. Bioassay methods used in resistance studies with organic pesticides include directly sprayed larvae (Kao et al. 1989, Liu et al. 1981), residue bioassays where the pesticide is distributed in uniformly sized droplets (Adams et al. 1990), disposable cup bioassay (Plapp et al. 1992), leaf dip or leaf residue (Tabashnik and Cushing 1987, Tabashnik et al. 1987, Magaro and Edelson 1990, Leibee and Savage 1992, Shelton et al. 1993b), and topical application (Tabashnik and Cushing 1987, Leibee and Savage 1992). Leaf residue and topical bioassays have been compared for their assessment of insecticide resistance in *P. xylostella* (Tabashnik and Cushing 1987). Bioassays with *B. thuringiensis* differ from those with synthetic insecticides because the insecticidal crystal proteins (ICPs) produced by this bacteria have to be ingested to be toxic to sensitive target species (de Barjac 1987).

The leaf residue bioassay (leaf-dip) has been the most common procedure for assessing *P. xylostella* resistance to commercial formulations of *B. thuringiensis* (Tabashnik et al. 1990, Shelton et al. 1993b), and spores and ICPs (Tang et al. 1996). Diet incorporation bioassays have been used to evaluate the biological activity of novel commercial preparations of *B. thuringiensis* in laboratory colonies of *P. xylostella* (Tsuchiyama 1978, Asano et al. 1993, Asano and Seki 1994), and in surface treated diet to distinguish between a susceptible and a resistant field population of diamondback moth (Ferré et al. 1991). Diet incorporation bioassays with *B. thuringiensis* have also been used to study behavioral responses and sublethal effects on other lepidoteran species (Gould et al. 1991, Johnson et al. 1991, Vail et al. 1992), and to evaluate resistance to *B. thuringiensis* in Indian meal moth and almond moth (Lepidoptera: Pyralidae) (McGaughey and Beeman 1990).

Determining the extent and geographic distribution of resistance to *B. thuringiensis* in field populations of *P. xylostella* is the 1st step toward developing resistance management strategies (Tabashnik 1994), because resistance to

Table 1. Crops and collection sites of *P. xylostella* populations

Population	Collection sites	Crop
Geneva 88	Geneva, Ontario County, New York, 1988	Cabbage
Belle Glade	Belle Glade, Palm Beach County, Florida, April 1994	Mustard
Honduras	San Juan del Rancho, Department of Francisco Morazan, Honduras, July 1993	Cabbage
Thailand	Rim Tai Subdistrict of Mae Rim District, Chiang Mai Province, Thailand, January 1994	Cauliflower
Zellwood	Zellwood, Orange County, Florida, April 1994	Cabbage
Georgia	Coastal Plain Experiment Station, Tifton, GA, January 1994	Mustard
Loxahatchee	Loxahatchee, Palm Beach County, Florida, April 1994	Chinese cabbage

insecticides in this insect may vary significantly in populations <10 km apart (Tabashnik et al. 1987). However, when the number of target populations is large, the required labor and infrastructure (i.e., environmental chambers, cages, laboratory space) will also be substantial. For instance, an area-wide insecticide resistance study done by Shelton et al. (1993b), required the shipment of *P. xylostella* larvae from 19 states within the United States, Mexico, and Canada to a laboratory where tests were conducted. An alternative method for assessing resistance in multiple insect populations was conducted by Bishop and Grafius (1991). In this study, Bishop and Grafius delivered resistance test kits to cooperators in several locations within the state of Michigan to evaluate the status of insecticide resistance in >60 populations of Colorado potato beetle (Coleoptera: Chrysomelidae). However, when resistance test kits are prepared for later use, loss of insecticide persistence may limit their use. Vail et al. (1992) reported that ICPs from *B. thuringiensis* subsp. *kurstaki* did not lose activity for 8 d in surface-treated diet stored at 26.7°C, and ICPs have remained stable for 7 mo when stored at 6°C in high pH buffer (pH = 10.5) (van Frankenhuyzen et al. 1993).

In this study, we compared the responses of field and laboratory populations of *P. xylostella* to *B. thuringiensis* subsp. *kurstaki* in leaf-dip and diet incorporation bioassays. We also studied the persistence of a commercial formulation of *B. thuringiensis* in stored diet, and assessed the effect of larval age on a potential discriminating concentration of this bioinsecticide also incorporated in diet.

Materials and Methods

Insects. We used 6 populations of *P. xylostella* collected in crucifer fields from different locations (Table 1). Our susceptible population (Geneva 88) was used as our standard for comparisons. This population has been reared continuously for >120 generations on artificial diet at the New York State Agricultural Experiment Station, Geneva. All other populations were reared on rape seedlings *Brassica napus* subsp. *oleifera*. All tests presented herein were done with larvae reared on rape seedlings

(Shelton et al. 1991) in the greenhouse until they reached 2nd instar (0.2–0.4 mg). Different generations of the populations collected in Florida (Belle Glade, Zellwood, and Loxahatchee) and Thailand were also used for other studies (Perez and Shelton 1996).

Insecticide. The bioassays were done with a commercial formulation of Javelin (*B. thuringiensis* subsp. *kurstaki*; WG 6.4% [AI], Sandoz, Des Plaines, IL; Lot No. 8611442). Genes producing the following ICPs are present in *B. thuringiensis* subsp. *kurstaki*: Cry IA(a), Cry IA(b), Cry IA(c), Cry IIA, and Cry IIB (Koziel et al. 1993).

Comparison of Leaf-Dip and Diet Incorporation Bioassays. We did a leaf-dip bioassay similar to that described by Shelton et al. (1993). For the bioassay with *B. thuringiensis* incorporated in diet, we used the artificial diet for *P. xylostella* reported by Shelton et al. (1991), with 2 modifications. First, no formaldehyde was added, and, 2nd, cabbage juice (65 mg cabbage leaves per 500 ml of water) autoclaved at 110°C for 20 min was used to replace 10% of the total water specified in the original diet. Javelin was serially diluted in 10 ml distilled water by using a 3.16-fold dilution factor to obtain concentrations ranging from 0.02 to 640 mg (AI)/liter of diet. A hot water bath (65°C) was used to keep the diet liquid before incorporating the bioinsecticide. After mixing, 30-ml clear plastic cups were filled with 3–4 ml of diet and allowed to cool for a period of 2 h. Five 2nd-instar (5 d old) *P. xylostella* were placed in each cup, and 5 replicates were used per concentration for a single population. Each population was exposed to 7 or 8 concentrations, and the range of concentrations used caused between 0 and 100% larval mortality in all populations tested. Five cups with an equal number of larvae were also used with untreated diet as controls. Larvae were allowed to feed for a period of 48 h at 26.7 ± 1°C, 30 ± 2% RH, and a photoperiod of 16:8 (L:D) h, after which mortality was recorded. A larva was considered dead if it did not move when prodded.

Persistence of *B. thuringiensis* Incorporated in Diet. The effect of storage on persistence of *B. thuringiensis* toxicity was tested with 2 bioassay procedures. In the 1st procedure, we used a single

Table 2. Concentration-mortality relationship of *P. xylostella* from various geographical origins exposed to *B. thuringiensis* subsp. *kurstaki* in leaf-dip and diet bioassays

Population ^a	Leaf-dip bioassay					<i>B. thuringiensis</i> incorporated in diet					<i>P</i> ^d
	<i>n</i>	Slope ± SE	LC ₅₀ ^b	95% CL	RR ^c	<i>n</i>	Slope ± SE	LC ₅₀ ^b	95% CL	RR ^c	
Geneva 88	174	2.17 ± 0.38	0.07	(0.05– 0.1)	1	171	2.23 ± 0.32	1.24	(0.9– 1.8)	1	0.90
Belle Glade	175	1.63 ± 0.21	0.18	(0.09– 0.4)	2.6	225	1.20 ± 0.14	2.13	(1.4– 3.4)	1.7	0.09
Honduras	205	1.12 ± 0.21	0.68	(0.3 – 1.2)	9.7	199	1.14 ± 0.16	8.76	(3.4–35.5)	7.3	0.93
Thailand	176	1.60 ± 0.20	0.87	(0.5 – 1.1)	12.4	202	1.50 ± 0.20	9.60	(5.7–12.7)	7.7	0.75
Zellwood	225	0.84 ± 0.26	10.70	(1.0 – 26.4)	152.8	225	1.16 ± 0.14	20.68	(8.1–54.0)	16.7	0.15
Georgia	154	2.63 ± 0.41	44.81	(19.0–106.0)	640.0	176	1.94 ± 0.27	54.50	(38.4–77.8)	41.9	0.14
Loxahatchee	225	0.88 ± 0.11	58.00	(21.2–240.4)	828.9	200	1.41 ± 0.20	42.20	(28.0–65.0)	34.0	0.008

^a Geneva 88, Honduras, and Thailand populations at generations 120, 9, and 3, respectively. All other populations at generation 2.

^b Milligrams (AI)/liter of water or diet.

^c Resistance ratios = LC₅₀ of resistant population per LC₅₀ of Geneva 88.

^d Slopes across bioassay are not significantly different if *P* > 0.05 (POLO, Russell et al. 1977).

concentration of Javelin (9.6 mg [AI]/liter of diet) based on the LC₅₀ of a *P. xylostella* population from Thailand (Table 2). Diet was prepared on 5 separate dates. After preparation, larvae were exposed to the diet after holding the diet for 0, 2, 6, 8, and 10 d. The diet was placed in 30-ml plastic cups as above. We used 10 cups of treated diet as replicates and 3 cups of untreated diet as controls. Except for the day of the test, each time the diet was formulated it was stored at 26.7 ± 1°C, 30 ± 2% RH, and 16:8 (L:D) h photoperiod with the cup lids on. Nine to 12 2nd instars of the Thailand population (at generation 3) were placed in each cup, for a total of 100 larvae per formulation date (treatment), and 30 larvae were used as controls for each treatment. Larvae were allowed to feed for 48 h before assessing mortality as described above.

In the 2nd procedure, persistence of *B. thuringiensis* subsp. *kurstaki* incorporated in diet was tested in a diet bioassay with concentrations (3.16-fold dilutions) ranging from 0.02 to 64 mg (AI) of Javelin per liter of diet. The diet and concentrations were formulated 14, 7, and 0 d before the test, and stored in a refrigerator at 5°C with the cup lids on. Five cups were used per concentration (replicates), including 5 cups with untreated diet being used as controls. Five 2nd instars of the Geneva 88 population (at generation 120) were placed in each plastic cup for a total of 25 larvae per concentration. Larvae were allowed to feed for a period of 48 h before assessing mortality.

Effect of Larval Age on Toxicity of a Diagnostic Concentration of *B. thuringiensis* Incorporated in Diet. We used a single concentration of *B. thuringiensis* subsp. *kurstaki* of 20.5 mg (AI)/liter of diet. The latter is a concentration that induced mortalities of *P. xylostella* highly correlated with mortalities induced by field applications with Javelin (Perez and Shelton 1996), and was used to assess resistance in diamondback moth populations from Central America (Perez and Shelton 1997). Eggs from 2 *B. thuringiensis* subsp. *kurstaki*-susceptible

populations, Geneva 88 and Belle Glade, were inoculated separately on rape seedlings on 3 separate dates to obtain larvae of 2nd, 3rd, and 4th instars or 5, 7, and 9 d old, respectively. Weights (mean ± SEM) of 15 individuals were 0.38 ± 0.02, 1.28 ± 0.11, and 4.70 ± 0.27 mg, for 2nd, 3rd, and 4th instars, respectively. On the day of the test, 10 larvae of each age group were placed in cups containing 3–4 ml of diet, and 10 cups were used as replicates for a total of 100 individuals per instar of each population. Three cups with untreated diet were also used as controls for each instar and population for a total of 30 larvae. The larvae were allowed to feed for a period of 48 h as above before assessing mortality.

Statistical Analyses. Mortality data from leaf-dip and diet-incorporated bioassays were analyzed with POLO (Russell et al. 1977, LeOra Software 1987) assuming the probit model. Median lethal concentrations (LC₅₀s) and their corresponding 95% CL were estimated. Resistance ratios were calculated by dividing the LC₅₀ of a field population by the LC₅₀ of our standard susceptible population (Geneva 88). Two LC₅₀s were significantly different if the corresponding 95% CLs did not overlap. Pearson correlation (SYSTAT 1992) was used to determine the correlation between LC₅₀s from both bioassay methods. We also compared the slopes of the concentration-mortality regressions of individual populations in the 2 bioassays by testing the hypothesis of parallelism (POLO; Russell et al. 1977). Two slopes were considered significantly different when the chi-square was associated with a probability *P* < 0.05. The *g* statistic is automatically estimated by POLO and was also used to compare the data from the 2 bioassay methods. If *g* > 0.5, confidence limits are not calculated (Finney 1971, Robertson and Preisler 1992).

The test for persistence of *B. thuringiensis* incorporated in diet (at 9.6 mg [AI] of Javelin per liter of diet) was arranged in a completely randomized design using 5 diet formulation dates as treatments and mortality as the dependent variable. We used analysis of variance (ANOVA)

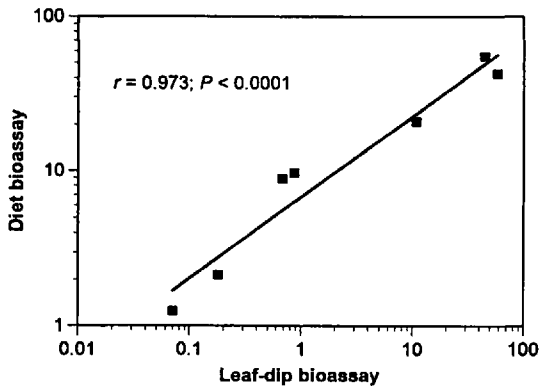


Fig. 1. Correlation between LC_{50} s of *B. thuringiensis* subsp. *kurstaki* from leaf-dip and diet-incorporated bioassays.

(SYSTAT 1992) to test for effect of diet formulation date on mortality of 2nd instar *P. xylostella*. Experimental data from this test were not transformed before ANOVA because percentage of mortality was always between 30 and 70% (Snedecor and Cochran 1989). Because the concentration used in this test was based on the LC_{50} of the population from Thailand, we also used the chi-square method (SYSTAT 1992) to test for significant departures from the expected 50% mortality in each treatment.

The test for effect of larval age on toxicity of a diagnostic concentration of *B. thuringiensis* subsp. *kurstaki* (20.5 mg [Al] of Javelin per liter of diet) was arranged as a fully factorial experiment with population (2 levels) and instars (3 levels) as factors and mortality as the dependent variable. The proportion (P = dead per total) of dead larvae on each cup was transformed to the arcsine of the square root of P before ANOVA (SYSTAT 1992). We used Abbott's (1925) formula to correct the mortality in the treatments. The Tukey honestly significant difference (HSD) procedure (P = 0.05; SYSTAT 1992) was used to separate the mean mortalities observed in the population by instar interaction term.

Results

Comparison of Leaf-Dip and Diet Bioassays.

In leaf-dip bioassays, the LC_{50} s of field populations of *P. xylostella* were 2.6- to \approx 829-fold higher than the LC_{50} of Geneva 88; in the diet-incorporated bioassay the LC_{50} s were 1.7- to 41.9-fold higher in the field populations than the LC_{50} of the Geneva 88 population (Table 2). Comparisons across bioassays indicate that the LC_{50} s of the least resistant populations were higher in diet bioassay. For instance, the LC_{50} of Geneva 88 in the diet-incorporated bioassay was 17.7-fold higher than the LC_{50} in the leaf-dip bioassay. That trend was similar in the populations from Belle Glade, Honduras, and Thailand, all of which displayed

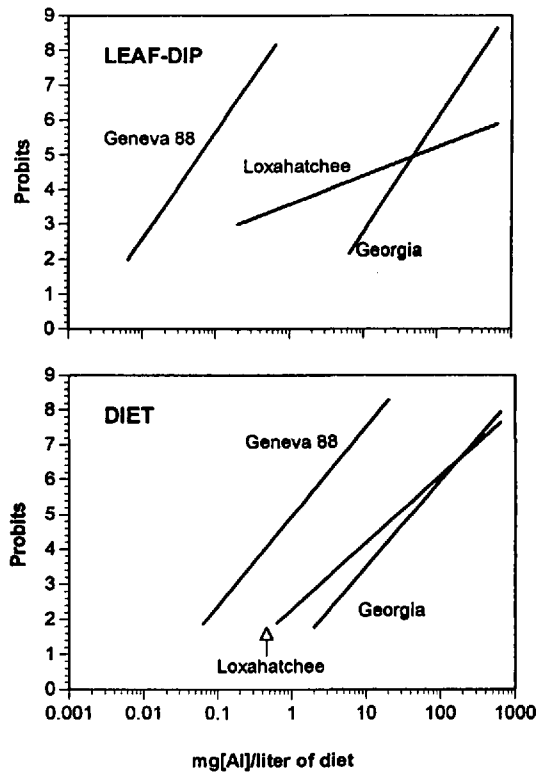


Fig. 2. Probit regression lines of susceptible (Geneva 88) and highly resistant (Loxahatchee and Georgia) *P. xylostella* in leaf-dip and diet-incorporated bioassays with *B. thuringiensis* subsp. *kurstaki*.

relatively moderate levels of resistance. The gap between LC_{50} s of individual populations across bioassay methods diminished with increasing levels of resistance. For instance, the LC_{50} in the diet bioassay of the population from Zellwood was \approx 2-fold higher than the corresponding LC_{50} in the leaf-dip bioassay, and the LC_{50} s of the populations from Georgia and Loxahatchee were similar in both bioassays.

Despite the differences in LC_{50} s of individual populations across bioassay methods, the LC_{50} s from both bioassays were significantly correlated (r = 0.973, P < 0.0001; Fig. 1), and both bioassays were effective in distinguishing between susceptible and resistant *P. xylostella*. For instance, 95% CL of the LC_{50} s of Belle Glade and Geneva 88 overlap in both bioassays, but the LC_{50} of the population from Honduras was significantly different from the LC_{50} of Geneva 88 regardless of the bioassay technique. Also, both bioassay methods indicated that the populations from Georgia and Loxahatchee were highly resistant (Table 2; Fig. 2).

In all but one population, slopes of the probit regression lines did not vary significantly (P > 0.05) by bioassay method, but the population from Loxahatchee displayed a significantly steeper slope in the diet bioassay when compared with the slope

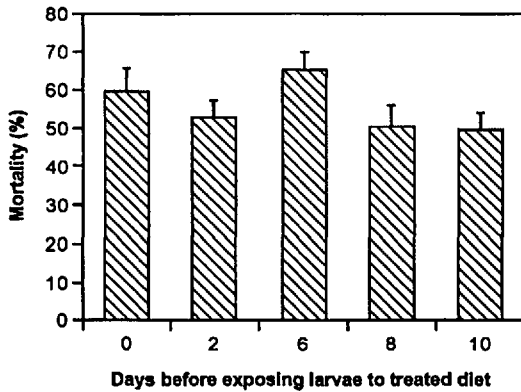


Fig. 3. Toxicity of *B. thuringiensis* subsp. *kurstaki* (at 9.6 mg [AI] of Javelin per liter of diet) incorporated in diet 0–10 d before exposing 2nd instars of *P. xylostella* from Thailand.

of the same population in the leaf-dip bioassay ($P = 0.008$; Table 2). The g values for the leaf-dip and diet bioassays ranged from 0.06 to 0.36 and 0.06 to 0.29, respectively, and in all cases permitted estimation of the 95% CL of the LC_{50} s. No mortality in the controls was observed in the diet incorporation method, whereas 4 and 8% mortality occurred in the control groups of the leaf-dip bioassay with populations from Honduras and Zellwood, respectively.

Persistence of *B. thuringiensis* Incorporated in Diet. Toxicity of diet containing *B. thuringiensis* subsp. *kurstaki* (9.6 mg [AI]/liter) did not decline over 10 d at room temperature ($F = 1.84$; $df = 4$, 45; $P = 0.14$; Fig. 3). Average mortalities ranged from 49.5 to 65.5%, with lowest and highest mortality observed on diet stored during 10 and 6 d, but deviations from the expected 50% mortality were not significant ($\chi^2 = 6.85$, $df = 4$, $P = 0.15$). In the bioassays with several concentrations of Javelin incorporated in diet and stored at 5°C for 0, 7, and 14 d, the LC_{50} s and LC_{90} s of Geneva 88 were not significantly different, and ranged from 1.07 to 1.25, and 11.10 to 12.91 mg (AI)/liter of diet, respectively (Table 3).

Effects of Larval Age on Toxicity of *B. thuringiensis* Incorporated in Diet. Average larval mortalities of populations from Geneva 88 and from Belle Glade were significantly different ($F = 41.9$; $df = 1$, 54; $P < 0.0001$; Table 4). The effects of instar ($F = 150.1$; $df = 2$, 54; $P < 0.0001$)

Table 4. Toxicity of a diagnostic concentration (20.5 mg [AI]/liter) of *B. thuringiensis* subsp. *kurstaki* incorporated in diet to 3 instars of *P. xylostella*

Population	Instar	n	Mean mortality (%) \pm SEM
Geneva 88	2nd	98	96.4 \pm 0.3a
	3rd	103	97.6 \pm 0.0a
	4th	100	59.5 \pm 1.9b
Belle Glade	2nd	100	91.3 \pm 1.8a
	3rd	100	92.1 \pm 0.5a
	4th	100	12.2 \pm 1.1c

Geneva 88 and Belle Glade populations were tested at generations 120th and 3rd, respectively. Means followed by the same letter are not significantly different by the Tukey HSD procedure (SYSTAT 1992).

and the population by instar interaction were also significant ($F = 11.9$; $df = 2$, 54; $P < 0.0001$). However, pairwise comparisons based on the population by instar interaction (Tukey HSD procedure [SYSTAT 1992]; Table 4) suggest that 4th instars accounted for most of the observed variation in mortality. For instance, mortality (mean \pm SEM) in 4th instars of Geneva 88 was significantly higher than that of 4th instars of the population from Belle Glade ($P < 0.0001$), but differences in mortality of 2nd and 3rd instars between and within populations were not significant ($P > 0.05$; Table 4). In addition, mortalities of 4th instars of both populations were significantly lower than the mortalities of 2nd and 3rd instars ($P > 0.302$), which displayed mortalities always $>91\%$. The lower mortality observed in 4th instars may be caused by decreased sensitivity of larvae with higher weight or to insufficient exposure to *B. thuringiensis* before pupation. After 48 h, 21 and 58% of 4th instars initially exposed pupated in Geneva 88 and Belle Glade populations, respectively. No pupae were observed in the controls of 2nd and 3rd instars, whereas pupation in the controls of 4th instars of Geneva 88 and Belle Glade was 90 and 76.7%, respectively. No mortality was observed in the control groups of 2nd and 3rd instars, but 3.2 and 2.5% died in the control groups of Geneva 88 and Belle Glade, respectively.

Discussion

Diamondback moth resistance to *B. thuringiensis* subsp. *kurstaki* has already been

Table 3. Concentration–mortality response of a susceptible *P. xylostella* population (Geneva 88) exposed to *B. thuringiensis* subsp. *kurstaki* incorporated in diet and stored at 5°C

Days ^a	n	Slope \pm SE	LC_{50} ^b	(95% CL)	LC_{90} ^b	(95% CL)
0	228	1.28 \pm 0.15	1.12	(0.60–2.15)	11.65	(5.70–28.90)
7	200	1.53 \pm 0.21	1.07	(0.70–1.70)	11.10	(5.12–29.13)
14	238	1.12 \pm 0.12	1.25	(0.70–2.40)	12.91	(6.47–30.82)

^a Days of diet storage before exposing the larvae.

^b Milligrams (AI)/liter of diet.

documented in field populations collected from Florida in 1992 (Shelton et al. 1993a). Our results with populations collected in 1994 confirmed continued high levels of resistance in field populations from Loxahatchee, intermediate levels of resistance in the population from Zellwood, and little to no resistance in a population from Belle Glade (Table 2). The data on field populations from Florida reported here were taken from 2nd-generation insects collected in 1994, although similar results with subsequent generations of the same populations have been reported elsewhere by Perez and Shelton (1996). Results from leaf-dip and diet bioassays also indicate that field populations of *P. xylostella* have evolved resistance to *B. thuringiensis* subsp. *kurstaki* in Georgia, Thailand, and Honduras (Table 2; Perez and Shelton 1997).

In other studies, different bioassay methods have led to different conclusions about the magnitude of resistance to insecticides in arthropods. Leibe and Savage (1992) reported that results from topical application and leaf-residue bioassays with fenvalerate on 2 *P. xylostella* populations were not correlated. A similar lack of correlation had been reported in spider mite by Dennehy et al. (1983) with leaf-residue and slide-dip bioassays. Three bioassay methods used to determine the magnitude of resistance to chlorpyrifos and cypermethrin in the German cockroach (Dictyoptera: Blattellidae) varied widely across bioassay technique (Scharf et al. 1995). Our studies were different from these in that we compared 2 bioassay techniques which essentially differed only in the feeding substrate. The leaf-dip and diet-incorporated bioassays were effective in distinguishing between susceptible and resistant *P. xylostella*; LC_{50} s from both bioassays were highly correlated, and both methods would be suitable for assessing evolution of resistance to *B. thuringiensis* in field populations of diamondback moth. However, the leaf-dip method was more sensitive compared with the diet-incorporated bioassay. If one wants to choose a discriminating concentration that kills $\approx 99\%$ of the susceptible individuals (probit value = 7.32; Fig. 2), the leaf-dip bioassay will be more effective in discriminating between susceptible and resistant populations because it will kill a lower proportion of the resistant individuals than a discriminating concentration based on our diet bioassay. However, the ideal bioassay method is one that is fast, labor efficient, and closely correlated with field control (French-Constant and Roush 1990). According to these criteria, the diet-incorporated assay would be preferable because the diet is easier to prepare and store.

In other studies, we have reported that a discriminating concentration of *B. thuringiensis* subsp. *kurstaki* incorporated in diet (20.5 mg [AI] of Javelin per liter of diet) was significantly correlated with mortality of *P. xylostella* exposed to

field applications of Javelin (Perez and Shelton 1996). The same discriminating concentration was effective in distinguishing between susceptible and resistant populations of *P. xylostella* from Central America (Perez and Shelton 1997). Recently, a diet-incorporated discriminating concentration was developed for area-wide evaluation of *Helicoverpa armigera* and *H. punctigera* (Lepidoptera: Noctuidae) susceptibility to *B. thuringiensis* in Australia (Forrester 1994). Our results in this study, combined with those reported elsewhere (Perez and Shelton 1996, 1997), show that we have developed a resistance detection system that can be used to predict efficacy of commercial formulations of *B. thuringiensis* subsp. *kurstaki* against *P. xylostella* in the field, and could be used in area-wide resistance studies that require delivery of the test kit to remote places. Another feature of this system is that the test kit can be stored for later use, whereas disks from cabbage leaves are difficult to maintain fresh beyond 72 h at room temperature (Asano et al. 1993). In addition, the diagnostic concentration we have proposed has provided reproducible results. In another set of 11 tests with Geneva 88, the susceptible population, mortality ranged from 97 to 99% ($n = 1,044$), and the overall mortality in the controls was 1.6% ($n = 289$) (C.J.P. and A.M.S., unpublished data). Another characteristic of our system is its flexibility in the size of larvae selected for resistance tests. In this study, we used a classification of test larvae based on days and average weight, but larval length may also be used as an alternative to select appropriate sizes (Bhalla and Dubeg 1986).

Our resistance detection system will assist growers, extension agents, and pest management consultants in rapid and easy determination of the causes of possible control failures following field applications with *B. thuringiensis*. The efficiency of resistance studies may also be increased because it is easier to use a single concentration test than a series of concentrations, which will often require more time and labor for preparation.

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