Field Applications, Leaf-Dip, and Diet Incorporated Diagnostic Assays Used Against *Bacillus thuringiensis*-Susceptible and Resistant Diamondback Moth (Lepidoptera: Plutellidae)

CARLOS J. PEREZ AND ANTHONY M. SHELTON

Department of Entomology, New York State Agricultural Experiment Station, Geneva, NY 14456

J. Econ. Entomol. 89(6): 1364–1371 (1996)

ABSTRACT The concentration–mortality responses of 6–8 populations of *Plutella xylostella* (L.) from leaf-dip bioassays were compared with the mortality of larvae caused by residues of field applications of *Bacillus thuringiensis* subsp. *kurstaki* Berliner (Javelin Wettable Granules 6.4% [AI]), and mortality of larvae in 3 diagnostic concentrations of Javelin (48.5, 32, and 20.5 mg [AI]/liter of diet) incorporated in an artificial diet. LC_{50}s of Javelin in leaf-dip assays ranged from 0.07–78.8 mg (AI)/liter of water. Populations with LC_{50}s of 0.07–0.15 mg (AI)/liter showed mortalities usually >80% in field applications at the recommended field rate of 1.12 kg/ha, and >90% at 2.24 kg/ha. Four populations with LC_{50}s of 0.6–1.8 mg (AI)/liter showed mortalities <60% when exposed to 1.12 kg/ha, but >75% when exposed to 2.24 kg/ha. In 3 other populations with LC_{50}s of 4.8–78.8 mg (AI)/liter mortality at 1.12 kg/ha was <7%, and <32% when exposed to 2.24 kg/ha. Mortalities of diamondback moth populations at the diagnostic concentrations of 32 and 20.5 mg (AI)/liter of diet were significantly correlated with mortalities at 1.12 kg/ha, but correlations between 1.12 kg/ha and the 20.5 mg (AI) concentration were higher. We concluded that LC_{50}s of Javelin >0.6 mg (AI)/liter in leaf dip bioassays can be associated with low levels of mortality in field applications, and that the diagnostic concentration of 20.5 mg can be used as an on-farm assay for monitoring *P. xylostella* resistance to *B. thuringiensis* subsp. *kurstaki*.

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

INSECTICIDE RESISTANCE IN *Plutella xylostella* (L.) has been documented in >46 commercial formulations of pesticides. Bioassay techniques used for resistance studies with synthetic insecticides include topical application, disposable cup bioassay, and leaf-dip bioassay (Tabashnik and Cushing 1987, Magaro and Edelson 1990). The leaf-dip bioassay technique has been used more frequently to assess *P. xylostella* resistance to microbial pesticides such as *B. thuringiensis* (Tabashnik et al. 1990, Tabashnik et al. 1993, Shelton et al. 1993). The LC_{50} and the corresponding 95% CL has also been the most frequent criterion used to compare the susceptibility of 2 or more populations of this insect to a particular pesticide (Tabashnik et al. 1990, Shelton et al. 1993, Kobayashi et al. 1992).

Resistance studies have emphasized the study of dose- or concentration-mortality relationships with doses or concentrations of pesticides designed to provide a good estimate of the probit regression line. However, it is necessary to correlate mortality observed in the laboratory with mortality from field applications. Shelton et al. (1993) combined the results of leaf-dip bioassays in the laboratory and field tests with *B. thuringiensis* against *P. xylostella*. They concluded that control can be achieved in the field when the LC_{50} for *B. thuringiensis* subsp. *kurstaki* is <0.5 mg (AI)/liter of water, and they suggested that the latter could be used as a diagnostic concetration. Resistance studies that rely on a single diagnostic concentration may be more efficient in discriminating between susceptible and resistant individuals when resistance is at low frequency, and permit less waste of test individuals exposed to the lowest concentrations (ffrench-Constant and Roush 1990).

Attempts have also been made to develop bioassay techniques to document *P. xylostella* resistance to pesticides based on a diagnostic concentration. A technique using liquid scintillation vials treated with diagnostic concentrations of several synthetic insecticides was used for resistance studies with *P. xylostella* in South Texas (Magaro and Edelson 1990). Other on-farm insecticide resistance techniques for *P. xylostella* have also been tested with synthetic pesticides (Zhao and Grafius 1993), and *B. thuringiensis* (Plapp et al. 1992). Resistance studies with *B. thuringiensis* δ-endotoxins using simple techniques are difficult because the toxins have to be ingested by the larvae to cause a physiological response. Leaf disks cut from crucifer leaves, and artificial diet may be used as substrate

0022-0493/96/1364–1371$02.00/0

to facilitate ingestion of *B. thuringiensis* δ-endotoxins by *P. xylostella* larvae (Shelton et al. 1991).

The purpose of our study was to determine the relationship between mortalities of *P. xylostella* populations in leaf-dip assays and field applications of *B. thuringiensis* subsp. *kurstaki*. Our primary goal was to find a LC_{50} that could be used as a threshold to predict field control failures when applying the recommended field application rate. In addition, we tested a low and a high field application rate to determine if the high dose approach could be used as a resistance management strategy in *P. xylostella* with low levels of resistance. Finally, we assessed which of 3 potential diagnostic concentrations of *B. thuringiensis* subsp. *kurstaki*, incorporated in artificial diet, was best correlated with mortality of *P. xylostella* when exposed to the recommended field application dose. We propose to eventually use this diagnostic concentration in an on-farm bioassay which would allow for *B. thuringiensis* resistance studies using higher numbers of *P. xylostella* populations than is currently feasible.

Materials and Methods

Populations. We used 8 populations in our experiments. The Geneva 88 population, susceptible to *B. thuringiensis* (Shelton et al. 1993), was collected in central New York in 1988. It has been continuously reared at the New York State Agricultural Experiment Station, Geneva, for >120 generations without exposure to pesticides. The other 7 populations were collected from commercial fields of crucifers in 1994. One population was collected from a cabbage field in Thailand in January. Other 2 populations (Rincon, Zamorano), were collected in April from commercial fields of cabbage in the Department of Francisco Morazan, Honduras. Four populations (Belle Glade, Zellwood, Loxahatchee '94A, and '94B) were collected in April in commercial fields of crucifers in central Florida, and were named after the nearest town to the collection site. *P. xylostella* collections in the state of Florida had been made by Shelton et al. (1993) at the same sites to document resistance to *B. thuringiensis* subspecies.

Bioassays. The above populations were part of a set of *P. xylostella* populations that were collected with the purpose of documenting resistance to *B. thuringiensis*. Before the field experiments and the tests with diagnostic concentrations in diet, we conducted leaf-dip bioassays with Javeling WG (wettable granules, 6.4% [AI] Sandoz, Des Plaines, IL), a commercial formulation of *B. thuringiensis* subsp. *kurstaki*. Within each population, we used insects from the same generation for each of the 3 bioassay techniques.

Leaf-dip bioassays with Javelin were done by using a method similar to that described by Shelton et al. (1993), with some modifications. In our tests, each disk was 3.2 cm in diameter, and larvae were allowed to feed at 26°C, 30% RH, and a photoperiod of 16:8 (L:D) h for a period of 48 h to increase time efficiency (Tabashnik et al. 1993). Javelin concentrations (mg [AI]/liter of water) were derived from 3.16-fold serial dilutions ranging from 0 to 640 mg. Data were analyzed assuming the probit model (Russell et al. 1977). LC_{50}s and their corresponding 95% CL and resistance ratios (RR) were estimated for each population. The susceptibility of 2 populations to Javelin was considered significantly different if the 95% CL of the corresponding LC_{50}s did not overlap.

Field Tests. Sixteen cabbage plots (variety 'Cheers') were planted at the Fruit and Vegetable Crops Research Farm of the New York State Agricultural Experiment Station, Geneva, NY, in the summer of 1994. Each plot was 3.6 m wide by 7 m long, and contained 60 plants spaced at 46 cm between plants and 90 cm between rows. Plots were arranged in a randomized complete block design with 4 treatments replicated 4 times.

Javelin WG (6.4% [AI]) was applied at 0.56, 1.12, or 2.24 kg actual formulation/ha by using a CO_2-assisted drop nozzle sprayer with 3 nozzles per row of cabbage; the sprayer was calibrated to deliver 200 liters/ha of spray mixture. The sprayer was equipped with 3 TXSV-6 nozzles (R&D Sprayers, Opeliusas, LA) and set at 2.8 kg/cm^2 pressure. All applications, (including those made in the control plots), included distilled water as the diluent. A spreader-sticker (Bond, Loveland Industries, Greeley, CO) was added to all applications at 0.2% vol:vol. Three applications of the above treatments were performed on the cabbage plots when the plants reached development stages 5, 6, and 7, respectively (Andaloro et al. 1983). After each application, leaves were allowed to dry for 2 h, and 1 leaf located in the middle part was collected from each of 3 randomly selected plants per plot.

In the laboratory, 6–8 disks (3.2 cm diameter) were cut from each leaf and placed individually into 30-ml plastic cups. Five 2nd-instar *P. xylostella* from each of the 8 populations were placed in each cup and allowed to feed on the leaf disk for 48 h at 26°C, 30% RH, and a photoperiod of 16:8 (L:D) h, after which mortality was recorded. A larvae was considered dead if did not move when prodded. At least 6 of the 8 populations were tested simulateously on each of 3 trials.

Diagnostic Concentrations. We used 3 potential diagnostic concentrations of Javelin incorporated in diet. Based on previous diet bioassays with *B. thuringiensis* subsp. *kurstaki*-susceptible and resistant populations (C.J.P. and A.M.S., unpublished data) we determined that 48.5, 32, and 20.5 mg (AI) of Javelin/liter of diet could be useful as diagnostic concentrations in resistance documentation with field populations of *P. xylostella*. These diagnostic concentrations were the LC_{99}, lower limit of the 95% CL of the LC_{99}, and twice the LC_{90}, respectively, of the Geneva 88 strain as estimated from 9 independent diet bioassays with

Table 1. Concentration–mortality relationship of *P. xylostella* used in simulated field applications with *B. thuringiensis* subsp. *kurstaki* in leaf-dip bioassays

Population	Generation	*n*	Slope ± SE	LC_{50}[a]	95% CL[b]	RR[c]
Geneva 88	120	199	2.2 ± 0.3	0.07a[d]	(0.05–0.1)	1
Belle Glade	3	175	1.6 ± 0.2	0.15ab	(0.08–0.27)	2.1
Rincon	3	175	1.5 ± 0.2	0.64bc	(0.20–3.0)	9.1
Thailand	6	189	1.5 ± 0.2	1.40c	(0.9–2.0)	20.0
Zamorano	3	172	1.8 ± 0.2	1.80cd	(0.6–6.2)	25.7
Zellwood	3	225	0.8 ± 0.1	4.80d	(2.7–8.6)	68.6
Loxahatchee '94A	3	223	2.0 ± 0.3	28.40e	(17.9–45.3)	405.7
Loxahatchee '94B	3	224	0.9 ± 0.1	78.80e	(24.5–527.1)	1,125.7

[a] mg (AI)/liter of water.
[b] 95% CL.
[c] Resistance ratios result from dividing the LC_{50} of resistant over the LC_{50} of the susceptible population (Geneva 88).
[d] LC_{50}s with different letters have nonoverlapping 95% CL.

Javelin. In this study, we compared the response of diamondback moth populations to these concentrations and to the response observed in the field applications with Javelin at 0.56, 1.12, and 2.24 kg/ha.

The diet contaning Javelin was prepared the day of the field tests. We reared sufficient quantities of 2nd-instar *P. xylostella* to take subsamples of a population, and expose the larvae to the field applications or the diagnostic concentrations. Five milliliters of treated or untreated diet were poured into 30-ml plastic cups. Ten cups with treated and 3 cups with untreated diet were used per population on each trial. Eight to 10, 2nd-instars per population were placed in each cup and allowed to feed on the diet for the same period and under the same environmental conditions as were larvae exposed to residues of *B. thuringiensis* from the field applications.

Data Analyses. For the simulated field tests and diagnostic concentrations, analysis of variance (PROC ANOVA; SAS Institute 1988) was done with the proportion of dead larvae recorded from each 30-ml plastic cup. The arcsine transformation was used to stabilize the variance (Snedecor and Cochran 1989). Mortality in the controls was always <5%; when appropriate, Abbott's (1925) formula was used to correct mortality in the treatments. ANOVA was performed separately on each of 3 trials because the number of populations tested was different at each trial. We used an ANOVA model that included *P. xylostella* populations and field rates of Javelin as factorial treatments. The 3 leaves collected per field plot were included as subsamples within each of the 4 replicates. One-way ANOVA with *P. xylostella* populations as the treatment was performed on mortality data recorded from the tests with diagnostic concentrations. The diagnostic concentration of 20.5 mg (AI) Javelin/liter of diet was tested twice, and ANOVA was performed on the pooled data; the other 2 concentrations were tested once. The Tukey honestly significant difference (HSD) procedure ($P = 0.05$; SAS Institute 1988) was used to separate the means. We used Pearson correlation analysis ($P = 0.05$; SYSTAT 1992) to estimate correlation between mortalities observed in the leaf dip bioassays, the field applications, and the diagnostic concentrations.

The analysis described above considers a *P. xylostella* population as indicator variable. Additional probit analysis was done by using the LC_{50} of each of 8 populations as an independent variable; the mortality data obtained from either the simulated field tests and the tests with different diagnostic concentrations of diet incorporated *B. thuringiensis* were response variables. Use of probit analysis (POLO; LeOra Software 1989), permitted estimating the LC_{50}s of *P. xylostella* populations that would be associated with 50–90% larva mortality in both the simulated field applications test and with the diagnostic concentrations.

Results and Discussion

Bioassays. The LC_{50}s of the 7 field populations of *P. xylostella* used in this studies were 2- to >1,000-fold greater than the LC_{50} for Javelin with Geneva 88 (Table 1). The resistance levels we observed in the populations collected in Florida in 1994 were similar to those reported by Shelton et al. (1993) for populations collected in 1992. From our results, selection with *B. thuringiensis* subsp. *kurstaki* apparently has been continuous, at least in cabbage growing areas of Florida state near Loxahatchee and Zellwood, because resistance levels did not decline 2 yr after the 1st resistance studies. The *P. xylostella* population collected near Belle Glade seemed to maintain susceptibility to *B. thuringiensis* subsp. *kurstaki* similar to that of Geneva 88. To our knowledge, no reports have been made on the extent of *P. xylostella* resistance to *B. thuringiensis* in Honduras or Central America, and Thailand. Our results suggest that 2 populations from Honduras and 1 from Thailand showed significantly lower levels of susceptibility to *B. thuringiensis* subsp. *kurstaki*, compared to our standard susceptible strain.

Field Tests. Response of *P. xylostella* to 3 field applications with Javelin are shown in Tables 2–4. Mortalities of Geneva 88 to field applications with Javelin at 1.12 and 2.24 kg/ha were typically >90%.

Table 2. Mortality of 2nd-instar *P. xylostella* at corresponding field application rates in simulated field applications of *B. thuringiensis* subsp. *kurstaki* (application 1, crop stage 5)

Population	*n*	% mortality ± SEM		
		0.56[a]	1.12[a]	2.24[a]
Geneva 88	226	70.9 ± 2.5bcd[b]	94.7 ± 0.3ab	95.6 ± 0.1a
Belle Glade	248	67.6 ± 3.5cd	84.7 ± 3.4abc	92.4 ± 0.3abc
Thailand	253	28.2 ± 4.9ef	48.5 ± 3.1de	90.7 ± 1.3abc
Zamorano	248	14.1 ± 1.7fg	19.7 ± 2.3efg	71.6 ± 1.7bcd
Zellwood	246	4.6 ± 0.4g	6.8 ± 1.1fg	31.9 ± 1.4ef
Loxahatchee '94A	226	4.2 ± 0.1g	4.1 ± 0.1g	3.9 ± 0.1g

[a] kg/ha of actual formulation of Javelin.
[b] Means followed by the same letters are not significantly different ($P = 0.05$) by Tukey HSD procedure; $\omega = 5.19$ and df = 51 (SAS Institute 1988). Each mean was estimated from 12 observations (4 blocks by 3 leaves per plot).

Except for the second application (Table 3), mortality of Geneva 88 from Javelin applied at 0.56 kg/ha was >70%. In other studies (Perez et al. 1995), we reported Geneva 88 mortality >90% to field applications with Javelin at rates ranging from 0.56 to 1.12 kg/ha, with a similar experimental method. We observed slight differences in mortalities in the 2 studies probably because the time for mortality assessment was modified from 72 to 48 h of exposure to *B. thuringiensis* residues.

In this study, a comparison of the mortalities observed in the Belle Glade and Geneva 88 populations indicated correspondence between the bioassay and field test results. Both the LC_{50}s and the average mortalities with the field applications were not significantly different ($P > 0.05$), although the Belle Glade population tended to have lower mortality (Tables 1–4). We also observed similar results from the bioassays and the field tests with the Thailand and Zamorano, and Thailand and Rincon populations (Tables 2 and 3). We were unable to do field tests with Zamorano and Rincon populations simultaneously. Mortality of the population from Zamorano was usually <72%, even at 2.24 kg/ha of Javelin. Mortality of the Thailand, Zamorano and Rincon populations at 1.12 kg/ha of Javelin, in 3 trials ranged from 19.7 to 56%. Mortalities <60% may not have any practical relevance in the field because famers are usually seeking control efficacy >90%. Average mortalities of populations collected near Zellwood and Loxahatchee were extremely low (<31%) at all field rates. Although the LC_{50}s of the Loxahatchee and Zellwood populations were significantly different, their mortalities observed on the field tests were not significantly different ($P > 0.05$; Tables 2 and 4).

The mortality observed in the Rincon population in the field test was of particular interest because its LC_{50} was not significantly different from the LC_{50} of the population from Belle Glade in the leaf-dip bioassay (Table 1), and mortality of both populations at 0.56 and 1.12 kg/ha of Javelin were not significantly different (Table 3). However, mortalities of both populations at 2.24 kg/ha, were significantly different from that of the Belle Glade population; they showed higher response levels with an increase in the application rate. These results suggest that field control failures with *B. thuringiensis* subsp. *kurstaki* are very likely when laboratory LC_{50}s are >0.6 mg (AI)/liter. The effect of resistance on efficacy may be more severe with populations with LC_{50}s >4.7 mg (AI)/liter. Shelton et al. (1993) concluded that *P. xylostella* populations can be controlled in the field when LC_{50} for *B. thuringiensis* in the laboratory is <0.5 mg (AI)/liter. This observation is consistent with our results because mortalities of *P. xylostella* with LC_{50}s from leaf-dip bioassays ranging from 0.3 to 0.7 mg (AI)/liter would yield a mortality of 60% when exposed to the recommended field rate of Javelin of 1.12 kg/ha (Table 5).

Table 3. Mortality of 2nd-instar *P. xylostella* at corresponding field application rates in simulated field applications of *B. thuringiensis* subsp. *kurstaki* (application 2, crop stage 6)

Population	*n*	% mortality ± SEM		
		0.56[a]	1.12[a]	2.24[a]
Geneva 88	252	54.8 ± 1.8bc[b]	93.4 ± 0.3a	94.9 ± 0.2a
Belle Glade	196	32.1 ± 2.6cde	76.9 ± 2.5ab	89.7 ± 0.8a
Thailand	224	29.9 ± 2.1cde	41.9 ± 2.8bcd	78.1 ± 2.1ab
Rincon	216	21.9 ± 0.7def	56.2 ± 2.6bc	52.7 ± 5.1bc
Zamorano	252	13.7 ± 0.3def	22.1 ± 0.9def	42.9 ± 2.5bcd
Loxahatchee '94A	229	3.8 ± 0.0f	4.0 ± 0.0f	4.9 ± 0.1f
Loxahatchee '94B	281	3.7 ± 0.1f	4.5 ± 0.2f	3.8 ± 0.1f

[a] kg/ha of actual formulation of Javelin.
[b] Means followed by the same letters are not significantly different ($P = 0.05$) by Tukey HSD procedure; $\omega = 5.19$ and df = 51 (SAS Institute 1988). Each mean was estimated from 12 observations (4 blocks by 3 leaves per plot).

Table 4. Mortality of 2nd-instar *P. xylostella* at corresponding field application rates in simulated field applications of *B. thuringiensis* subsp. *kurstaki* (application 3, crop stage 8)

Population	*n*	% mortality ± SEM		
		0.56[a]	1.12[a]	2.24[a]
Geneva 88	279	71.8 ± 9.3bc[b]	88.9 ± 4.2ab	96.8 ± 0.1a
Belle Glade	201	51.7 ± 5.0cd	71.5 ± 11.7bc	91.5 ± 1.3ab
Zamorano	172	38.7 ± 6.0de	43.3 ± 13.4cde	68.0 ± 9.6bcd
Zellwood	246	10.6 ± 3.1f	9.4 ± 1.6f	17.2 ± 6.6ef
Loxahatchee '94A	230	3.9 ± 0.1f	5.2 ± 0.5f	4.8 ± 0.4f
Loxahatchee '94B	214	5.8 ± 0.6f	6.6 ± 2.1f	7.4 ± 1.8f

[a] kg/ha of actual formulation of Javelin.
[b] Means followed by the same letters are not significantly different ($P = 0.05$) by Tukey HSD procedure; $\omega = 5.19$ and df = 51 (SAS Institute 1988). Each mean was estimated from 12 observations (4 blocks by 3 leaves per plot).

Tests with Diagnostic Concentrations. Mortality of *P. xylostella* populations exposed to a diagnostic concentration of 48.5 mg (AI)/liter of diet varied significantly ($F = 36.5$; df = 5, 54; $P < 0.0001$; Table 6; Fig. 1). However, this concentration does not appear reliable in predicting the response of diamondback moth to field applications with Javelin for 3 reasons. First, it causes relatively high mortality (>50%) in highly resistant populations such as Loxahatchee '94A; in simulated field applications at a rate of 2.24 kg/ha, a rate 2-fold higher than the recommended field rate of Javelin, mortality of Loxahatchee '94A was always <5.2% (Tables 2–4). Second, mortalities at 48.5 mg were significantly correlated ($P = 0.001$) only with mortalities at 2.24 kg/ha of Javelin, but were not significantly correlated with mortalities at 0.56 and 1.12 kg/ha ($P = 0.09$; Table 7). Finally, despite significant correlations between LC_{50}s and mortalities at 48.5 mg, the separation of average mortalities by the Tukey HSD procedure ($P = 0.05$) did not agree with significant differences in LC_{50}s of Thailand and Zamorano populations compared to Geneva 88 (Table 6).

The diagnostic concentrations of 32 and 20.5 mg (AI)/liter of diet were tested simultaneously in a single trial. An additional test was conducted with 20.5 mg (AI)/liter of diet alone. Mortality of *P. xylostella* populations varied significantly at 32 mg ($F = 30.7$; df = 6, 62; $P < 0.0001$), and at 20.5 mg ($F = 44.1$; df = 7,110; $P < 0.001$; Table 6). Average mortalities on both concentrations were significantly correlated ($r \geq 0.86$, $P \leq 0.01$), with average mortalities at 0.56, 1.12, and 2.24 kg/ha of Javelin in the field applications (Tables 8 and 9).

Mortalities on both diagnostic concentrations were also highly correlated ($r = 0.99$, $P < 0.0001$; Table 8). This relationship, and the fact that correlations between LC_{50}s and mortalities at 20.5 mg were higher compared with 32 mg, were used as criteria to conduct additional experiments with the diagnostic concentration of 20.5 mg. We considered the latter to be more reliable indicator of field control failures with *B. thuringiensis* subsp. *kurstaki* because mortalities of highly resistant populations such as Loxahatchee '94A and '94B were lower than mortalities of the same populations at 32 mg (Table 6).

A comparison of the overlap among the 95% CL of the corresponding LC_{50}s (Table 1), versus separation of average mortalities by the Tukey HSD procedure, were consistent at 20.5 mg. For in-

Table 5. Results of leaf-dip bioassays and estimated mortalities (90–50%) of *P. xylostella* when exposed to simulated field applications or diet-incorporated *B. thuringiensis* subsp. *kurstaki*

Field rate or concentration	% estimated mortality				
	90[a]	80	70	60	50
0.56 kg/ha	0.008[b] (0.002–0.017)[c]	0.024 (0.01– 0.04)	0.05 (0.03– 0.09)	0.1 (0.06– 0.2)	0.2 (0.12– 0.29)
1.12 kg/ha	0.07 (0.04 –0.12)	0.2 (0.1 – 0.2)	0.3 (0.2 – 0.4)	0.5 (0.3 – 0.7)	0.8 (0.6 – 1.0)
2.24 kg/ha	0.3 (0.1 –0.5)	0.6 (0.3 – 0.9)	0.9 (0.6 – 1.34)	1.4 (1.0 – 2.1)	2.2 (1.5 – 3.2)
48.5 mg[d]	3.4 (1.8 –6.0)	7.8 (4.3 –13.6)	14.2 (8.1 –25.0)	23.6 (13.6 –43.0)	38.1 (21.8 –72.5)
32 mg[d]	1.7 (0.8 –3.4)	4.16 (2.1 – 8.0)	7.8 (4.0 –15.2)	13.5 (7.0–26.6)	22.4 (11.6 –45.3)
20.5 mg[d]	0.4 (0.2 –0.8)	1.2 (0.6 – 2.04)	2.7 (1.6 – 4.4)	5.2 (3.2– 8.8)	9.8 (6.0 –17.7)

[a] Expected mortality (%) of 2nd-instar *P. xylostella*.
[b] LC_{50} (mg [AI]/liter of water) of *B. thuringiensis* subsp. *kurstaki* from leaf-dip bioassays.
[c] 95% CL of corresponding LC_{50}.
[d] mg (AI) of Javelin/liter of diet.

Table 6. Mortality (mean % ± SEM) of *P. xylostella* populations exposed to 3 diagnostic concentrations of *B. thuringiensis* subsp. *kurstaki* incorporated in diet

Population	mg (AI)/liter of diet		
	48.5	32	20.5
Geneva 88	99.8 ± 0.0a	96.9 ± 0.0a	97.1 ± 0.02a
Belle Glade	98.9 ± 2.8a	92.8 ± 1.1ab	88.4 ± 3.1ab
Thailand	99.4 ± 0.7a	84.7 ± 4.2bc	80.6 ± 5.9bc
Rincon	NT	97.5 ± 0.0a	87.6 ± 1.6b
Zamorano	97.9 ± 2.5a	73.1 ± 4.1c	76.4 ± 3.4c
Zellwood	85.2 ± 7.3b	NT	59.1 ± 6.7d
Loxahatchee '94A	58.2 ± 4.7c	42.0 ± 5.4d	34.1 ± 5.7e
Loxahatchee '94B	NT	45.4 ± 4.5d	15.7 ± 4.5e

Means within a column followed by the same letter are not significantly different ($P = 0.05$) by the Tukey test (SAS Institute 1988). NT, not tested.

stance, LC_{50}s of Geneva 88 and Belle Glade are not significantly different, nor are the average mortalities separated by the Tukey test (Table 6). However, the LC_{50}s of Geneva 88 and Rincon were significantly different (Table 1), whereas their average mortalities at 32 mg were not significantly different. At 20.5 mg the response of Geneva 88 and Rincon differed significantly by both methods. We did not observe agreement in separation of the responses of Zamorano versus Rincon and Zellwood in the 2 bioassay techniques. Based on the Tukey test, mortality of Zamorano and Zellwood differed significantly, but the LC_{50}s did not vary significantly. However, LC_{50}s of Zamorano and Rincon were not significantly different; average mortalities at 20.5 mg (AI)/liter were significantly different.

We observed agreement in the response of Zellwood, Loxahatchee '94A, and Loxahatchee '94B in both bioassays. Despite high correlation between the 20.5 mg diagnostic concentration and the field application rate of 1.12 kg/ha, the probit regressions (Fig. 1) indicated that this diagnostic concentration is somewhat conservative because it yields mortalities consistently higher than those observed in the 1.12 kg/ha field rate. However, our main objective was not to find a diagnostic concentration that would yield a response that was exactly that resulting from exposure to field residues, but a concentration that would permit distinguishing between field susceptible and resistant *P. xylostella* populations. Our results suggest that a diagnostic concentration of 20.5 mg ([AI] of Javelin)/liter of diet was effective in distinguishing *P. xylostella* populations with various levels of resistance to *B. thuringiensis* subsp. *kurstaki*.

The methods that we used to study the intensity of resistance in *P. xylostella* populations, shows results comparable with results of resistance assessment done directly in the field. Shelton et al. (1993) did field experiments in which larvae mortality was directly assessed in the field with naturally occurring populations of *P. xylostella*. When Javelin was sprayed at 1.12 kg/ha in that study, only the population susceptible to *B. thuringiensis* subsp. *kurstaki* (Geneva 88) had mortality >85%; mortality of the field populations with higher levels of resistance were <27%, compared with the untreated controls. Although Shelton et al. (1993)

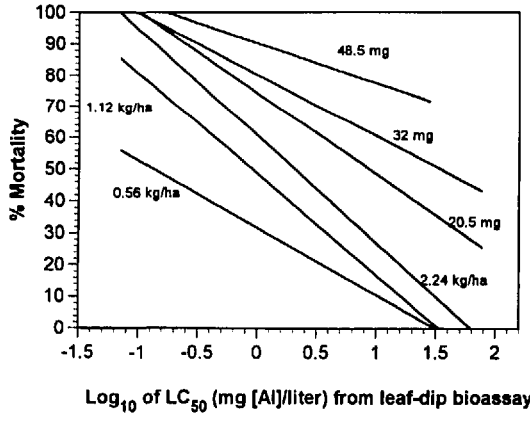

Fig. 1. Relationship between $LC_{50}$from laboratory bioassays and *P. xylostella* response to simulated field applications and diet incorporated B. thuringiensis subsp. *kurstaki*.

Table 7. Pearson correlation coefficients of *P. xylostella* mortality in simulated field applications and mortality from a diagnostic concentration incorporated in diet

Field rate and concentration	$Log_{10}[LC_{50}]$[a]	Field rate (kg/ha)		
		0.56[b]	1.12[b]	2.24[b]
0.56[b]	−0.96[b] (0.003)[c]	—	—	—
1.12[b]	−0.95 (0.004)	0.99 (<0.0001)	—	—
2.24[b]	−0.91 (0.011)	0.84 (0.03)	0.85 (0.03)	—
48.5 mg[c]	−0.86 (0.03)	0.74 (0.09)	0.75 (0.09)	0.98 (0.001)

[a] In parts per million.
[b] kg/ha of actual formulation of Javelin.
[c] mg (AI) of Javelin/liter of diet.
[d] Pearson correlation coefficient.
[e] Probability. If $P > 0.05$, the correlation is not significant (SYSTAT 1992).

Table 8. Pearson correlation coefficients of *P. xylostella* mortality in simulated field applications and mortality from 2 diagnostic concentrations of *B. thuringiensis* subsp. *kurstaki* incorporated in diet

Field rate and concentration	$Log_{10}[LC_{50}]^a$	Field rate and concentration			
		0.56[b]	1.12[b]	2.24[b]	20.5 mg[c]
0.56[b]	−0.71[d] (0.07)[e]	—	—	—	—
1.12[b]	−0.7 (0.08)	0.97 (<0.0001)	—	—	—
2.24[b]	−0.78 (0.04)	0.97 (<0.0001)	0.94 (0.001)	—	—
20.5 mg[c]	−0.85 (0.016)	0.86 (0.01)	0.90 (0.006)	0.89 (0.008)	—
32 mg[c]	−0.77 (0.04)	0.88 (0.009)	0.93 (0.003)	0.89 (0.008)	0.99 (<0.0001)

[a] In parts per million.
[b] kg/ha of actual formulation of Javelin.
[c] mg (AI) of Javelin/liter of diet.
[d] Pearson correlation coefficient.
[e] Probability. If $P > 0.05$, the correlation is not significant (SYSTAT 1992).

demonstrated that control failures in the field were correlated with results in the laboratory, the study required a great amount of effort because the field tests were done at several locations. Besides reliable determination of response of *P. xylostella* to field applications of *B. thuringiensis,* the methods used in our study has the advantage of allowing tests with several populations collected from commercial field plots from a wide geographical range to be done simultaneaously.

Although mortalities at 20.5 mg (AI)/liter of diet were highly correlated with mortalities at 1.12 kg/ha (the recommended field rate), prediction of control at the field level from this diagnostic concentration is still dependent on the criteria we use to define a good control. If the criteria were >90% mortality in the field, tolerance levels to *B. thuringiensis* subsp. *kurstaki* beyond that of the Belle Glade population would justify changes in pest management practices. If 80% mortality in the diagnostic concentration were used as threshold, then good control efficacy might be achieved on populations with LC_{50}s <0.60 mg (AI)/liter, especially if the field rate of Javelin is doubled or applied with an improved application technique such as the electrostatic spraying system (Perez et al. 1995). Other studies have empirically set mortality thresholds of 80 and 70% in a discriminating concentration developed to assess spider mite resistance to propargite (Grafton-Cardwell et al. 1989).

Table 9. Pearson correlation coefficients of *P. xylostella* mortality in simulated field applications and mortality on a diagnostic concentration of *B. thuringiensis* subsp. *kurstaki* incorporated in diet

Field rate and concentration	$Log_{10}[LC_{50}]^a$	Field rate		
		0.56[b]	1.12[b]	2.24[b]
0.56[b]	−0.97[d] (0.007)[e]	—	—	—
1.12[b]	−0.97 (0.005)	0.99 (0.001)	—	—
2.24[b]	−0.97 (0.006)	0.99 (0.001)	0.98 (0.002)	—
20.5 mg[c]	−0.97 (0.007)	0.99 (<0.0001)	0.99 (0.001)	0.98 (0.002)

[a] In parts per million.
[b] kg/ha of actual formulation of Javelin.
[c] mg (AI) of Javelin/liter of diet.
[d] Pearson correlation coefficient.
[e] Probability. If $P > 0.05$, the correlation is not significant (SYSTAT 1992).

Our results raise concern about the limitations of resistance management tactics based on a high-rate approach. Depending on the level of resistance from leaf-dip assays, *P. xylostella* populations responded differently to increased Javelin rates (i.e., 1.12–2.24 kg/ha). Except for one case (the Rincon population, Table 3), mortality of populations with LC_{50}s <4.8 mg (AI)/liter, tended to increase when exposed to field applications with Javelin at 2.24 kg/ha. However, populations such as Zamorano and Zellwood (Tables 2–4) with 1.8 and 4.8 mg (AI)/liter, respectively (Table 1), showed low mortality at the highest field rate of Javelin. Under special circumstances (i.e., recent release of an introduced natural enemy) the field rate could be increased to control *P. xylostella* populations with LC_{50}s ≤1.4 mg (AI)/liter of water.

We conclude that any of the 3 methods tested in this study can be used for *B. thuringiensis* resistance monitoring programs. Our results suggest that the simulated field applications, and the on-farm bioassay with *B. thuringiensis* incorporated in diet seem to be promising methods for resistance studies with populations from several locations. The leaf-dip bioassay should continue to provide valuable information about differences in susceptibility to *B. thuringiensis,* but it alone does not provide insight into respone in the field.

Acknowledgments

We are grateful to John Barnard (Computer and Statistical Services of the New York State Agricultural Ex-

periment Station at Geneva), for his valuable help in the data analysis. We thank Juliet Tang, William T. Wilsey, and the 'Shelton 1994 Summer Crew' for their valuable help with laboratory and field experiments. This research was supported in part by the *Bacillus thuringiensis* Management Working Group and the World Bank Graduate Scholarship Program. This work was part of a dissertation submitted to the Graduate School of Cornell University by C.J.P. in partial fulfillment of the requirements for a Ph.D. degree.

References Cited

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

Andaloro, J. T., K. B. Rose, A. M. Shelton, C. W. Hoy, and R. F. Becker. 1983. Cabbage growth stages. N.Y. Food Life Sci. Bull. 101.

ffrench-Constant, R. H., and R. T. Roush. 1990. Resistance detection and documentation: the relative role of pesticidal and biochemical assays, pp. 4–38. *In* R. T. Roush and B. E. Tabashnik [eds.], Pesticide resistance in arthropods. Chapman & Hall, New York.

Grafton-Cardwell, E. E., J. Granett, T. F. Leigh, and S. M. Normington. 1989. Development and evaluation of a rapid bioassay for monitoring propargite resistance in *Tetranychus* species (Acari: Tetranichidae) on cotton. J. Econ. Entomol. 82: 706–715.

Kobayashi, S., S. Aida, M. Kobayashi, and K. Nonoshita. 1992. Resistance of diamondback moth to insect growth regulators, pp. 383–390. *In* N. S. Talekar [ed.], Management of diamondback moth and other crucifer pests. AVRDC, Shanhua, Taiwan.

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

Magaro, J. J., and J. V. Edelson. 1990. Diamondback moth (Lepidoptera: Plutellidae) in south Texas: a technique for resistance monitoring in the field. J. Econ. Entomol. 83: 1201–1206.

Perez, C. J., A. M. Shelton, and R. C. Derksen 1995. Effect of application technology and *Bacillus thuringiensis* subspecies on management of *B. thuringiensis* subsp. *kurstaki*-resistant diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 88: 1113–1119.

Plapp, F. W., J. J. Magaro, and J. V. Edelson. 1992. Diamondback moth in South Texas: a technique for resistance monitoring in the field, pp. 443–446. *In* N. S. Talekar [ed.], Management of diamondback moth and other crucifer pests. AVRDC, Shanhua, Taiwan.

Russell, R. M., J. L. Robertson, and N. E. Savin. 1977. POLO: a new computer program for probit analysis. Bull. Entomol. Soc. Am. 23: 209–213.

SAS Institute. 1988. SAS users guide: statistics, 6th ed. SAS Institute, Cary, NC.

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. 1993. Resistance of diamondback moth (Lepidoptera: Plutellidae) to *Bacillus thuringiensis* subspecies in the field. J. Econ. Entomol. 86: 697–705.

Snedecor, G. W., and W. G. Cochran. 1989. Statistical methods, 8th ed. Iowa State University Press, Ames.

SYSTAT. 1992. Statistics, version 5 ed. SYSTAT, Evanston, IL.

Tabashnik, B. E., and N. L. Cushing. 1987. Leaf residue vs. topical bioassays for assessing insecticide resistance in the diamondback moth, *Plutella xylostella* L. F.A.O. Plant Prot. Bull. 35: 11–14.

Tabashnik, B. E., N. L. Cushing, N. Finson, and M. W. Johnson. 1990. Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 83: 1671–1676.

Tabashnik, B. E., N. Finson, C. F. Chilcutt, N. L. Cushing, and M. W. Johnson. 1993. Increasing efficiency of bioassays: evaluating resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 86: 635–644.

Zhao, J. Z., and E. Grafius. 1993. Assessment of different bioassay techniques for resistance monitoring in the diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 86: 995–1000.

Received for publication 2 May 1996; accepted 9 August 1996.