Monitoring and Characterization of Diamondback Moth (Lepidoptera: Plutellidae) Resistance to Spinosad

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ABSTRACT Fourteen populations of the diamondback moth, *Plutella xylostella* (L.), were collected from fields of crucifer vegetables in the United States, Mexico, and Thailand in 1999 and 2000 for susceptibility tests with spinosad. Most populations were susceptible to spinosad and similar to earlier baseline values, but populations from Thailand and Hawaii showed high levels of tolerance. A statewide survey in Hawaii in 2000 and 2001 indicated resistance problems on several islands. One colony collected in October 2000 from Pearl City, HI, was subjected to further selection pressure, using spinosad in the laboratory, and then was used as the resistant strain (Pearl-Sel) for other tests. Spray tests using the recommended field rates of spinosad on potted broccoli plants in the greenhouse confirmed that field control failures due to resistance were possible in the areas of these collections. Analysis of probit lines from F_1 reciprocal crosses between the Pearl-Sel and S strain indicated that resistance to spinosad was inherited autosomally and was incompletely recessive. A direct test of monogenic inheritance based on the $F_1 \times$ Pearl-Sel backcrosses suggested that resistance to spinosad was probably controlled by one locus. The synergists S,S,S-tributyl phosphorotrithioate and piperonyl butoxide did not enhance the toxicity of spinosad to the resistant colony, indicating metabolic mediated detoxification was probably not responsible for the spinosad resistance. Two field colonies in Hawaii that were resistant to spinosad were not cross-resistant to emamectin benzoate or indoxacarb. Resistance developed in Hawaii due to the continuous cultivation of crucifers in which as many as 50 applications of spinosad per year may have been made to a common population of *P. xylostella* in sequential plantings, although each grower might have used the labeled restrictions for resistance management. Resistance management strategies will need to address such cropping and pest management practices.

KEY WORDS *Plutella xylostella*, spinosad, resistance, inheritance, synergism

THE DIAMONDBACK MOTH, *Plutella xylostella* (L.), is a key insect pest of crucifers, particularly cabbage, broccoli, and cauliflower in many parts of the world (Talekar and Shelton 1993). Resistance to major classes of insecticides in field populations has evolved worldwide, especially in tropical areas. In the mid-1990s in Hawaii, resistance in *P. xylostella* to all available insecticides was so severe that marketable yields of crucifers were reduced, and crucifers were imported to supplement local production (Mau and Gusukuma-Minuto 2001).

Spinosad is the first member of the Naturalyte class of insecticides developed by Dow AgroSciences. It is comprised primarily of two macrocylic lactones, spinosyn A and spinosyn D, which are secondary metabolites produced by S*accharopolyspora spinosa* Mertz & Yao under natural fermentation conditions (Sparks et al. 1995). Spinosad has a unique chemistry and mode of action, a high level of activity against economically important pests, a short half-life, and large margins of safety for mammals, birds, fish and even most beneficial insects (Thompson et al. 2000). Spinosad has been registered on over 180 crops in the United States and in over 35 countries for the control of Lepidoptera insects, beetles, leafminers and thrips. In Hawaii, it was commercialized for pest control in crucifers in April 1998 with excellent initial control of *P. xylostella* (Mau and Gusukuma-Minuto 1999). However, in some areas in Hawaii control failures became evident in 2000.

The objectives of this study were to investigate the geographic variation in susceptibility of *P. xylostella* to spinosad in three countries, examine whether control failures in Hawaii were due to resistance to spinosad, determine the inheritance of spinosad resistance, examine cross-resistance patterns to two other recently introduced insecticides (emamectin benzoate and indoxacarb), and test the effects of synergists to help explain possible mechanisms involved in the resistance to spinosad.

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Table 1. Susceptibility of second instars of *P. xylostella* to spinosad

Population	Generation	*n*	Slope (±SE)	LC_{50}, mg (AI)/liter	95% CL	χ^2	TR	% survival[a]
			1999					
Geneva 88 (S)	Pooled	330	1.74 (0.25)	0.032	0.024–0.042	2.69	1.0	0
Coach, CA	F_2	150	2.14 (0.31)	0.041	0.027–0.062	2.24	1.3	0
Santa Maria, CA	F_1	180	2.24 (0.27)	0.138	0.100–0.192	1.55	4.3	0
Shrine, CA	F_1	150	2.57 (0.35)	0.357	0.250–0.508	1.98	11.2	0
Homestead, FL	F_1	155	2.13 (0.31)	0.289	0.192–0.434	4.14	9.0	0
Immokalee, FL	F_1	150	1.97 (0.33)	0.110	0.065–0.185	1.64	3.4	0
Celamanca, Mex.	F_2	150	1.02 (0.21)	0.209	0.108–0.412	0.98	6.5	0
San Luis Potosi, Mex.	F_2	180	0.99 (0.24)	0.072	0.006–0.187	7.62	2.3	0
			2000					
Geneva 88 (S)	Pooled	340	2.36 (0.32)	0.045	0.033–0.057	1.80	1.0	0
Guadalupe, CA	F_2	210	1.21 (0.16)	0.107	0.037–0.535	7.60	2.4	0
Oxnard, CA	F_2	150	2.17 (0.36)	0.485	0.251–0.940	3.92	10.8	0
Kunia, HI	F_4	180	2.43 (0.38)	14.2	10.5–18.3	1.98	316.0	56.7
Weslaco, TX	F_1	180	0.76 (0.18)	0.042	0.013–0.104	2.03	0.9	0
La Minita, Mex.	F_2	300	0.73 (0.13)	0.082	0.042–0.175	1.67	1.8	0
Villagran, Mex.	F_2	150	2.76 (0.37)	0.486	0.393–0.605	2.09	10.8	0
Bangbuathong, Thailand	F_1	180	1.79 (0.36)	7.96	4.21–12.07	2.35	177.0	40.0

TR, toxicity ratio = LC_{50} of field population/LC_{50} of Geneva 88.
[a] Survival at the discriminating concentration of 10 mg (AI)/liter (n = 30–40).

Materials and Methods

Insects. In 1999 and 2000, 14 populations of *P. xylostella* were collected from fields of crucifer vegetables in three countries, including 11 populations from four states of the United States (California, Florida, Hawaii and Texas), four populations from Mexico (Guanajuato state), and one population from Thailand (Nonthaburi province). About 300 *P. xylostella* larvae and pupae were collected in each location, and transported to the New York State Agricultural Experiment Station (NYS-AES) where bioassays were performed. 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 over 200 generations at the same laboratory and was used in bioassays for comparison. Populations collected from fields in 1999 and 2000 were cultured on rape seedlings in a greenhouse at 25–30°C (Shelton et al. 1991). The second instars of generations one or two for most populations were used in the bioassays. Larvae of Geneva 88 and all field colonies were reared on rape plants for bioassays.

In the state of Hawaii, a *P. xylostella* population was collected from a farm in Ewa, Oahu, in March 1998 for baseline susceptibility tests. Spinosad was subsequently commercialized for use in April 1998 after baseline susceptibility tests and a field efficacy test were completed. A collection of *P. xylostella* was made again from the same farm in November 2000 after complaints of control failure with spinosad. Twelve populations in total were established from three islands (Oahu, Maui and Hawaii) between September 2000 and April 2001. The larvae of a susceptible laboratory (S) colony were field collected from a head cabbage farm located in Kamuela (Lalamilo), HI, in December 1994 before the introduction of spinosad. Subsequent generations were reared on greenhouse grown head cabbage at ≈24°C. All colonies were reared on cabbage or rape at the University of Hawaii at Manoa, and third instars of generations 1–5 were used for bioassays.

Based on bioassay results in Hawaii, one colony collected on 17 October 2000 from Pearl City, Oahu, was found to be highly resistant to spinosad, and was transported to NYS-AES for further study. The Pearl colony was in F_6 when it reached NYS-AES. Larvae were reared on rape seedlings in the greenhouse as noted above. A selection was made on F_7 larvae with spinosad at 100 ppm and F_8 larvae at 200 ppm using a cabbage leaf dip method as previously reported (Shelton et al. 1993, 2000; Zhao et al. 2000) and described in the following section. After the selections this resistant colony was named the Pearl-Sel strain.

Insecticide and Chemicals. Spinosad (SpinTor 2 SC and Success, 240 g [AI]/liter) was supplied by Dow AgroSciences (Indianapolis, IN). Emamectin benzoate (Proclaim, 5% SG) was supplied by Syngenta Crop Protection (Greensboro, NC) and indoxacarb (Avaunt 30%WDG) was supplied by DuPont Crop Protection (Wilmington, DE). Synergists were technical grade S,S,S-tributyl phosphorotrithioate (DEF) (98%, Chem Service, West Chester, PA) and piperonyl butoxide (PBO) (98%, Chem Service).

Bioassays. Cabbage leaf dip bioassays, as previously reported (Shelton et al. 1993, 2000, Zhao et al. 2000), were used at NYS-AES for each strain of *P. xylostella.* Before each formal bioassay, we conducted a preliminary assay using 0.01, 0.1, 1.0 and 10 mg (AI)/liter of spinosad solutions to determine the proper dilutions for the formal assay. Each bioassay included five to six concentrations at the ratio of 1:2 (1, 2, 4, 8, 16, 32) or 1:3.16 (1, 3.16, 10, 31.6, 100) plus a control, using five leaf disks for each concentration. Six or eight 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

Table 2. Susceptibility of third instars of *P. xylostella* from Hawaii to spinosad

Population	Year	Generation	*n*	Slope (±SE)	LC_{50}, mg (AI)/liter	95% CL	χ^2	TR
Lab (S)	1998	F_{60}	575	1.48 (0.34)	0.68	0.06–2.13	18.9	1[a]
Ewa, Oahu	1998	F_1	669	2.08 (0.26)	0.52	0.31–0.78	64.3	0.8[a]
Lab (S)	2000	F_{119}	120	2.53 (0.66)	0.14	0.05–0.21	14.5	1
Ewa, Oahu	2000	F_2	244	1.68 (0.38)	187.0	137–279	19.4	1,340
Kunia, Oahu	2000	F_4	378	3.16 (0.43)	89.9	74.16–15	53.7	6,422
Pearl City, Oahu	2001	F_5	484	0.65 (0.17)	151.0	0.12–756	14.0	1,080
Poamoho, Oahu	2001	F_2	400	2.08 (0.46)	3.28	2.51–5.54	20.6	23
Waianae, Oahu	2001	F_2	420	2.76 (0.80)	34.7	0.38–51.8	12.0	248
Waimanalo, Oahu	2001	F_2	562	1.48 (0.13)	1.56	1.16–2.15	72.6	12
Lower Kula, Maui	2000	F_3	153	1.76 (0.23)	4.95	3.00–7.49	60.0	35
Middle Kula, Maui	2001	F_3	291	0.72 (0.12)	83.0	40.8–149	37.0	592
Lalamilo, Hawaii	2000	F_1	176	2.49 (0.65)	3.60	1.00–6.00	14.9	25
Pahala, Hawaii	2001	F_2	450	3.48 (0.67)	0.43	0.38–0.50	26.8	3
Puukapu, Hawaii	2000	F_1	249	0.83 (0.20)	28.5	0.18–203	17.7	204
Volcano, Hawaii	2001	F_2	420	4.76 (0.82)	0.27	0.19–0.34	33.8	2

TR, toxicity ratio = LC_{50} of field population/LC_{50} of Lab (S) colony.
[a] LC_{50} of 1998 lab colony was used to calculate TR. For other colonies, LC_{50} of 2000 laboratory colony was used.

at 0.1% to all test concentrations and to the water control. Mortality was determined after 72 h at 27 ± 1°C. A similar cabbage leaf dip bioassay method was used in Hawaii, but third instars were used and assays were conducted in 50 by 9-mm petri dishes (Mau and Gusukuma-Minuto 2001).

Spray Tests. At NYS-AES we conducted laboratory spray tests to evaluate control of populations of *P. xylostella* on plants. Small pieces of egg sheets (Shelton et al. 1991) containing either the S (F_{284}) or Pearl-Sel (F_9) strain were infested on potted broccoli plants ('Green Comet') with six to eight true leaves. Once the insects had developed to the second instar, spinosad was sprayed. There were ≈45–70 larvae on each plant before spraying. The application rates were based on those commonly used in the field, 26 g (AI)/ha for the S strain, and 26 and 53 g (AI)/ha for the Pearl-Sel strain. Based on the spray volume of 281 liter/ha, the concentrations used in spraying were 94 and 188 mg (AI)/liter (ppm), respectively. For each treatment, all six plants (replicates) were placed in a line with 46-cm plant spacing. A CO_2 backpack sprayer with a one-row boom, having three nozzles per row and delivering 281 liter/ha at 2.8 kg/cm^2 and 3.2 km/h, was used to apply treatments. Insects were counted on each plant on the same day before spraying and two and 5 d after spraying. Plants were kept in a greenhouse at 25–30°C.

A supplemental test was also conducted with the sprayed plants. Two leaf disks were cut from each plant 24 h after spraying. Each leaf disk was placed into a 30-ml plastic cup and infested with 20 neonates or 10 second instars, respectively, of the corresponding strain as in the spray treatments. Cups with larvae were held for 3 d at the same conditions described for bioassays, before assessing mortality.

Inheritance Studies. To study the inheritance of spinosad resistance, insects of the F_7 Pearl-Sel colony were used as the resistant strain for the reciprocal crosses with the S strain. Because the inheritance of the resistance was incompletely recessive, reciprocal backcrosses (BCs) were made between F_1 and the resistant Pearl-Sel strain (F_8). Following Stone's (1968) method, the degree of dominance for resistance was calculated using the reciprocal F_1 crosses and the pooled data. The chi-square goodness-of-fit test was used to determine how well the backcross mortality data of the second instars, observed at each concentration (pooled data for the reciprocal BCs), fit mortality predicted by each model of inheritance. For a direct test of monogenic inheritance, calculations of expected mortality for the backcross offspring were based on experimental data (Preisler et al. 1990, Tabashnik 1991).

Synergism Tests. *P. xylostella* larvae were immersed in water solutions of DEF and PBO for 10 s, 2 h before the spinosad bioassay (Zhao et al. 1994). Technical grade of DEF and PBO was dissolved in acetone to obtain a 1% master solution that was used to prepare different concentrations in distilled water. Based on preliminary tests, one ppm for either DEF or PBO was the highest rate that caused minimal mortality of either the

Table 3. Cross-resistance tests of *P. xylostella* from Hawaii to emamectin benzoate and indoxacarb

Insecticide	Population	Generation	*n*	Slope (±SE)	LC_{50}, mg (AI)/liter	95% CL	χ^2	TR[a]
Emamectin benzoate	Lab (S)	F_{126}	320	2.83 (0.53)	0.01	0.008–0.01	28.7	1.00
	Pearl City	F_2	397	1.67 (0.17)	0.04	0.03–0.05	95.5	4.00
Indoxacarb	Lab (S)	F_{126}	200	2.73 (0.49)	0.69	0.47–0.91	31.4	1.00
	Ewa	F_5	105	2.62 (0.61)	0.88	0.43–1.30	18.7	1.28

[a] TR, toxicity ratio = LC_{50} of field population/LC_{50} of Lab (S) colony.

Table 4. Susceptibility of *P. xylostella* larvae from Pearl, Hawaii to spinosad

Population	Generation	*n*	Slope (±SE)	LC_{50}, mg (AI)/liter	95% CL	χ^2	Ratio
Geneva 88 (S)	Pooled	340	2.36 (0.32)	0.045	0.033–0.057	1.80	1
Pearl, HI	F_7	420	0.55 (0.07)	44.6	3.34–198	16.8	991
Pearl-Sel	F_8	300	1.69 (0.26)	590.0	402–789	1.23	13,100
	F_9	200	0.95 (0.18)	837.0	469–1,610	2.00	18,600

S or Pearl-Sel strain, so it was selected for the synergism tests. Bioassays were performed as noted above.

Statistical Analysis. The POLO program was used for probit analysis of dose–response data (Russell et al. 1977, 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 LC50 of the susceptible strains. SAS programs were used for analysis of variance (ANOVA) (SAS Institute 1985). Insect density data were transformed using $\log(x + 1)$ before each ANOVA was performed. Treatment means were compared and separated by Tukey's studentized range test honestly significant difference (HSD) at $P = 0.05$ (SAS Institute 1985).

Results

Geographic Variation of the Susceptibility to Spinosad in Three Countries. In the 14 populations of *P. xylostella* collected in 1999 and 2000 from the United States, Mexico, and Thailand, most populations were susceptible to spinosad, but two colonies (one from Bangbuathong District, Thailand and the other from Hawaii) showed significant tolerance compared with the susceptible Geneva 88 strain (Table 1).

After we finished the inheritance tests as described in the following section, we found that spinosad at 10 mg (AI)/liter killed all susceptible (S) and S × R heterozygous individuals but caused no mortality to the homozygous resistant strain (Pearl-Sel). This concentration is equal to 23 times the LC_{99} of the S strain, so we used 10 mg (AI)/liter as the discriminating concentration for a diagnostic assay. Survivors at this concentration were designated the homozygous resistant individuals. The surviving homozygous resistant individuals were 40 and 56.7% in the Bangbuathong and Hawaii populations, respectively, as determined by the discriminating concentration (Table 1). Geographic variation in susceptibility to spinosad occurred in other populations (0.9–11.2 in TR), but no homozygous resistant individual was detected at the discriminating concentration using limited individuals for each population ($n = 30–40$).

Resistance to Spinosad in Hawaii and Cross-Resistance Tests. Previous field efficacy tests using spinosad at 52.5 g (AI)/ha in1998, before its commercial use in Hawaii, provided excellent control of *P. xylostella* (Mau and Gusukuma-Minuto 1999). Baseline susceptibility tests of a *P. xylostella* population from Ewa in March 1998 showed spinosad susceptibility similar to the laboratory susceptible strain (Table 2). After ≈2.5 yr of commercial applications, some complaints of control failure for spinosad by growers were reported in a few areas including Ewa. A population collected in November 2000 from the same location in Ewa as in 1998 was significantly less sensitive to spinosad (Table 2). A state-wide survey in three islands of Hawaii in 2000 and 2001 indicated that six of the 12 populations were highly tolerant to spinosad (TR > 100-fold) (Table 2). *P. xylostella* field populations from Pearl and Ewa that were resistant to spinosad were not resistant to emamectin benzoate and indoxacarb, respectively (Table 3), indicating lack of cross-resistance.

Selection of Pearl Colony from Hawaii. The F_7 larvae of one of the Hawaiian populations collected from Pearl City showed 991 times less susceptibility to spinosad than the S strain (Table 4). One selection using spinosad at the lower field rate (100 ppm) resulted in the population being 13,100 times less susceptible than the S strain (Table 4).

Spray Tests. One application of spinosad at the lower rate of 26 g (AI)/ha caused 94.1% mortality to the S strain 2 d after treatment, while spraying at 26 and 53 g (AI)/ha caused only 15.3 and 17.1% mortality

Table 5. Efficacy of spinosad on susceptible (S) and resistant (R) *P. xylostella* larvae

Strain	Rate, g (AI)/ha	Actual concn, ppm	Initial larvae per plant	2 d		5 d	
				Larvae per plant	% mortality	Larvae per plant	% mortality
S	1.5	94	51.2 ± 1.8a	3.0 ± 1.3b	94.1	0.2 ± 0.2c	99.6
	CK	0	58.0 ± 3.0a	48.5 ± 4.5a	16.4	28.3 ± 2.4b	51.2
R	1.5	94	55.3 ± 3.2a	46.8 ± 5.1a	15.3	42.5 ± 6.5ab	23.1
	3	188	55.5 ± 2.2a	46.0 ± 1.9a	17.1	39.0 ± 2.6ab	29.4
	CK	0	57.7 ± 1.5a	49.0 ± 1.9a	15.1	43.5 ± 2.6a	24.6

Mean ± SEM within a column followed by same letters are not significantly different ($P > 0.05$, HSD). For initial larvae per plant: $F = 1.33$; df = 4, 25; $P = 0.2868$; 2 d: $F = 75.72$; df = 4, 25; $P = 0.0001$; 5 d: $F = 303.24$; df = 4, 25; $P = 0.0001$.

Table 6. Efficacy of spinosad on susceptible (S) and resistant (R) *P. xylostella* infested on the leaf disks in the laboratory 24 h after spraying

Strain	Rate, g (AI)/ha	Actual concn, ppm	Neonates, 3 d		Second instars, 3 d	
			Larvae per plant	% mortality	Larvae per plant	% mortality
S	1.5	94	0.7 ± 0.5b	96.5	0b	100
	CK	0	14.0 ± 0.9a	30.0	7.5 ± 0.6a	25.0
R	1.5	94	14.5 ± 1.2a	22.5	8.8 ± 0.3a	12.0
	3	188	15.5 ± 0.6a	22.5	8.7 ± 0.3a	13.0
	CK	0	16.2 ± 1.0a	23.5	8.8 ± 0.3a	12.0

$n = 120$ for neonates and $n = 60$ for second instars for each treatment. Mean ± SEM within a column followed by same letters are not significantly different ($P > 0.05$, HSD). For neonates per plant: $F = 82.56$; df = 4, 25; $P = 0.0001$; second instars: $F = 666.7$; df = 4, 25; $P = 0.0001$.

to the Pearl-Sel strain, respectively, which was not significantly different from the mortality of the control (15.1%) (Table 5). The supplemental tests in the laboratory using leaf disks from the sprayed plants also indicated control failure of spinosad to the Pearl-Sel strain. (Table 6).

Inheritance Studies. No significant differences in LC_{50} values were observed between the F_1 progeny of the two reciprocal crosses between the resistant (Pearl-Sel) and S strain (Table 7), indicating that resistance to spinosad was autosomally inherited. The degree of dominance of the resistance was −0.83 based on pooled F_1 results (Table 7), indicating incompletely recessive resistance. A direct test of monogenic inheritance based on responses of $F_1 \times$ Pearl-Sel backcrosses suggested that the spinosad resistance was probably controlled by one locus (Table 8).

Synergism Tests. The synergists DEF and PBO did not have a significant effect on the toxicity of spinosad to the resistant colony (Table 9), indicating that metabolic-mediated detoxification was probably not responsible for the spinosad resistance.

Discussion

Our results indicated that most populations of *P. xylostella* were susceptible to spinosad, and similar to earlier baseline values (Shelton et al. 2000). But control failures occurred in several locations of Hawaii due to resistance development after ≈2.5 yr of commercial use. The spinosad resistance in the Pearl-Sel strain collected from Hawaii was inherited as an autosomal and incompletely recessive factor, and metabolic mediated detoxification was not responsible for the resistance. More studies on other mechanisms (penetration, target sites) for spinosad resistance are needed.

A Thailand population of beet armyworm (*Spodoptera exigua*) was 85 (second instar) and 58 times (third instar) less sensitive to spinosad than a susceptible strain, and the resistance was inherited autosomally and incompletely dominant (Moulton et al. 2000). The different conclusions on the dominance of spinosad resistance between *P. xylostella* and *S. exigua* species may have resulted from the difference in species and/or the large difference in levels of resistance (13,100-fold TR in *P. xylostella* compared with 85-fold TR in *S. exigua*). We could find a discriminating concentration (10 mg [AI]/liter) to separate the SS and RS genotype from RR genotype in *P. xylostella*, but for *S. exigua* there was no such concentration due to overlap in the LD-p lines between the three genotypes (Figure 2 inMoulton et al. 2000).

Insecticide resistance management (IRM) strategies were incorporated into the label for spinosad before its introduction. The manufacturer recommended an IRM strategy that limited use to ≤3 applications in a 30-d period, followed by at least 30 d of non-use, and a maximum of six applications per crop. Such IRM restrictions were probably helpful in maintaining spinosad susceptibility in populations of *P. xylostella* in most areas. However, in some areas of Hawaii resistance developed despite labeled restrictions designed to prevent overuse. We believe the guidelines were generally followed, but they did not take into account a more 'regional' approach for resistance management where as many as 50 applications per year might have been made to a common *P. xylostella* population due to continuous sequential plantings on adjacent farms. Crucifers were planted and harvested to meet fresh market needs every week of the year. Although each grower might have used the IRM restrictions, the practices were farm-focused and not coordinated between the small and medium-sized

Table 7. Inheritance of spinosad resistance in the Pearl-Sel colony of *P. xylostella*

Strain	*n*	Slope (±SE)	LC_{50}, mg (AI)/liter	95% CL	χ^2	TR	D[a]
Geneva 88 (S)	340	2.36 (0.32)	0.045	0.033–0.057	1.80	1.0	
Pearl-Sel (R)	300	1.69 (0.26)	590	402–789	1.23	13,100	
S(f) × R	180	0.90 (0.16)	0.128	0.053–0.249	2.61	2.8	−0.78
R(f) × S	240	0.98 (0.15)	0.088	0.041–0.156	1.63	2.0	−0.86
Pooled F_1	420	0.94 (0.11)	0.103	0.060–0.161	2.77	2.3	−0.83

[a] Degree of dominance.

Table 8. Direct test of monogenic inheritance for spinosad resistance in *P. xylostella* based on the responses of backcrosses (pooled $F_1 \times R$)

Concn, mg (AI)/liter	No. of deaths		χ^2 (df = 1)	$P > \chi^2$
	Observed	Expected		
0.1	6	14.9	7.02	0.01[a]
0.316	14	20.3	2.95	0.09
1	26	24.7	0.12	0.73
3.16	29	27.6	0.14	0.71
10	34	29.1	1.59	0.21
31.6	35	30.2	1.55	0.21
100	39	32.8	2.57	0.11
316	48	39.7	2.97	0.08
1,000	54	49.5	2.32	0.13

$n = 60$ for each concentration.

[a] Observed mortality significantly deviated from the model prediction ($P < 0.05$).

farms in the region. We believe that this situation is common in many of the tropical regions of the world where *P. xylostella* is a pest and has often contributed to the rapid development of resistance to new insecticides. Resistance management strategies will need to address such cropping practices.

Regional rather than farm-focused resistance management programs should be developed. Such a program was instituted in Hawaii in February 2001 by the University of Hawaii with the assistance of the Insecticide Resistance Action Committee (IRAC) (Mau and Gusukuma-Minuto 2001). In Hawaii, regional committees of growers and extension advisors developed integrated programs that included improved crop hygiene, mating disruption pheromones, conservation of naturally occurring biological agents, pest monitoring and rotation of insecticide classes. However, this is a voluntary program based on education and peer pressure and not on regulatory management, which likely would be difficult if not impossible to enforce (Mau and Gusukuma-Minuto 2001). All committees voluntarily removed spinosad from their programs when control failures occurred. Fortunately, as resistance to spinosad was developing, two other products with modes of actions different than spinosad became registered. Emamectin benzoate (Proclaim), a second-generation member of the avermectin class of compounds, and indoxacarb (Avaunt) have shown to be highly effective against *P. xylostella* in previous trails in Hawaii (Mau and Gusukuma-Minuto 1999), and growers were fortunate to have them become available. The resistance to spinosad was not cross-resistant to either insecticide. If all three products had been available simultaneously and were used in a more carefully designed program, we believe that resistance to spinosad would not have been as likely to develop.

We do not have such detailed use patterns for the Bangbuathong region of Thailand, but company reports indicate that spinosad was used beginning in the second quarter of 2000. Our field population was collected on 26 August 2000 and had a TR value of 177 compared with the Geneva 88 colony. However, locally produced LC_{50} values have not changed and they were conducted before the compound introduction (G.D.T., unpublished data). Similarly, high native tolerance was reported in Thailand by Moulton et al. (2000) in a population of *S. exigua* from the same district. We are not sure if the TR value of 177 for *P. xylostella* was native tolerance, but higher field rates were established in Thailand due to a high level of native tolerance and local cultural factors (G.D.T., unpublished data).

The percentage of homozygous resistant individuals to spinosad (RR genotype) were 40 and 56.7%, respectively, in the Bangbuathong and Kunia populations (Table 1). As spinosad resistance was controlled by one incompletely recessive gene, the estimated allele frequency was 63.2% for the Bangbuathong and 75.3% for the Kunia population. The estimated resistance allele frequency in the Pearl population before selection was the same as Kunia, as shown by the same survival rates at 10 mg (AI)/liter. It was hence predictable that a single selection on the Pearl population in the laboratory using100 ppm (equivalent to 28 g [AI]/ha in field spray rate) could create a homozygous resistant strain (Pearl-Sel) to spinosad. The discriminating concentration of spinosad at 10 mg (AI)/liter in the leaf disk assay can be used in global resistance monitoring programs in the future. If a 1% survival rate of *P. xylostella* was consistently detected in a given region using this concentration, an allele frequency of 10% would be predicted, and more careful resistance monitoring and management would be needed to delay, and possibly prevent, control failures.

The recessive nature of spinosad resistance may allow continued use, but such use must be carefully monitored. However, more importantly, such an insecticide resistance management program should be implemented before a resistance crisis. Areas with unique use patterns that result in high risk should be given special attention. Growers need multiple cost effective products to use in a resistance management program within the context of an overall integrated pest management program, which should also emphasize cultural and biological controls.

Table 9. Susceptibility of the Pearl-Sel resistant strain of *P. xylostella* to spinosad with and without synergists

Synergist pretreatment	*n*	Slope (±SE)	LC_{50}, mg (AI)/liter	95% CL	χ^2	Synergism ratio[a]
None	300	1.69 (0.26)	590	402–789	1.23	1.0
DEF (1 ppm)	150	1.91 (0.44)	660	362–984	1.59	0.9
PBO (1 ppm)	150	1.71 (0.40)	541	279–833	1.88	1.1

[a] LC_{50} without synergist/LC_{50} with synergist.

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