Assessment of Insecticide Resistance After the Outbreak of Diamondback Moth (Lepidoptera: Plutellidae) in California in 1997

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ABSTRACT During an outbreak of the diamondback moth, *Plutella xylostella* (L.), in California in 1997, nine populations were collected from the major broccoli areas throughout the state. Populations were assayed for their susceptibility to currently used materials (*Bacillus thuringiensis* subsp. *kurstaki*, permethrin, and methomyl) and to newer materials that had not yet been commercially used in California (spinosad, emamectin benzoate, and chlorfenapyr). For the currently used insecticides, elevated levels of resistance were seen only with permethrin and seven of the nine populations had tolerance ratios (TR) of >100. With the newer chemistries, TR values were all <15. To compare potential cross-tolerance, TR values of the currently used insecticides were compared with TR values of the newer insecticides. There were significant relationships found between: methomyl and emamectin benzoate, methomyl and spinosad, and permethrin and spinosad. Further biochemical studies are needed to confirm the actual mechanisms that lead to these relationships and field tests are needed to determine what impact, if any, such TR levels would have on control in the field. These data indicate that resistance to at least one of the commonly used insecticides (permethrin) may have played a role in the outbreak during 1997. However, other factors may have been at least equally important. The winter of 1996–1997 was warmer than normal, and during the period from February through August of 1997 the amount of rainfall was <50% of normal. Hot and dry conditions are known to be conducive to outbreaks of *P. xylostella.* These data add to an overall knowledge about the geographic variation of resistance in *P. xylostella* populations within the United States. They also serve as a baseline for monitoring changes in susceptibility to these newer insecticides and can also help explain the occurrence of outbreaks caused by factors other than insecticide resistance.

KEY WORDS *Plutella xylostella,* resistance, diamondback moth

Broccoli was grown on 49,815 ha in California and had a farm gate value of approximately $450 million in 1997, thus making California the leading U.S. producer (USDA-NASS 1997). The major production areas extend from Monterey Bay to the Imperial Valley (Fig. 1). In 1997 there was an outbreak of diamondback moth, *Plutella xylostella* (L.), that resulted in crop losses estimated to be more than $6 million (Sances 1997).

Plutella xylostella is a recurring pest on cole crops in southern California, but 1997 was an unusual year because of its abundance. In the central valley and north coast regions of California it is not considered a major pest, although it was in 1997. In many regions of the world it is the principal pest of cole crops, and the annual cost of managing it throughout the world is estimated to be U.S. $1 billion (Talekar 1992). Much of the problem in control is the result of insecticide resistance, and populations of *P. xylostella* have a long history of eventually becoming resistant to every insecticide used extensively against them (Talekar and Shelton 1993). This includes many of the currently used carbamate, organophosphate, and pyrethroid insecticides as well as *Bacillus thuringiensis* products. However, in a national survey for insecticide resistance conducted in 1988 (Shelton et al. 1993a), we were not able to collect populations of *P. xylostella* from California that had elevated levels of resistance to methomyl, permethrin or methamidophos, three representatives of the commonly used classes of carbamate, pyrethroid and organophosphate insecticides, respectively. In an additional survey of *P. xylostella* collected in 1990 we did not detect resistance in *P. xylostella* populations from California to commonly used products of *B. thuringiensis* (Shelton et al. 1993b). This was in contrast to other areas in the United States we surveyed that had resistance levels up to 780 times for methomyl, 81 times for permethrin, and 1,641 times for *B. thuringiensis* subsp. *kurstaki.* In both surveys, only limited collections were done in California because of the difficulty in finding enough insects. Thus, although it is possible that resistance was also occurring in California during those surveys, a

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Fig. 1. Major production areas for cole crops in California and the nine collection sites of *P. xylostella* in 1997.

more likely explanation is that *P. xylostella* was controlled with standard insecticides because there was no widespread evidence of *P. xylostella* outbreaks until 1997.

To help assess the possible causes of the 1997 outbreak we conducted a survey of *P. xylostella* in the principal broccoli growing regions of the state. The survey consisted of evaluating populations for their susceptibility to three commonly used insecticides (methomyl, permethrin, and *B. thuringiensis* subsp. *kurstaki*) but also provided an opportunity to gather baseline information on the populations' responses to three novel insecticides that had not yet been used commercially (spinosad, emamectin benzoate, and chlorfenapyr). Spinosad is derived from the bacterium *Saccharopolyspora spinosa* and has been shown to be highly effective against several important Lepidoptera (Bret et al. 1997). It kills an insect through both contact and ingestion and is considered relatively safe to most beneficial insects and has a good safety profile for humans. Emamectin benzoate is a second-generation member of the avermectin class of compounds, a class that has been shown to be potent against Lepidoptera but has a narrow spectrum of activity (Lasota and Dybas 1991). Like spinosad it is also rapidly incorporated into plant tissue and has a good safety profile for humans and beneficial insects. In field studies it has been effective against *P. xylostella* (Leibee et al. 1995) and laboratory studies have indicated a lack of cross or multiple tolerance between it and methomyl and permethrin (Lasota et al. 1996). Chlorfenapyr has a novel mode of action (Bret et al. 1997), kills by both contact and ingestion, and is effective against a wide range of insects. Previous trials have shown all three insecticides to be very effective against *P. xylostella* (Mau and Gusukuma-Minuto 1999). At the time of this study these three insecticides were not registered for use in California, although spinosad and emamectin benzoate had pending registrations for broccoli.

Materials and Methods

A single collection of ≈300 *P. xylostella* larvae was made from each of nine locations (Fig. 1) between October and November of 1997. All insects were transported to the New York State Agricultural Experiment Station where bioassays were performed. An insecticide susceptible population of *P. xylostella*, Geneva 88 (Shelton et al. 1993a), was also reared at the same laboratory for comparison. Populations were cultured on rape seedlings (Shelton et al. 1991) and toxicity of the insecticides was measured using a cabbage leaf dip bioassay similar to that reported previously (Shelton et al. 1993a, 1993b). Generations 199–206 of Geneva 88 and generations 2–6 of the other populations were used in the bioassays. On each day, six to eight concentrations of the insecticides were prepared in a 1.76 or 3.16× dilution series (depending on results from an initial test) including a control. Each concentration was replicated at least four times. Five leaf discs (75 mm diameter) were dipped for 10 s in each concentration and allowed to air dry. Leaf discs were placed into 30-ml clear plastic cups (one disc per cup) and six second instars were placed into each cup. Mortality was determined after 72 h at 27°C and a larva was considered dead if it did not move when prodded. Mortality in the controls was always <3%.

We tested six commercial formulations of insecticides against *P. xylostella:* permethrin (Ambush 2E, Zeneca, Wilmington, DE); methomyl (Lannate LV, DuPont, Wilmington, DE; *Bacillus thuringiensis* subsp. *kurstaki* (Javelin WG, Novartis, Greensboro, NC); emamectin benzoate (Proclaim 5SG, Novartis, Greensboro, NC); spinosad (Success, Dow Agrosciences, Indianapolis, IN); chlorfenapyr (Alert, American Cyanamid, Parsippany, NJ). In all cases, the insecticides were diluted in distilled water for the tests. A solution containing distilled water was also used as control. A spreader-sticker (Bond, Loveland Industries, Loveland, CO) was added to each solution and the control at a concentration of 0.02% (vol:vol).

The concentration or dose-mortality relationship was estimated assuming a probit model by using POLO (LeOra Software 1987). The median concentration (LC_{50}) and the corresponding 95% fiducial limits (FL) were estimated for each insecticide and population. The responses of two populations were considered different if the corresponding 95% FL did not overlap. The tolerance ratios (TR) were calculated by dividing the LC_{50} of a field population by the corresponding LC_{50} of the Geneva 88 population. We used the more neutral term 'TR' rather than RR (resistance ratio) because of the latter's potentially unfounded implications. Values for both terms, however, are equal.

When a new product is being developed for the market, there is always concern about the potential for cross-tolerance with currently used products. To examine this question we used the Pearson correlation (SYSTAT 1992). For this test we compared the TR value of one of the currently used insecticides to the TR of one of the newer insecticides. For analysis we excluded the TR values of the standard Geneva 88 population because it was always treated as 1. We did not compare TR values of one of the currently used insecticides to another currently used insecticide because the populations may have been selected simultaneously for resistance to two or more of the insecticides and, thus, significant correlations would not provide evidence of true cross-tolerance.

In addition to the assays, insecticide records for 1997 were collected for each field and weather data were collected from stations near the sites identified in Fig. 1. The latter was done to assess whether climatological data may help explain the population outbreaks observed in 1997. Precipitation and average daily temperatures were summarized for each month for the following locations, which constitute the principal broccoli production areas where outbreaks occurred: Watsonville, Salinas, King City, Coalinga, Santa Maria, and Oxnard. To provide a comparison of the climatic conditions in 1997 with previous years we averaged the monthly temperature and precipitation data of these five sites and compared these averages to the 30-yr averages (1961–1990). All data were compiled using information from weather observing sites supervised by the National Oceanic and Atmospheric Administration/National Weather Service and received at the National Climatic Data Center (Ashville, NC).

Results and Discussion

For the standard insecticides (older chemistries), elevated levels of resistance were seen only with permethrin and seven of the nine populations had TR levels >100 (Table 1). Only the two populations collected from the Imperial Valley area had LC_{50} values similar to Geneva 88, indicating that these populations had not been intensively selected for resistance and also that the Geneva 88 population was a realistic standard for these assays. Traditionally, cole crops are grown for only 3–4 mo in the Imperial Valley, compared with year-round in the rest of California, so selection pressure would be reduced. A significant difference, based on nonoverlapping of the 95% FL of the LC_{50} values, was observed between Imperial Valley #1 and Geneva 88 populations, but this translated to a TR value of only 1.6. Based on previous reports, this difference would not result in control failures in the field, although TR values for permethrin of >100 would (Shelton et al. 1993a). For methomyl the three most susceptible populations (Imperial Valley #1, Imperial Valley #2, and Geneva 88) had LC_{50} values not significantly different from each other, but these three were significantly different from the other populations. However, all populations had TRs <10 and, based on previous reports (Shelton et al. 1993a), these levels would probably not result in control failures in the field. For *B. thuringienis* there were significant differences between some populations, but all populations except one had TR values <10. The Santa Maria population had a TR value of 11.1 and this value is probably borderline for control problems in the field (Perez and Shelton 1996). Considerably less variation was seen with the newer chemistries, all of which had TR values <15. For chlorfenapyr, TR values were all ≤1.8. Although there were some differences between populations, these would probably be insignificant for field performance. More variation was seen with emametin benzoate whose TR values varied by up to 13 times and there were signifcant differences between several populations. Similar variation was also seen with spinosad where TR values varied by up to 14.8 times. However, without detailed data relating performance in the field to these TR values, we are uncertain of their real significance. Preliminary observations in the fall of 1997, when spinosad was used under a Section 18 label, indicated no apparent problems. Because the populations reported in this article were collected before the Section 18 label and subsequent widespread use of spinosad, these data will be useful as baseline data to monitor changes in susceptiblity for this and the other new products.

A correlation analysis of the TR values indicated some interesting trends (Table 2). No significant relationship was found between TR values of *B. thuringiensis* and TR values of any of the three new insecticides. This would be expected because the only known mechanism of resistance to *B. thuringiensis* in *P. xylostella* is reduced binding of the *B. thuringiensis* toxin to the brush border membrane of the gut (e.g., Tang et al. 1996). There were significant relationships found between methomyl and emamectin benzoate, methomyl and spinosad, and permethrin and spinosad. However, when these nine data points were plotted, it became evident that some data points were either high or low and few fell in the middle range. Although it would not be justified to eliminate any points in our analysis, having populations with more values in the middle range would have improved the analysis and perhaps given more insight into possible cross-tolerance. Still, the relationships we found would not be anticipated because the target sites for methomyl, permethrin, spinosad, and emamectin benzoate are different (Bret et al. 1997). Besides target site insensitivity, resistance can also occur because of reduced penetration through the cuticle or enzymatic detoxification of the insecticide before reaching the target site. Further biochemical studies would be needed to elucidate the actual mechanism(s), but these results should provide some caution about possible cross-tolerance of these new insecticides based on prior use of pyrethroids and carbamates.

Weather data provide some insight into possible causes for the outbreak in 1997. Hot, dry conditions are known to favor outbreaks of *P. xylostella* (Talekar and Shelton 1993) and the mild winter of 1996/1997

Table 1. Toxicity of selected insecticides against populations of *P. xylostella* collected from California in 1997

Population	*n*	Slope ± SE	LC_{50} ppm (AI)	95% CL	TR
		Older insecticides			
Bacillus thuringiensis subsp. kurstaki					
G88	537	1.90 ± 0.19	0.319	0.237–0.418	1.0
Imperial Valley #2	450	1.56 ± 0.15	1.619	1.200–2.138	5.1
Soledad	483	1.27 ± 0.15	2.146	1.434–3.023	6.7
Guadalupe	510	0.95 ± 0.16	1.921	0.746–3.615	6.0
Oxnard	480	0.99 ± 0.08	1.522	0.598–4.235	4.8
Santa Maria	360	1.89 ± 0.23	3.544	1.009–7.497	11.1
Spreckles	445	1.41 ± 0.15	1.693	0.860–2.946	5.3
Coalinga	475	1.16 ± 0.10	0.439	0.316–0.602	1.4
Ocean Cliff	513	1.35 ± 0.21	1.25	0.222–2.467	3.9
Imperial Valley #1	450	1.66 ± 0.21	1.155	0.604–1.903	3.6
Methomyl					
G88	955	3.86 ± 0.26	0.183	0.158–0.210	1.0
Imperial Valley #2	360	2.76 ± 0.27	0.122	0.075–0.206	0.7
Soledad	356	2.83 ± 0.33	0.708	0.419–0.979	3.9
Guadalupe	481	2.66 ± 0.24	0.707	2.860–0.237	3.9
Oxnard	323	3.05 ± 0.32	1.191	0.973–1.457	6.5
Santa Maria	418	2.51 ± 0.27	1.216	0.857–1.586	6.6
Spreckles	420	2.54 ± 0.25	0.817	0.667–0.972	4.5
Coalinga	361	2.11 ± 0.22	1.290	1.017–1.603	7.0
Ocean Cliff	481	2.60 ± 0.23	1.300	1.015–1.621	7.1
Imperial Valley #1	388	3.10 ± 0.29	0.143	0.123–0.166	0.8
Permethrin					
G88	418	3.48 ± 0.44	0.003	0.002–0.003	1.0
Imperial Valley #2	329	2.66 ± 0.30	0.004	0.003–0.006	1.3
Soledad	420	3.44 ± 0.30	0.332	0.295–0.375	110.7
Guadalupe	380	3.96 ± 0.42	0.365	0.270–0.459	121.7
Oxnard	360	4.57 ± 0.56	0.619	0.506–0.736	206.3
Santa Maria	571	3.22 ± 0.25	0.331	0.263–0.410	110.3
Spreckles	630	4.38 ± 0.48	0.436*	0.259–0.573	145.3
Coalinga	300	4.78 ± 0.55	0.463	0.412–0.521	154.3
Ocean Cliff	388	3.51 ± 0.35	0.378	0.300–0.462	126.0
Imperial Valley #1	420	1.76 ± 0.15	0.005	0.004–0.005	1.7
		Newer insecticides			
Emamectin Benzoate					
G88	478	2.92 ± 0.39	0.002	0.002–0.003	1
Imperial Valley #2	359	2.25 ± 0.24	0.007	0.005–0.009	3.5
Soledad	419	2.12 ± 0.19	0.008	0.007–0.010	4.0
Guadalupe	482	3.11 ± 0.42	0.009	0.006–0.011	4.5
Oxnard	461	1.42 ± 0.114	0.012	0.006–0.025	6.0
Santa Maria	480	2.41 ± 0.29	0.012	0.007–0.019	6.0
Spreckles	480	2.07 ± 0.22	0.014	0.009–0.020	7.0
Coalinga	466	2.09 ± 0.20	0.018	0.012–0.027	9.0
Ocean Cliff	355	1.83 ± 0.21	0.026	0.015–0.043	13.0
Imperial Valley #1	300	2.84 ± 0.31	0.005*	0.002–0.009	2.5
Chlorfenapyr					
G88	412	5.70 ± 0.93	3.916	2.937–4.663	1
Imperial Valley #2	360	5.10 ± 0.54	5.351	3.381–8.058	1.4
Soledad	354	5.51 ± 0.77	5.344	3.313–7.192	1.4
Guadalupe	360	6.85 ± 0.75	5.032	3.706–6.670	1.3
Oxnard	168	6.76 ± 0.99	6.571	4.013–8.598	1.7
Santa Maria	360	4.94 ± 0.47	5.93	5.369–6.550	1.5
Spreckles	355	6.21 ± 0.69	5.141*	3.748–6.687	1.3
Coalinga	389	4.97 ± 0.49	6.144	4.813–8.029	1.6
Ocean Cliff	480	3.58 ± 0.35	3.997	3.530–4.495	1.0
Imperial Valley #1	420	4.26 ± 0.38	3.826	3.017–4.815	1.0
Spinosad					
G88	415	1.61 ± 0.15	0.015	0.008–0.29	1
Imperial Valley #2	347	3.34 ± 0.36	0.008*	0.004–0.014	0.5
Soledad	330	3.14 ± 0.38	0.159	0.132–0.190	10.6
Guadalupe	359	3.48 ± 0.53	0.184	0.150–0.220	12.3
Oxnard	287	3.27 ± 0.48	0.192	0.154–0.233	12.8
Santa Maria	329	2.92 ± 0.32	0.19	0.159–0.229	12.7
Spreckles	360	2.97 ± 0.31	0.22	0.116–0.429	14.7
Coalinga	330	2.72 ± 0.29	0.222	0.148–0.336	14.8
Ocean Cliff	390	2.37 ± 0.26	0.218	0.173–0.270	14.5
Imperial Valley #1	360	3.93 ± 0.48	0.011	0.009–0.012	0.7

TR, tolerance ratio = LC_{50} of field population/LC_{50} of Geneva 88 *90% CL.

Table 2. Coefficients of correlation and *P* values for TR values ($n = 9$)

Insecticide	*B. thuringiensis*	Methomyl	Permethrin
Emamectin Benzoate	−0.276 (0.473)	0.773 (0.014)	0.524 (0.147)
Chlorfenapyr	0.196 (0.613)	0.437 (0.239)	0.576 (0.104)
Spinosad	0.074 (0.849)	0.901 (0.001)	0.905 (0.001)

Pearson's correlation coefficient *r* (probability of a greater value under H_O: $P = 0$).

and the below normal rainfall and warmer conditions in the growing season of 1997 met these criteria. Temperatures during the winter of 1996/1997 were above normal and this continued through until the normal harvest time of late August of 1997 (Fig. 2). During the month of May when much of the broccoli was in the ground, daily mean temperatures were ≈110% of normal. Rainfall during the main production period, February–August, was well below normal (Fig. 3). In fact, only in August did the precipitation exceed 50% of the 30-yr average and then it only reached 67% of the norm. Rainfall is a major mortality factor for *P. xylostella* (Harcourt 1963), and in Hawaii overhead irrigation of watercress fields is used to control *P. xylostella* (Tabashnik and Mau 1986).

Our surveys indicated that growers used an array of insecticides in each of the fields from which we obtained populations. At one time or another, however, most growers used either methomyl or a *B. thuringiensis* product. Based on our assays, these materials should have provided adequate control under normal circumstances, if applied correctly. However, because of favorable climatic conditions for *P. xylostella* that resulted in much higher than normal populations throughout the year, it appears that the insects could not be controlled to the level required regardless of which insecticides were used unless, perhaps, they were used more frequently than they were in 1997.

These data add to our overall knowledge about the geographic variation of resistance in *P. xylostella* populations within the United States and suggest that favorable climatic conditions along with permethrin resistance may have caused the 1997 outbreak. They also provide baseline information on some newer products, some of which entered the market after this study was performed. There should be some concern about these newer products because there appears to be some cross-tolerance. A previous study (Scott 1998) evaluated possible cross-tolerance with spinosad and strains of house flies resistant to the major classes of synthetic insecticides. That study reported only a low level (4.3-fold) decrease in susceptibility in a strain of pyrethroid-resistant flies. A previous laboratory study with *P. xylostella* (Lasota et al. 1996) indicated a lack of cross-tolerance between avermectins and methomyl and permethrin. Based on our data, higher levels of cross-tolerance with permethrin may be occurring in *P. xylostella* with spinosad and emamectin benzoate because some populations had TR values >10 times to 1 or both of these insecticides. Further biochemical studies are needed to confirm the actual mechanisms that lead to these relationships and field tests are needed to determine what impact, if any, such levels may have on control in the field.

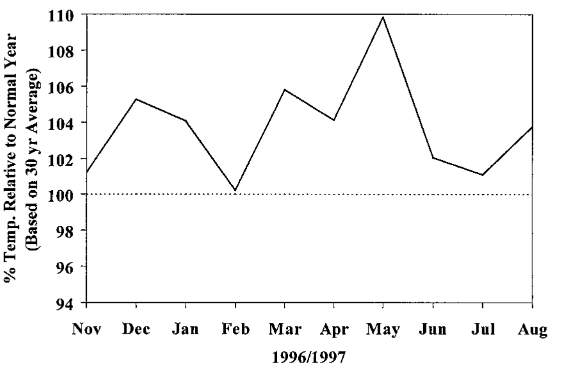

Fig. 2. Monthly temperatures, in relation to the 30-yr average, near the sites where *P. xylostella* was collected.

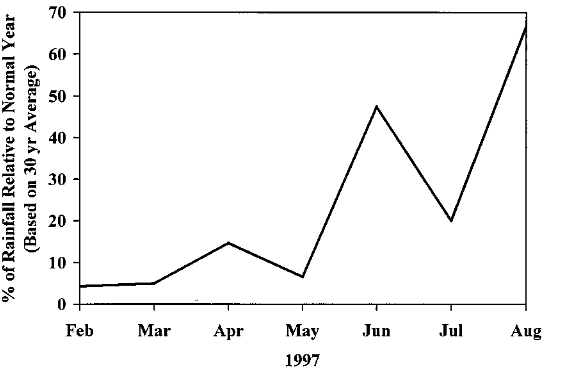

Fig. 3. Monthly rainfall, in relation to the 30-yr average, near the sites where *P. xylostella* was collected.

In 1998 there were no reported significant outbreaks of *P. xylostella* in California cole crops. Most likely this was primarily caused by the considerably higher rainfall (300% in some areas) that reduced populations especially during the winter and spring. However, it could also be caused by the fact that spinosad became registered in the fall of 1997 and was used in 1998, or that growers were able to get control with other materials such as methomyl or *B. thuringiensis.* Most likely it was a combination of reduced insect pressure and the use of effective insecticides. In years in which populations will not be so suppressed by environmental conditions, it will be important to know the geographic distribution of resistance to specific classes of insecticides so they can be avoided. At the same time, older, but still effective, insecticides can be used in a resistance management program with the newer insecticides, if cross-tolerance is not a problem.

In the past 50 yr, *P. xylostella* has become one of the most difficult insects in the world to control, primarily because it has become resistant to every class of insecticide used extensively against it. With the recent registration of at least one of these new insecticides in

California (spinosad), there is even more incentive for implementing a resistance management program within the context of an overall integrated pest management program that stresses the use of regular field sampling, thresholds, and biological and cultural controls.

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