Teflubenzuron Resistance in Diamondback Moth (Lepidoptera: Plutellidae)

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ABSTRACT Laboratory selection of susceptible and field strains of diamondback moth, *Plutella xylostella* (L.), larvae for 20 generations or more resulted in only 8–12-fold resistance to teflubenzuron, a benzoylphenylurea (BPU) that interferes with chitin synthesis. Cross-resistance with another BPU, chlorfluazuron, or a number of conventional insecticides was not apparent. Selection of larvae with teflubenzuron caused the diamondback moth to develop considerable resistance to the ovicidal effect of this compound. Piperonyl butoxide, an inhibitor of microsomal oxidases, restored the effectiveness of teflubenzuron against larvae and eggs of the selected strains, indicating that microsomal oxidation was the major resistance mechanism. Larvae selected with teflubenzuron exhibited much higher aldrin epoxidase and aryl hydrocarbon hydroxylase activities than those of the susceptible and field strains. Lack of cross-resistance between conventional insecticides and teflubenzuron–chlorfluazuron, and between teflubenzuron and chlorfluazuron suggested that different microsomal oxidases in diamondback moth might be involved.

KEY WORDS Insecta, teflubenzuron resistance, diamondback moth

LARVAE OF DIAMONDBACK MOTH, *Plutella xylostella* (L.), feed on the foliage of cruciferous plants from the seedling stage to harvest, and greatly reduce the yield and quality of the produce. Farmers often use large quantities of insecticides and spray cocktails of chemicals to control this insect. This, coupled with the rapid turnover of generations in the tropical climate, has resulted in the development of resistance in diamondback moth to practically all categories of synthetic insecticides (Sun et al. 1986). Therefore, insecticides with novel modes of action are being sought constantly as a means to cope with these resistance problems.

In the past few years, benzoylphenylureas (BPUs) have received much attention in the management of this insect pest because of their unique action in interfering with chitin synthesis. Laboratory and field experiments have indicated that some of these newly developed compounds, such as teflubenzuron and chlorfluazuron, are effective on resistant diamondback moth (Becker 1986, Kohyama 1986, Lim & Khoo 1986, Sagenmueller & Rose 1986). Perng & Sun (1987) recently reported that no significant cross-resistance between conventional insecticides and these two BPUs was detected in seven field strains of diamondback moth collected throughout the island of Taiwan.

Pimprikar & Georghiou (1979) found that microsomal oxidases played an important role in diflubenzuron resistance in the housefly. These enzymes in the diamondback moth were involved in resistance to pyrethroids and pyrethroid–synergist mixtures (Chen & Sun 1986). Sun et al. (1986) considered microsomal oxidases in this insect to be unusually versatile in dealing with new types of xenobiotics. Therefore, diamondback moth may become resistant to BPUs once they are widely used. In early 1987, unofficial reports from Thailand indicated that diamondback moth has developed significant resistance to several BPUs only 2–3 yr after they were introduced to control this pest, which by then had become resistant to almost all other conventional insecticides.

We conducted our study to select for teflubenzuron resistance in the laboratory and attempt to identify the major resistance mechanism in the diamondback moth.

Materials and Methods

At least 500 mature larvae were collected from each of seven locations throughout Taiwan and reared as one (MD) strain in the laboratory on mustard seedlings (Liu & Sun 1984). The susceptible (FS) strain was obtained from L'Institut National de la Récherche Agronomique, France.

The BPUs tested were teflubenzuron (15% suspension concentrate, Celamerck GmbH S., Ingelheim am Rhein, Federal Republic of Germany); chlorfluazuron (5% emulsifiable concentrate, Ishihara Sangyo Kaisha, Japan); diflubenzuron (25% wettable powder, Duphar B.V., Graveland, the Netherlands); PH 70-23 and PH 60-51 (10% liquid formulation, Duphar). Formulations and sources of other insecticides and synergists were reported earlier by Chen & Sun (1986).

To determine the susceptibility to BPUs, third instars were fed sprayed mustard seedlings, and adult emergence was used as the criterion for effectiveness (Perng & Sun 1987). Whenever syner-

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Table 1. Susceptibility to several chitin synthesis inhibitors of the larvae of susceptible (FS) and teflubenzuron-selected (FSR) strains of diamondback moth

Insecticide	FS			FSR			
	n	LC_{50} (95% FL) (μg/ml)	Slope ± SE	*n*	LC_{50} (95% FL) (μg/ml)	Slope ± SE	RR[a]
Teflubenzuron	384	0.068 (0.058–0.075)	3.83 ± 0.61	225	0.80 (0.67–0.96)	3.21 ± 0.44	12
Chlorfluzuron	216	0.12 (0.10–0.14)	3.46 ± 0.62	225	0.16 (0.12–0.25)	2.38 ± 0.59	1.4
PH 70-23	448	0.67 (0.52–0.78)	3.69 ± 0.22	225	0.52 (0.42–0.63)	2.57 ± 0.47	0.8
PH 60-51	216	0.11 (0.086–0.13)	3.15 ± 0.52	270	0.57 (0.41–0.80)	1.44 ± 0.47	5.2

[a] Resistance ratio, LC_{50} of FSR strain/LC_{50} of FS strain.

gists were tested, the seedlings were sprayed with an acetone solution of the synergist before treatment with the BPU and then fed to larvae. For selection, >3,000 third instars of the FS or MD strains were treated similarly in each generation with selection pressure maintained between 70 and 85% mortality. Susceptibility to conventional insecticides was determined by spraying an acetone solution of the insecticide onto fourth instars and recording 24 h mortality (Chen & Sun 1986).

To test ovicidal activity of BPUs, four pairs of 1-d-old moths were allowed to mate in a Petri dish. Between 50 and 150 eggs were collected within 24 h. The eggs were sprayed with acetone solution of the test compounds for 5 s (Shandon spraygun, Astmoor, UK). Five to seven concentrations were tested in each of three replicates. Eggs in the Petri dish were kept at 90 ± 2% relative humidity (RH). Mortality was recorded 4 d later (unfertilized eggs were excluded). Only those that completed eclosion were considered unaffected. Data were corrected for control mortality (<10%) by Abbott's (1925) formula and subjected to probit analysis (Finney 1971).

For 4 h, 100 early fourth instars were allowed to ingest mustard seedlings that contained 0.25 μCi (Curie) of D-(U-^{14}C) glucose (specific activity 274 mCi/mmol, Amersham International, UK). They were then fed untreated mustard seedlings for various intervals up to 36 h. The experiment was replicated twice for each time interval. The larvae were then homogenized in water and centrifuged at 15,000 × *g* for 10 min. The precipitate was extracted with acetone, and treated with sodium hydroxide and then with hydrochloric acid (Ishaaya & Casida 1974). Extracted chitin was combusted (Horrocks 1974), and the $^{14}CO_2$ was absorbed in ethanolamine/ethylene glycol monoethyl ether (1:8). A Beckman LS 5800 liquid scintillation system (Beckman Instruments, Palo Alto, Calif.) with proper quenching correction was used to count ^{14}C activity. Incorporation of ^{14}C-glucose into cuticle increased with time and became constant after 24 h. Therefore, to measure the effect of teflubenzuron on the incorporation of glucose into cuticle, larvae were fed treated seedlings before being given seedlings treated with ^{14}C-glucose. The ^{14}C activity in chitin was determined 24 h after the larvae ingested ^{14}C-glucose.

Aldrin epoxidase activity was determined as follows. Twenty fourth instars were homogenized in 10 ml sodium phosphate buffer (0.1 *M*, pH 7.2). This buffer contained 1 m*M* phenylmethanesulfonyl fluoride, 1 m*M* phenylthiourea, 1 m*M* EDTA (ethylenediaminetetraacetate), 0.1 m*M* 1,4-dithioerythritol, and 20% glycerol. The homogenate was filtered through several layers of cheesecloth and centrifuged at 1,000 × *g* for 10 min. The supernatant was used as enzyme source. Enzyme solution (0.5 ml) was added to 1.5 ml sodium phosphate buffer, which contained 0.75 mg NADPH (reduced nicotinamide adenine dinucleotide phosphate) and 0.5 mg NADH (reduced nicotinamide adenine dinucleotide). After 5 min incubation at 34°C, 5 nmol aldrin in 0.1 ml ethylene glycol monoethyl ether was added to initiate the reaction. The reaction was terminated 10 min later by the addition of 4 ml n-hexane. The amount of dieldrin was determined using a gas–liquid chromatograph (Tracor 222, Tracor, Austin, Tex.) (Wolf et al. 1979).

For aryl hydrocarbon hydroxylase determination, the incubation system was the same. To initiate the reaction, 40 nmol benzo[α]pyrene in 0.1 ml acetone (containing 0.692 μCi ^{3}H as (G-^{3}H) benzo[α]pyrene from Amersham International, UK) was added. After 15 min, the reaction was stopped by adding 1 ml 0.05 *M* NaOH in 80% ethanol solution. The unreacted benzo[α]pyrene was removed with n-hexane and ^{3}H activity in water layer was determined with the Beckman liquid scintillation counter (dePierre et al. 1978).

Results and Discussion

After 29 generations of selection with teflubenzuron, the susceptible FS strain gained a 12-fold resistance to the selecting agent (Table 1). Susceptibility to teflubenzuron of the field strain decreased approximately 8-fold after selection for 20 generations (Table 2). The rate and extent of development of teflubenzuron resistance in these two strains were slower than expected. Chen & Sun

Table 2. Susceptibility to several chitin synthesis inhibitors of the larvae of field (MD) and teflubenzuron-selected (MDR) strains of diamondback moth

Insecticide	MD			MDR			
	n	LC_{50} (95% FL) (μg/ml)	Slope ± SE	*n*	LC_{50} (95% FL) (μg/ml)	Slope ± SE	RR[a]
Teflubenzuron	320	0.14 (0.13–0.15)	5.30 ± 0.66	320	10.05 (0.94–1.17)	3.87 ± 0.49	7.5
Chlorfluazuron	320	0.15 (0.14–0.25)	4.57 ± 1.01	315	0.18 (0.15–0.21)	3.33 ± 0.45	1.2
PH 70-23	320	0.89 (0.74–1.04)	2.62 ± 0.39	270	3.15 (2.17–4.35)	1.59 ± 0.28	3.5
PH 60-51	315	0.99 (0.86–1.13)	4.31 ± 0.32	324	2.41 (1.56–3.46)	1.44 ± 0.24	2.4

[a] Resistance ratio, LC_{50} of MDR strain/LC_{50} of MD strain.

(1986) reported the development of extremely high levels of fenvalerate resistance in field strains of diamondback moth after only a few generations of selection in the laboratory. Sun et al. (1986) observed >100-fold carbofuran resistance in a susceptible strain of this insect after 10 generations of selection.

Roush & McKenzie (1987) suggested that the rate of resistance development in the field is a function of initial frequency and dominance of resistance alleles, relative fitness of resistant genotypes, and population structure. Diamondback moth was not exposed to teflubenzuron or related compounds (with the possible exception of diflubenzuron) in Taiwan. Perng & Sun (1987) did not find significant cross-resistance from conventional insecticides to this group of compounds. Thus, the individuals collected from the field and used in the selection program in this study might not have contained the major resistance genes for a high level of teflubenzuron resistance, such as occurred in Thailand.

The FS and MD resistant strains selected with teflubenzuron did not show apparent cross-resistance to another BPU resistant, chlorfluazuron, which was also very effective against the larvae of diamondback moth (Tables 1 and 2). However, some cross-resistance to PH 60-51 was apparent in the FS strain.

Larvae of the MDR strain selected with teflubenzuron had no substantial cross-resistance to several conventional insecticides, including pyrethroids, organophosphorous, and carbamate compounds, except a slight increase of tolerance to mevinphos (Table 3).

Piperonyl butoxide (PB), a known inhibitor of microsomal oxidases, almost completely restored the susceptibility of the MDR strain to teflubenzuron and PH 60-51 (Table 4), suggesting that microsomal oxidation was the primary cause for the observed resistance to teflubenzuron and PH 60-51. Another microsomal oxidase inhibitor, MGK 264, was less effective in synergizing teflubenzuron in diamondback moth, as Chen & Sun (1986) reported for pyrethroid resistance in this insect. Tributyl phosphorotrithioate (an esterase inhibitor) and carbaryl, reported to be an inhibitor of amidase in plants (Herrett 1969), had no effect on response to teflubenzuron, suggesting that hydrolysis via esterases or amidase was not involved in the teflubenzuron resistance in the MDR strain.

In terms of teflubenzuron inhibition of in vivo ^{14}C-glucose incorporation into cuticle, larvae of the MDR strain selected with teflubenzuron were less

Table 3. Susceptibility to several conventional insecticides of field (MD) and teflubenzuron-selected (MDR) strains of diamondback moth

Insecticide	MD			MDR			
	n	LC_{50} (95% FL) (μg/ml)	Slope ± SE	*n*	LC_{50} (95% FL) (μg/ml)	Slope ± SE	RR[a]
Permethrin	294	0.27 (0.20–0.33)	2.62 ± 0.38	357	0.34 (0.29–0.39)	2.73 ± 0.27	1.3
Fenvalerate	206	0.81 (0.55–1.05)	1.69 ± 0.27	329	1.38 (1.06–1.75)	1.65 ± 0.20	1.7
Mevinphos	306	0.11 (0.084–0.13)	1.91 ± 0.26	305	0.33 (0.25–0.45)	1.31 ± 0.21	3.0
Prothiophos	229	0.29 (0.24–0.35)	2.34 ± 0.27	284	0.45 (0.36–0.59)	2.01 ± 0.26	1.6
Carbofuran	358	0.50 (0.41–0.61)	2.17 ± 0.24	299	0.54 (0.42–0.71)	1.52 ± 0.21	1.1

[a] Resistance ratio, LC_{50} of MDR strain/LC_{50} of MD strain.

Table 4. Synergism of teflubenzuron and PH 60-51 by several synergists in larvae of a teflubenzuron-selected (MDR) strain of diamondback moth

Treatment	*n*	LC_{50} (95% FL) (μg/ml)	Slope ± SE	SR[a]
Teflubenzuron				
Alone	320	1.05 (0.94–1.17)	3.87 ± 0.49	
+ PB	378	0.12 (0.098–0.14)	2.12 ± 0.29	8.8
+ MGK 264	315	0.33 (0.28–0.40)	2.49 ± 0.30	3.2
+ carbaryl	315	1.08 (0.75–1.83)	1.15 ± 0.27	1.0
+ TBPT	320	1.28 (0.52–33.1)	0.84 ± 0.32	0.8
PH 60-51				
Alone	315	2.66 (2.01–3.91)	1.51 ± 0.26	
+ PB	225	0.35 (0.25–0.46)	1.91 ± 0.34	7.7

[a] Synergism ratio, LC_{50} unsynergized/LC_{50} synergized.

susceptible than the FS and the unselected MD strains (Fig. 1). Synergism of teflubenzuron by PB was also observed in this experiment.

While chlorfluazuron and diflubenzuron had practically no effect on the eggs of diamondback moth, teflubenzuron showed high ovicidal activity against the FS strain (Table 5). Embryos of the affected eggs developed normally as determined by examination with a light microscope, but they could not complete eclosion and leave the eggshell. Kohyama (1986) also reported that teflubenzuron significantly inhibited egg hatch in this insect.

FSR and MDR strains of diamondback moth selected with teflubenzuron developed considerable ovicidal resistance to this chitin synthesis inhibitor (Table 5). Grosscurt (1980) reported a similar case; upon larval selection with diflubenzuron, larvicidal as well as ovicidal resistance developed in houseflies (*Musca domestica* L.).

Diflubenzuron and penfluron injected into female houseflies were deposited without degradation in the eggs (Chang & Borkovec 1980). When present in sufficient quantities in the eggs, these BPUs may affect their hatching. Therefore, if a sufficient amount of teflubenzuron is passed from larvae through adults to eggs, and if the resistance mechanism selected in the larval stage expresses itself in the egg stage, selection pressure also might occur in the egg stage. Therefore, we would anticipate the appearance of ovicidal resistance. Our attempt to determine the amount of teflubenzuron transmitted from larvae to eggs was unsuccessful because of the low specific activity of available ^{14}C-teflubenzuron and the high efficacy of this compound against even the resistant strains. Table 6 shows that PB increased ovicidal activity of teflubenzuron in the selected MDR strain. Thus, the same resistance mechanism (i.e., enhanced microsomal oxidative detoxication of teflubenzuron) might exist in larvae and eggs of MDR strain of diamondback moth.

Table 6. Synergism of teflubenzuron by piperonyl butoxide (PB) against the eggs of a teflubenzuron-selected (MDR) strain of diamondback moth

Teflubenzuron concentration μg/ml	Mortality %	
	No PB	With PB (100 μg/ml)
10	9.8	12.3
20	5.4	56.5
60	4.7	50.2
100	10.7	71.9
200	34.4	79.1
300	95.7	100

Very low levels of microsomal oxidases were detected in eggs of diamondback moth, but meaningful data could not be obtained with the available methods. Larvae of the selected FSR and MDR strains of diamondback moth exhibited higher aldrin epoxidase and aryl hydrocarbon hydroxylase activities than those of the susceptible FS and field MD strains (Table 7).

Enhanced microsomal oxidation was suggested to be a major mechanism of pyrethroid resistance in diamondback moth (Liu et al. 1984, Chen & Sun 1986). However, no cross-resistance was de-

Table 5. Toxicity of teflubenzuron, chlorfluazuron, and diflubenzuron to the eggs of susceptible (FS), field (MD) and two teflubenzuron-selected (FSR and MDR) strains of diamondback moth

Insecticide	Strain	*n*	LC_{50} (95% FL) (μg/ml)	Slope ± SE	RR[a]
Teflubenzuron	FS	1,608	0.44 (0.40–0.48)	2.61 ± 0.23	—
	MD	986	0.48 (0.43–0.53)	3.97 ± 0.31	1.1
	FSR	895	20.1 (18.0–22.4)	2.33 ± 0.15	46
	MDR	895	27.2 (23.7–30.9)	2.32 ± 0.18	57
Chlorfluazuron	FS	1,243	>100		
Diflubenzuron	FS	1,579	>100		

[a] Resistance ratio, LC_{50} of each strain/LC_{50} of FS strain.

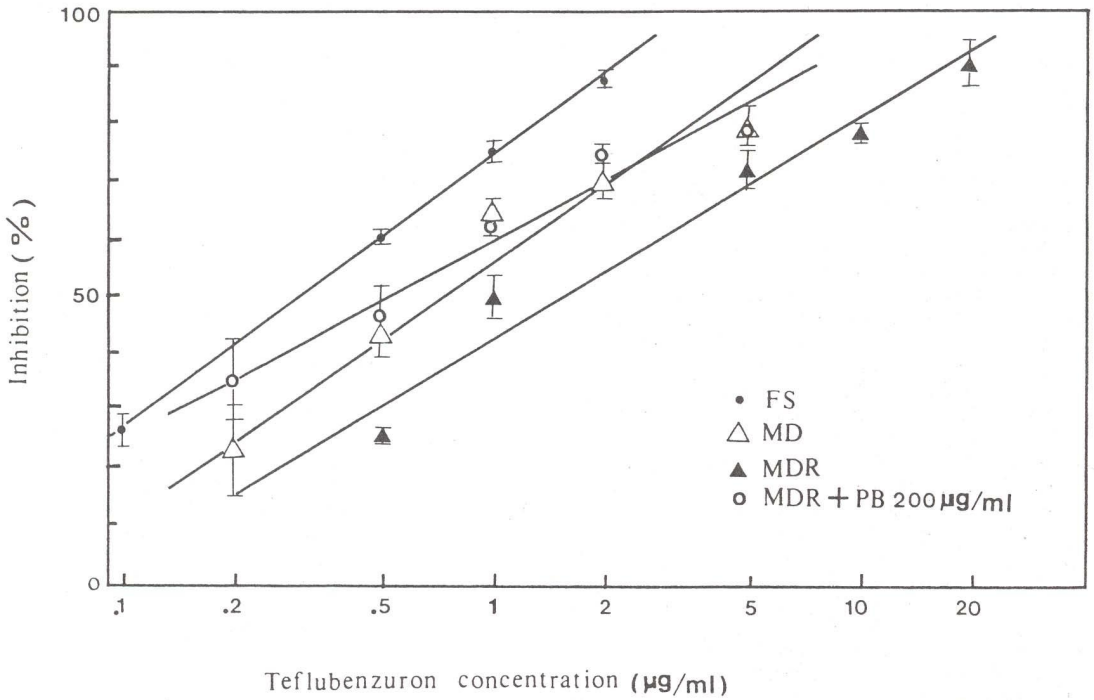

Fig. 1. The inhibition by teflubenzuron of in vivo ^{14}C-glucose incorporation into the cuticle of larvae of susceptible (FS), field (MD), and teflubenzuron-selected (MDR) strains of diamondback moth. PB: piperonyl butoxide.

tected between pyrethroids and teflubenzuron or chlorfluazuron (Perng & Sun 1987). Thus, microsomal oxidases in the field strains of diamondback moth were able to detoxify pyrethroids, but not teflubenzuron or chlorfluazuron. The lack of cross-resistance to chlorfluazuron in FSR and MDR strains suggested that the microsomal oxidases evolved in the selected strains with low levels of teflubenzuron resistance could not oxidize chlorfluazuron.

Sun et al. (1986) proposed use of a series of synergists, pyrethroids, and organophosphorus insecticides in the management of resistance in diamondback moth. They suggested that applying BPUs between organophosphorus and pyrethroid insecticides might lessen the selection pressure. This suggestion is supported by our results; no cross-resistance was observed between teflubenzuron and conventional insecticides, nor between teflubenzuron and chlorfluazuron. The significance of ovicidal resistance to teflubenzuron merits further assessment.

Table 7. Aldrin epoxidase (AE) and aryl hydrocarbon hydroxylase (AHH) activities in the larvae of susceptible (FS), field (MD), and two teflubenzuron-selected (FSR and MDR) strains of diamondback moth

Strain	AE (pmol/min/mg protein)	AHH (pmol/min/mg protein)
FS	32.6 ± 5.3	28.1 ± 1.5
MD	84.1 ± 6.7	46.7 ± 5.8
FSR	161 ± 16.3	220 ± 10.5
MDR	103 ± 15.2	151 ± 7.0

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