Monitoring of Diamondback Moth (Lepidoptera: Plutellidae) Resistance to Spinosad, Indoxacarb, and Emamectin Benzoate

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J. Econ. Entomol. 99(1): 176–181 (2006)

ABSTRACT Six to nine populations of the diamondback moth, *Plutella xylostella* (L.), were collected annually from fields of crucifer vegetables in the United States and Mexico from 2001 to 2004 for baseline susceptibility tests and resistance monitoring to spinosad, indoxacarb, and emamectin benzoate. A discriminating concentration for resistance monitoring to indoxacarb and emamectin benzoate was determined based on baseline data in 2001 and was used in the diagnostic assay for each population in 2002–2004 together with a discriminating concentration for spinosad determined previously. Most populations were susceptible to all three insecticides, but a population from Hawaii in 2003 showed high levels of resistance to indoxacarb. Instances of resistance to spinosad occurred in Hawaii (2000), Georgia (2001), and California (2002) as a consequence of a few years of extensive applications in each region. The collaborative monitoring program between university and industry scientists we discuss in this article has provided useful information to both parties as well as growers who use the products. These studies provide a baseline for developing a more effective resistance management program for diamondback moth.

KEY WORDS *Plutella xylostella*, resistance, spinosad, indoxacarb, emamectin benzoate

THE DIAMONDBACK MOTH, *Plutella xylostella* (L.), is the main insect pest of crucifers, particularly cabbage, broccoli, and cauliflower (Talekar and Shelton 1993). In the past 50 yr, *P. xylostella* has become one of the most difficult insects in the world to control, primarily because of resistance evolution in some parts of the world to every class of insecticide used extensively against it (Shelton et al. 2000, Sarfraz and Keddie 2005).

Spinosad, indoxacarb, and emamectin benzoate are members of three different classes of insecticides with novel modes of action introduced in recent years. In some areas, such as Hawaii, they are the most important insecticides to control *P. xylostella* and are used in areawide insecticide resistance management programs (Zhao et al. 2002, Mau and Gusukuma-Minuto 2004). Spinosad is the first member of the Naturalyte class of insecticides developed by Dow AgroSciences (Sparks et al. 1995), with a high level of activity against many economically important insect pests and low environmental and human risk (Thompson et al. 2000, Thompson and Sparks 2002). Spinosad was granted organic status by the USDA–National Organic Program (USDA–NOP 2002). However, resistance development has been found in field populations of two species and in laboratory-selected strains of two other species. After the first documented case of spinosad resistance in field populations of *P. xylostella* in the United States (Zhao et al. 2002), a field population of *P. xylostella* from Malaysia (Sayyed et al. 2004) and two populations of the leafminer *Liriomyza trifolii* (Burgess) collected from commercial ornamental production greenhouses in the United States also were found to be resistant to spinosad (Ferguson 2004). Laboratory selection of field-collected house flies, *Musca domestica* L. (Shono and Scott 2003) and tobacco budworm, *Heliothis virescens* (F.) (Young et al. 2003), produced a highly spinosad-resistant strain of each species.

Indoxacarb, discovered and developed by E.I. DuPont and Co., is the first commercialized pyrazoline-type sodium channel blocker with activity against a wide range of lepidopteran, coleopteran, and sucking insect pests (McCann et al. 2001). Results from laboratory bioassays indicate that indoxacarb was highly toxic to *P. xylostella* larvae through food ingestion, and in the field, one application of indoxacarb suppressed *P. xylostella* larvae below an economic threshold for 14–21 d (Liu et al. 2003). Selection of field-collected house flies with indoxacarb produced an indoxacarb-resistant strain with >118-fold resistance after three generations (Shono et al. 2004). One strain of oblique-banded leafroller, *Choristoneura rosaceana* (Harris)

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collected from Michigan exhibited a very high level of resistance/tolerance (>700-fold) to indoxacarb, although it has never been used in Michigan nor is it labeled to control this insect pest (Ahmad et al. 2002). No field-evolved insect resistance to indoxacarb has been reported; however, decreased control efficacy of *P. xylostella* was observed in certain farms in Hawaii in 2003 (R.F.L.M., unpublished data).

Emamectin benzoate is a semisynthetic and second generation avermectin insecticide. It is registered to control lepidopteran pests and/or leafminers in cole crops, leafy and fruiting vegetables at low use rates (Syngenta 2004). Geographic variations (five-fold) in the susceptibility of *P. xylostella* in nine field populations collected from California have been detected (Shelton et al. 2000), but no laboratory or field-evolved insect resistance to emamectin benzoate has been reported. However, *P. xylostella* populations from China (Liang et al. 2003) and Malaysia (Sayyed et al. 2004) displayed resistance to avermectin, suggesting that proactive resistance monitoring and management programs are important for all insecticides of the same class.

The objectives of this study were to investigate the geographic variation in susceptibility of *P. xylostella* to spinosad, indoxacarb, and emamectin benzoate in the United States and Mexico and to monitor potential resistance evolution soon after field introductions.

Materials and Methods

Insects. A susceptible (S) strain of *P. xylostella*, Geneva 88 (Shelton et al. 1993, 2000), has been maintained on a wheat germ-casein artificial diet (Shelton et al. 1991) for ≈300 generations and was used in bioassays for comparison. In 2001–2004, six to nine populations of *P. xylostella* were collected each year from fields of crucifer vegetables in five states of the United States and the state of Guanajuato in Mexico. These sites were selected because they are important crucifer production areas with a history of intensive use of insecticides for management of *P. xylostella.* Approximately 100–300 *P. xylostella* larvae and pupae were collected in each location and transported to Cornell University's New York State Agricultural Experiment Station where bioassays were performed. After spinosad resistance was detected in Camilla, GA, in 2001, eight colonies were collected in January and April 2002 from collards on farms from five counties (Colquitt, Echols, Grady, Mitchell, and Tift) in southwestern Georgia close to Camilla (3–140 km) for additional diagnostic tests of spinosad resistance. For most populations, second instars of generation 1 or 2 were used in the bioassays. Geneva 88 larvae and all field colonies were reared on broccoli plants for bioassays (Zhao et al. 2002).

Insecticides. Spinosad (SpinTor 2 SC, 240 g [AI]/liter) was supplied by Dow AgroSciences (Indianapolis, IN), indoxacarb (Avaunt 30% WDG) by DuPont Crop Protection (Newark, DE), and emamectin benzoate (Proclaim, 5% SG) by Syngenta Crop Protection (Greenshoro, NC).

Bioassays. Cabbage leaf dip bioassays, as reported previously (Shelton et al. 1993, 2000; Zhao et al. 2002), were used for each strain of *P. xylostella.* Before each formal bioassay, we conducted a preliminary assay using 0.01, 0.1, 1.0, 10, and 100 mg ([AI])/liter (=ppm) solutions of the three insecticides to determine the proper dilutions for the formal assay. Each bioassay included five to six concentrations at the ratio of 1:2 or 1:3.16 plus a control, with five leaf disks for each concentration. Ten second instars (0.2–0.3 mg per larva) were placed on each of the leaf disks inside 30-ml plastic cups. Bond spreader/sticker (Loveland Industry, Loveland, CO) was added at 0.1% to all test concentrations and the water control. Mortality was determined after 72 h at 27 ± 1°C.

In a previous study, we found that spinosad at 10 mg ([AI])/liter killed all susceptible (S) and heterozygous individuals (RS genotype) but caused no mortality to the homozygous resistant (R) strain (Zhao et al. 2002), so we used 10 mg ([AI])/liter as the discriminating concentration for diagnostic assays in 2001–2004. Based on baseline data in 2001, indoxacarb at 50 mg ([AI])/liter and emamectin benzoate at 1 mg ([AI])/liter caused 100% mortality for the susceptible strain and ≥99% mortality for most (indoxacarb) or all (emamectin benzoate) field populations. These concentrations were used as an additional and discriminating concentration for diagnostic assay of each population in 2002–2004. Subsequently, we found that indoxacarb at 50 mg ([AI])/liter caused a high mortality (>90%) of RS individuals and low mortality (<10%) of a resistant population (J.-Z.Z. et al., unpublished data).

Statistical Analysis. The POLO program was used for probit analysis of dose–response data (LeOra Software 1997). Mortality was corrected using Abbott's formula (Abbott 1925) for each probit analysis. Differences in susceptibility were considered significant when the 95% CL of LC_{50} values did not overlap. The toxicity ratio (TR) (=resistance ratio, RR) was calculated by dividing the LC_{50} of a field population by the corresponding LC_{50} of the susceptible strains.

Results

Baseline Data of the Susceptibility of *P. xylostella* to Three Insecticides (2001). Nine populations of *P. xylostella* collected in 2001 from the United States and Mexico were evaluated for baseline susceptibility data for indoxacarb and emamectin benzoate. Such data were already available for spinosad (Zhao et al. 2002). The TRs to indoxacarb and emamectin benzoate relative to the susceptible strain were 1.4–140- and 2.1–60.5-fold, respectively (Table 1), indicating large geographic variations in different populations. The geographic variations to spinosad were 1.2–11.2-fold in most field populations, similar to the results reported previously (Shelton et al. 2000, Zhao et al. 2002). However, a population collected from collards in Camilla, GA, in 2001, where spinosad had been spayed extensively on collards, showed an extremely high level of resistance to spinosad (>20,000-fold)

Table 1. Susceptibility of diamondback moth to spinosad, indoxacarb, and emamectin benzoate (2001)

Population	G[a]	n	Slope (SE)	LC_{50}, ppm	95% CI	LC_{90}, ppm	95% CI	χ^2 (df)	TR[b]
Spinosad									
Geneva 88 (S)	F298	160	1.20 (0.23)	0.040	0.022–0.063	0.463	0.233–1.75	1.0 (2)	1.0
Nipomo, CA	F1	200	1.85 (0.34)	0.351	0.206–0.505	1.73	1.14–3.51	1.9 (3)	8.8
Teix, CA	F1	200	1.69 (0.30)	0.083	0.045–0.126	0.479	0.306–0.995	1.5 (3)	2.1
Apopka, FL	F1	200	1.19 (0.26)	0.061	0.016–0.140	0.720	0.255–38.0	4.7 (3)	1.5
Vero, FL	F1	240	0.71 (0.13)	0.051	0.020–0.105	3.30	1.08–27.9	1.6 (4)	1.2
WC, FL	F2	200	1.83 (0.35)	0.448	0.254–0.655	2.26	1.46–4.91	0.8 (3)	11.2
Camilla, GA	F2	240	0.90 (0.21)	961	462–2,018	25,945		0.8 (4)	24,025
Rio Grande, TX	F1	200	2.09 (0.50)	0.294	0.168–0.421	1.21	0.768–3.37	0.9 (3)	7.4
Mora, Mexico	F1	200	1.90 (0.31)	0.091	0.058–0.128	0.432	0.292–0.810	1.1 (3)	2.3
Poblano, Mexico	F1	160	1.63 (0.25)	0.189		1.16		3.0 (2)	4.7
Indoxacarb									
Geneva 88 (S)	F299	240	0.90 (0.13)	0.134	0.073–0.226	3.63	1.68–12.5	3.8 (4)	1.0
Nipomo, CA	F1	240	1.44 (0.32)	1.25	0.575–1.96	9.66	5.53–31.5	2.3 (3)	9.3
Teix, CA	F1	200	1.32 (0.21)	0.388	0.206–0.616	3.63	2.14–8.23	1.4 (3)	2.9
Apopka, FL	F1	240	0.72 (0.12)	0.194	0.048–0.604	11.7	2.45–943	5.0 (4)	1.4
Vero, FL	F1	200	1.38 (0.22)	0.602	0.069–1.59	5.10	1.88–170	5.6 (3)	4.5
WC, FL	F2	200	1.31 (0.25)	0.482	0.255–0.771	4.57	2.45–15.1	3.0 (3)	3.6
Camilla, GA	F2	240	1.57 (0.32)	18.8	10.5–28.3	124	72.4–365	1.4 (3)	140
Rio Grande, TX	F1	240	1.23 (0.32)	4.06	2.15–7.71	45.1	17.8–602	0.3 (3)	30.3
Mora, Mexico	F1	200	1.44(0.20)	0.820	0.526–1.20	6.33	3.87–13.5	0.8 (3)	6.1
Poblano, Mexico	F1	240	1.43 (0.34)	1.40	0.632–2.18	11.0	6.15–42.3	3.3 (4)	7.8
Emamectin Benzoate									
Geneva 88 (S)	F299	200	1.40 (0.20)	0.0020	0.001–0.003	0.019	0.012–0.042	2.3 (3)	1.0
Nipomo, CA	F1	200	1.89 (0.32)	0.0156	0.010–0.022	0.074	0.049–0.148	2.0 (3)	7.8
Teix, CA	F1	200	1.53 (0.22)	0.0042	0.003–0.006	0.029	0.018–0.056	2.3 (3)	2.1
Apopka, FL	F1	240	1.07 (0.27)	0.0149	0.003–0.031	0.233	0.088–8.09	5.5 (4)	7.5
Vero, FL	F1	240	1.00 (0.17)	0.0159	0.001–0.053	0.308	0.084–53.1	8.9 (4)	8.0
WC, FL	F2	200	1.76 (0.26)	0.0113	0.008–0.016	0.060	0.040–0.116	0.6 (3)	5.7
Camilla, GA	F2	200	2.00 (0.34)	0.121	0.085–0.162	0.525	0.354–1.05	1.3 (3)	60.5
Rio Grande, TX	F1	160	1.49 (0.30)	0.0062	0.003–0.010	0.045	0.025–0.144	1.1 (2)	3.1
Mora, Mexico	F1	200	1.45 (0.26)	0.0065	0.002–0.015	0.050	0.020–1.49	3.0 (3)	3.3
Poblano, Mexico	F1	240	1.49 (0.50)	0.0345		0.249		6.7 (4)	17.3

[a] Generation.

[b] TR, toxicity ratio = LC_{50} of field pop/LC_{50} of the susceptible colony.

(Table 1). Data from all three insecticides indicated that use of the Geneva 88 strain was justified as a standard because at least one field population tested for each insecticide had a similar LC_{50} value.

Resistance Monitoring to Three Insecticides (2002–2004). Resistance monitoring results based on toxicity ratios and diagnostic tests indicated that most populations collected in 2002–2004 were susceptible to all three insecticides (Table 2). However, high levels of resistance to spinosad in populations collected from collards in Georgia were confirmed in 2002 and 2003, and a population from Hawaii with decreased control efficacy by indoxacarb (R.F.L.M., unpublished data) in 2003 showed high levels of resistance to indoxacarb (TR = 859-fold with 68% survival in the diagnostic test). A low level of resistance to spinosad was detected in 2002 in a population collected from collards in Oxnard, CA (TR = 27.3-fold with 20% survival in the diagnostic test), but this increased rapidly into 2004 (TR > 15,000-fold and 93% survival in diagnostic test) (Table 2).

In seven of the eight populations collected from collards in five counties in southwestern Georgia in 2002, various resistant individual frequencies to spinosad (2.5–29%) were detected (Table 3). Highest survival was detected in a farm (29%, Lewis) that is 30 km east of Camilla, whereas no survival occurred at the Coggins farm of Echols County, which is 140 km southeast of Camilla.

Discussion

After several years of commercial use of spinosad, indoxacarb, and emamectin benzoate, our results indicated that most populations of *P. xylostella* in the United States and Mexico remain susceptible to all three insecticides. However, resistance to spinosad occurred in Hawaii (2000), Georgia (2001), and California (2002), and resistance to indoxacarb was detected in Hawaii in 2003, all as a consequence of a multiple years of extensive applications. We did not intend to compare the relative speed of resistance evolution to each of the three insecticides used in this study because the application history and selection pressure are much different for each insecticide in each location. It is well documented that *P. xylostella* has the potential to develop resistance in the field to most insecticides sooner or later after extensive applications (Talekar and Shelton 1993).

A major reason for the rapid resistance development to spinosad in Hawaii was the lack of suitable alternatives and the unsynchronized use of insecticide classes that led to continuous population exposure to all three insecticides (Mau and Gusukuma-Minuto

Table 2. Resistance monitoring of diamondback moth based on toxicity ratio and diagnostic tests of spinosad, indoxacarb, and emamectin benzoate (2002–2004)

Pop and generation	Spinosad		Indoxacarb		Emamectin benzoate	
	TR[a]	% survival[b]	TR[a]	% survival[c]	TR[a]	% survival[d]
2002						
Gonzales, CA (F2)	4.3	0	4.4	0	10.0	0
Oxnard, CA (F2)	27.3	20.0	5.0	0	33.3	0
Omega, GA (F2)	23.8	12.5	40.7	0	47.7	0
Irwin, GA (F1)	9,193	93.0	44.4	6.0	35.7	0
Donna, TX (F1)	2.3	0	8.7	0	2.9	0
Cortazar, Mex. (F2)	4.2	0	7.0	0	10.5	0
Florencia, Mex. (F1)	5.4	0	15.8	0	24.7	0
2003						
Barat, CA (F2)	3.9	0	2.6	0	4.4	0
Ysidro, CA (F2)	2.7	0	1.9	0	3.1	0
Indian River, FL (F4)	4.9	0	13.0	0	11	0
Quincy, FL (F2)	2.9	0	2.4	0	2.5	0
Mitchell, GA (F2)	8,317	90.0	8.0	0	36	0
Berlin, GA (F3)	>244	43.0	–	0	–	0
Waipio, HI (F2)	–	82.0	859	68	–	0
Bajio, Mex. (F2)	5.1	0	5.2	0	4.4	0
2004						
Oxnard, CA (F2)	15,922	93.0	3.6	0	3.6	0
Gonzales, CA (F2)	6.0	0	6.2	0	1.3	0
Indian River, FL (F2)	1.0	0	1.9	0	1.1	0
Donna, TX (F2)	6.8	0	2.4	0	1.4	0
Florencia, Mexico (F3)	6.0	0	1.8	0	1.7	0
T. Landa, Mexico (F2)	6.2	0	2.8	0	1.7	0

[a] TR, toxicity ratio = LC_{50} of field pop/LC_{50} of the susceptible colony (0.023–0.041 ppm for spinosad, 0.128–0.162 ppm for indoxacarb, and 0.002–0.007 for emamectin benzoate).

[b] Percentage of survival at discriminating concentration of 10 ppm (n = 100–200).

[c] Percentage of survival at discriminating concentration of 50 ppm (n = 100–200).

[d] Percentage of survival at discriminating concentration of 1 ppm (n = 100–200).

2004). After spinosad resistance became evident in Hawaii, region-focused resistance management plans were implemented in February 2001 by growers and University of Hawaii extension advisers in an Insecticide Resistance Action Committee-sponsored program (Mau and Gusukuma-Minuto 2001, 2004). Spinosad was voluntarily removed from the program to mitigate resistance levels, and indoxacarb (labeled for use in December 2000) and emamectin benzoate (labeled for use in June 1999) became the major insecticides and were highly effective against *P. xylostella.* Within 6–8 mo after spinosad was suspended, susceptibility to spinosad increased significantly and more rapidly than anticipated. For example, the resistance ratio decreased from >1000-fold in October 2000 to two-fold in June 2002 in Pearl City (Mau and Gusukuma-Minuto 2004), and growers in such regions were allowed again to use spinosad in 2002. In the locations in Georgia and California where spinosad resistance was detected, the insects were collected from collards in both states. In these areas, extensive applications of spinosad per year had been made to a common *P. xylostella* population because of continuous sequential plantings on adjacent fields or farms. Thus, although growers may have followed label restrictions on the number of applications allowed per planting, the movement of *P. xylostella* between plantings exposed the common population of *P. xylostella* and enhanced selection pressure for resistance, a situation similar to what occurred in Hawaii (Zhao et al. 2002).

Table 3. Diagnostic test of spinosad resistance in samples collected from collards in southwestern Georgia in 2002

County	Pop	% survival at 10 ppm (SEM) (n = 200)	% mortality of CK (n = 40)
Colquitt	Lewis	29.0 (3.3)	0
Colquitt	Williams	8.5 (2.3)	0
Echols	Coggins	0 (0)	0
Grady	Hopkins	3.0 (1.3)	7.5
Grady	Powe	2.5 (1.2)	2.5
Mitchell	Eubanks	2.5 (1.0)	0
Mitchell	Martin	3.0 (1.3)	0
Tift	Omega	18.0 (1.7)	0

Two field colonies in Hawaii that were resistant to spinosad were not cross-resistant to indoxacarb or emamectin benzoate (Zhao et al. 2002). However, the population collected from Camilla, GA, in 2001 that was resistant to spinosad had the highest toxicity ratio for both indoxacarb and emamectin benzoate (Table 1). We do not know the reason for the higher tolerance in this population. Although there was 10 and 6% survival at the discriminating concentration of indoxacarb for a *P. xylostella* population from Georgia collected in 2001 and 2002, respectively, additional selection experiments using the discriminating concentration did not result in any insects able to survive to pupation, indicating that there were no homozygous resistant individuals in the collections in Georgia. However, resistance of *P. xylostella* to indoxacarb developed in Hawaii after 3 yr of commercial use (2000–2003), a time period similar to spinosad resistance development in several locations in Hawaii (Zhao et al. 2002, Mau and Gusukuma-Minuto 2004). The time to resistance development for each insecticide would likely have increased if all three products had been available simultaneously and were used in a more carefully designed resistance management program.

Proactive resistance monitoring and management programs are vital for the sustainable use of a new insecticide for control of *P. xylostella.* The collaborative monitoring program between university and industry scientists we discuss in this article provides useful information to both parties as well as growers who use the products. This program was initiated at an opportune time when three new products with different modes of action were introduced within relatively the same time period. Such a situation has been rare in the history of insecticide development. Resistance is a temporal and spatial phenomenon that changes (Shelton and Zhao 2003). In this report, when resistance developed to one or more products in a particular location at a particular time, we were fortunate that at least one other product effective against *P. xylostella* remained. Additionally, when resistance developed to a product and it was withdrawn from the market, susceptibility returned (e.g., spinosad in Hawaii) and the product was reintroduced. In this situation, reintroduction of spinosad would decrease selection pressure to the other products, although even more careful monitoring of the reintroduced product is necessary. Implementation of areawide grower practices, including crucifer-free periods, also could significantly mitigate the development of insecticide resistance.

This report illustrates a useful example of how a proactive monitoring program can be developed before the widespread use of a new insecticide. Besides providing some baseline information on geographic and temporal variation, regular and proactive monitoring provided industry and extension personnel with the necessary information to help growers switch products before a major economic loss of the crop. However, a more sophisticated resistance management program that makes use of the thoughtful and cooperative rotation of products on an areawide basis would further enhance the longevity of each product.

Acknowledgments

We thank Y. M. Cheung and J. D. Plate for technical assistance. This research was supported in part by Cornell University–New York State Agricultural Experiment Station, University of Hawaii at Manoa, Dow AgroSciences, DuPont Crop Protection, and Syngenta Crop Protection.

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Received 17 August 2005; accepted 3 October 2005.