

Resistance of Diamondback Moth to Insect Growth Regulators

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Abstract

Susceptibility of diamondback moth *Plutella xylostella* (L.) from Thailand to various insecticides was determined. Results showed that the diamondback moth was resistant to synthetic pyrethroids, organophosphorus insecticides, carbamates, as benzoylphenylurea well as insect growth regulators such as chlorfluazuron, diflubenzuron, hexaflumron, PH 70-23, NK-081 and NI-18. The degree of resistance to the last group varied widely among the chemical groups. The insect was sensitive to *Bacillus thuringiensis* Berliner, abamectin, NC-176 and juvenile hormone mimic. An addition of piperonyl butoxide to benzoylphenylurea insecticides resulted in no synergism, indicating that the resistance mechanism of this strain does not include microsomal oxidation of insecticides. In order to determine the mode of inheritance of insect growth regulator resistance, we conducted crossing and reciprocal tests using resistant and susceptible strains. The gene responsible for resistance seems to be completely recessive because the dose-mortality regression curve of F₁ progeny coincided with the susceptible strain. The result of backcrosses suggested that the inheritance of resistance was monofactorial. Reciprocal crossing test results showed that there was no sex linkage in this inheritance. During 40 generations of rearing without insecticide pressure no recovery of sensitivity to insect growth regulators was observed. Insect growth regulator-resistant strains of the diamondback moth emerged in 1989 at Kagoshima and Okinawa in Japan. The Kagoshima strain showed low levels of resistance to insect growth regulators.

Introduction

Diamondback moth (DBM) *Plutella xylostella* (L.) (Lepidoptera:Yponomeutidae) has become the most important insect pest of crucifers, and has developed resistance to organophosphorus insecticides (Noppun et al. 1986), carbamates (Sun et al. 1978), synthetic pyrethroids (Liu et al. 1981, 1982; Hama 1986; Horikiri et al. 1987, Makino et al. 1985, Noppun et al. 1986), *Bacillus thuringiensis* Berliner preparations, and others. In Southeast Asian countries, including Thailand, this increased resistance to insecticides is causing major problems for the growers.

Benzoylphenylurea insect growth regulators (IGR) with superior toxicity to the resistant DBM have been developed. However, Perng (1987), Perng et al. (1988), Kohyama et al. (1989), and Lin et al. (1989) found resistance in DBM even to these IGRs.

Susceptibility tests on various IGRs were performed on the DBM collected in Thailand. The results showed that the DBM had developed resistance to IGRs. To clarify the features of the resistance to IGRs, we carried out a number of investigations on the susceptibility, synergists, genetic mechanisms responsible for resistance, stability of IGR resistance, and others.

Materials and Methods

Test insect

The following four strains of DBM were used in this study: a BBT strain collected at Bang Bua Thong near Bangkok in January 1988; a BK strain collected at Bangkhae in the suburbs of Bangkok in January 1988, an MZB strain collected at Mizobe Kagoshima, Japan, in April 1990, and a susceptible strain maintained at Agricultural Technical Center, Zen-Noh, Japan.

The BBT and BK strains are presumed to have been exposed to DDT, malathion, dichlorvos, methomyl, carbofuran, fenvalerate, permethrin, cypermethrin, teflubenzuron, and since 1985 chlorfluazuron and *B. thuringiensis*. MZB strain was exposed to the plant foot application of benfuracarb, acephate granules, fluvalinate wettable powder and a *B. thuringiensis* preparation. However, the toxicity of these insecticides to MZB strain has been decreasing in recent years. For this reason, when chlorfluazuron was registered in October 1988, it was supposed to have been used for its superior efficacy.

In our tests, the S strain was designated the standard strain. The test insects were the 3rd instar larvae reared without IGR pressure at $25 \pm 2^\circ\text{C}$, 16L:8D (Koshihara and Yamada 1978). The test of juvenile hormone mimic (JHM) used 4th instar larvae.

Test chemicals

The following insecticides were used in this study: IGRs: chlorfluazuron 5% emulsifiable concentration (EC), diflubenzuron 23.5% wettable powder (WP), hexaflumron 5% EC, PH 70-23 25% liquid formulation (L), NK-081 5% EC, NI-18 2.5% EC and synthetic pyrethroids; fenvalerate 96.3% technical (Tech.), permethrin 96.4% technical and ethofenprox 96% technical. Carbamates: carbofuran 97.3% technical and methomyl 91% technical. Organophosphorus insecticides: dichlorvos 99% technical, dimethylvinphos 96% technical, trichlorfon 99.7% technical, vamidothion 54.2% technical, fenitrothion 96.5% technical, diazinon 96% technical, salithion 95.5% technical, pirimiphos methyl 91.5% technical, EPN 92.7% technical, phenthoate technical, prothiophos 94.1% technical, malathion 95% technical, acephate 97% technical and *B. thuringiensis* preparations; Toarrow-CT 7% WP, Dipel 10% WP and cartap 50% soluble powder (SP), JHM NC-184, abamectin (MK-936) and NC-176.

These formulated chemicals were diluted with deionized water, and the spreader Neo-Esterin was added. Technical grades of chemicals were dissolved in a small amount of acetone before dilution. Synergist tests were carried out by adding 100 ppm piperonyl butoxide equivalent to each chemical solution.

Susceptibility tests

Each test was conducted by dipping a cabbage leaf in an aqueous solution of chemicals for 1 min. Five to seven concentrations of each chemical with three replications were tested. After being air-dried, the leaf was put in the cup (diameter 9 cm, height 5 cm) and 10 DBM larvae were released. The larvae were maintained in a chamber controlled at $25 \pm 2^\circ\text{C}$, 16L:8D. Mortality and inhibition of adult eclosion were recorded as follows: 5 days after treatments on IGRs for mortality, 7 days after treatments on JHM for inhibitor of adult eclosion and 2 days after treatments of other chemicals for mortality.

The results were analyzed by the probit method (Bliss 1935) on connected mortality (Abbott 1925). LC_{50} values were calculated.

Crossing tests

Pupae from the BBT, S strain and their F₁ were put in a glass tube 8 mm diameter and 50 mm high. A day after adult eclosion, 20 male and female adults from the respective strains were put into a 15 cm diameter cup for the reciprocal crossing, back-crossing between the BBT strain and F₁ that sprang from the reciprocal crossing, and the crossing of F₂. The susceptibility test in this crossing was carried out with the leaf dipping method using 3rd instar larvae.

The mode of inheritance of DBM resistance to IGRs was investigated using degree of dominance (D) and chlorfluazuron concentration-mortality curves. The degrees of dominance were calculated using Stone's (1968) formula.

Results and Discussion

Susceptibility of DBM to various IGRs

Susceptibility of BBT and BK strains of DBM to various IGRs decreased, but the degree of resistance varied widely among the chemical groups belonging to IGRs (Table 1). For example, resistance ratio (RR) of PH 70-23 was 49, 080-fold on BBT strain, while RR of NI-18 was 8 and that of hexaflumron 12. Among organophosphorus compounds, it is known that cross-resistance is usually not detected between phenthoate and dimethylvinphos. It is also assumed that resistance of IGRs has a tendency similar to the organophosphorus resistance. Therefore, IGR resistance is presumed to have no cross-resistance, largely due to their unique mode of action. Future studies on the IGRs mode of action should clarify this.

DBM from chlorfluazuron-used area showed less susceptibility to other IGRs. A certain difference in the susceptibility to BBT and BK strains was noticeable in the chlorfluazuron, but it remained quite similar to the other chemicals.

Table 1. Susceptibility to several chitin synthesis inhibitors of the larvae of susceptible (S) and resistant (BBT, BK) strains of DBM.

Insecticide	BBT-strain		BK-strain		S-strain
	LC ₅₀ (ppm)	RR ^a	LC ₅₀ (ppm)	RR ^a	LC ₅₀ (ppm)
chlorfluazuron 5%EC	13	130	1.4	14	0.1
diflubenzuron 23.5%WP	6910	28	6544	26	248
hexaflumron 5%EC	1.8	12	— ^b	—	0.15
PH 70-23 25%L	18945	49080	—	—	0.39
NK-081 5%EC	160	5517	206	7103	0.029
NI-18 2.5%EC	1.2	8	2.0	13	0.16

^aRR: Resistance Ratio, (LC₅₀ of resistant strains/LC₅₀ of susceptible strain). ^b—: Not available.

Susceptibility of DBM to various insecticides

Synthetic pyrethroids and carbamates have not been used recently in Thailand, however the BBT and BK strains showed decreased susceptibility. DBM also showed a lowered susceptibility to organophosphorus compounds, but dimethylvinphos, pirimiphos methyl and phenthoate which have higher toxicity to DBM, were still effective on these strains. These results are similar to other recent reports. The relationship between structure and activity of three organophosphorus compounds was carefully investigated. The three insecticides belonged to three classes of organophosphorus compounds, indicating no structural similarity to each other. A difference in the susceptibility to BBT and BK strains was noticeable in phenthoate, but it remained similar in the other chemicals. It is not clear whether cartap was used in the area investigated, however, a certain lowering of susceptibility was observed indicating sufficient efficacy with the conventional application (Table 2). Among the two *B. thuringiensis* preparations, Dipel showed a lower susceptibility than Toarrow-CT. It is reported that Thuricide was used in Thailand, so it is necessary to look into the cause and effect in more detail.

These results confirmed that the BBT and BK strains collected in Thailand showed a multiple resistance to various insecticides.

Table 2. Susceptibility to several groups of insecticides of the larvae of susceptible (S) and resistant (BBT, BK) strains of DBM.

Insecticide	Formulation	BBT-strain		BK-strain		S-strain
		LC ₅₀ (ppm)	RR ^a	LC ₅₀ (ppm)	RR ^a	LC ₅₀ (ppm)
fenvalerate	96.3% Technical	190	100	186	98	1.9
permethrin	96.4% Technical	122	37	120	36	3.3
ethofenprop	96% Technical	421	13	435	13	33
carbofuran	97.3% Technical	177	12	219	15	15
methomyl	91% Technical	1907	10	1724	9	183
dichlorvos	99% Technical	> 1000	ND ^d	≤ 1000	ND	60
dimethylvinphos	96% Technical	133	27	147	30	4.9
trichlorfon	99.7% Technical	> 1000	ND	> 1000	ND	178
vamidothion	54.2% Technical	> 1000	ND	≤ 1000	ND	9503
fenitrothion	96.5% Technical	> 1000	ND	> 1000	ND	196
diazinon	96% Technical	> 1000	ND	≤ 1000	ND	15
salithion	95.5% Technical	> 1000	ND	< 1000	ND	20
pirimiphos methyl	91.5% Technical	138	24	89	16	5.7
EPN	92.7% Technical	> 1000	ND	> 1000	ND	693
phenthoate	Technical	88	33	303	112	2.7
prothiophos	94.1% Technical	812	677	< 1000	ND	1.2
malathion	95% Technical	> 1000	ND	> 1000	ND	251
acephate	97% Technical	> 1000	ND	< 1000	ND	37
cartap	50% SP	113	10	121	11	11
Toarrow-CT ^b	7% WP	3	3	2.9	3	0.97
Dipel ^b	10% WP	18.1	10	- ^c	-	1.77

^aRR: Resistance Ratio (LC₅₀ of resistant strains/LC₅₀ of susceptible strain). ^bTrade marks valid in Japan. ^cNot available. ^dND: Not determined.

The effect of new insecticides

Abamectin 2% WP and NC-176 10% EC showed extremely high activity against S and BBT strains. The RR for abamectin was 11, and that for NC-176, 3.4, and the concentration of conventional application was considered effective.

JHM of NC-184 showed extremely high activity against S strain. However, the inhibition concentrations for over 80% adult eclosion was 10 ppm in BBT as against 1 ppm in S strain.

This suggests that both IGRs and JHM acted similarly in the metamorphosis, resulting in the possibility of cross resistance between two. However, since NC-184 is highly active, it should work effectively at conventional concentrations.

The NC-184 is slow to produce effect because of its activity only at pupation and adult eclosion stages. For this reason, the feeding should continue until the effect begins. Regardless of its unique activity, its practical application will necessitate further research.

Synergistic effect

No synergism with addition of up to 100 ppm piperonyl butoxide (PB) was observed in a chlorfluazuron-resistant DBM strain having an RR of 405. Perng et al. (1988) have reported that PB has synergism (SR:8.8) to teflubenzuron in the teflubenzuron-resistant DBM (RR:12). In our test, however, PB did not show any synergism to chlorfluazuron in DBM having a resistance of 405. This suggests that the microsomal oxidases did not play any major role in the mechanism of chlorfluazuron resistance.

It will therefore be necessary to study the treatment method of PB and its concentration, and use of other inhibitors. Through a detailed study of synergists, some clues may be obtained about the mechanism of IGR resistance.

The mode of inheritance in IGR resistance

From the crossing tests between S and BBT strains, it was observed that the susceptibility of F₁ progeny coincided with that of S strain. Furthermore, judging from the degree of dominance ($D = -1$), it was suggested that the mode of inheritance in the IGR resistance was completely recessive. Since no difference in susceptibility in the reciprocal crossing tests was noticed in each F₁ progeny, it was confirmed that the IGR resistance is not sex linked. Also, from the dose-mortality curves of F₂ and back-crossing, it was confirmed that the inheritance originated from the monofactorial major gene (Fig. 1).

Recovery of susceptibility in absence of IGR pressure

An investigation was done on the fluctuation of susceptibility to chlorfluazuron during 40 generations. Every LC₅₀ value remained within 95% confidence limit to chlorfluazuron, and almost no sign of recovery in the susceptibility was observed (Fig 2).

The gene frequency in the population, or the relative merits of gene, is an important factor for development of IGR resistance. Such a factor has, however, almost nothing to do with the possibility for recovery of susceptibility that was once reduced. Hardy-Weinberg's law stipulates that the gene frequency in the population and the ratio of gene type do not vary even during generations. The law is supposedly applicable under the rearing conditions we used.

The recovery of susceptibility in a resistant population is presumably attributed to the case where the existential frequency of susceptible gene goes up in the population. The recovery of susceptibility is a result of inferior intrinsic rate of natural increase and inferior fitness, and the recovery is also supposedly caused by blended genes of foreign populations or by obtaining the resistant gene. Furthermore, the recovery of susceptibility largely depends upon whether the population is homogeneous or heterogeneous with regard to resistance.

From the above, we assumed that the BBT strain remained no less able for the above intrinsic rate of natural increase and fitness, regardless of the resistant gene carried by the BBT. Also it was suggested that the population in question was possibly a homogeneous group regarding resistance.

IGR-resistant DBM in Kagoshima, Japan

A problem arose in Japan in 1989 when DBM's susceptibility to IGR suddenly decreased. To study this, the susceptibility to various chitin synthesis inhibitors was examined. The MZB

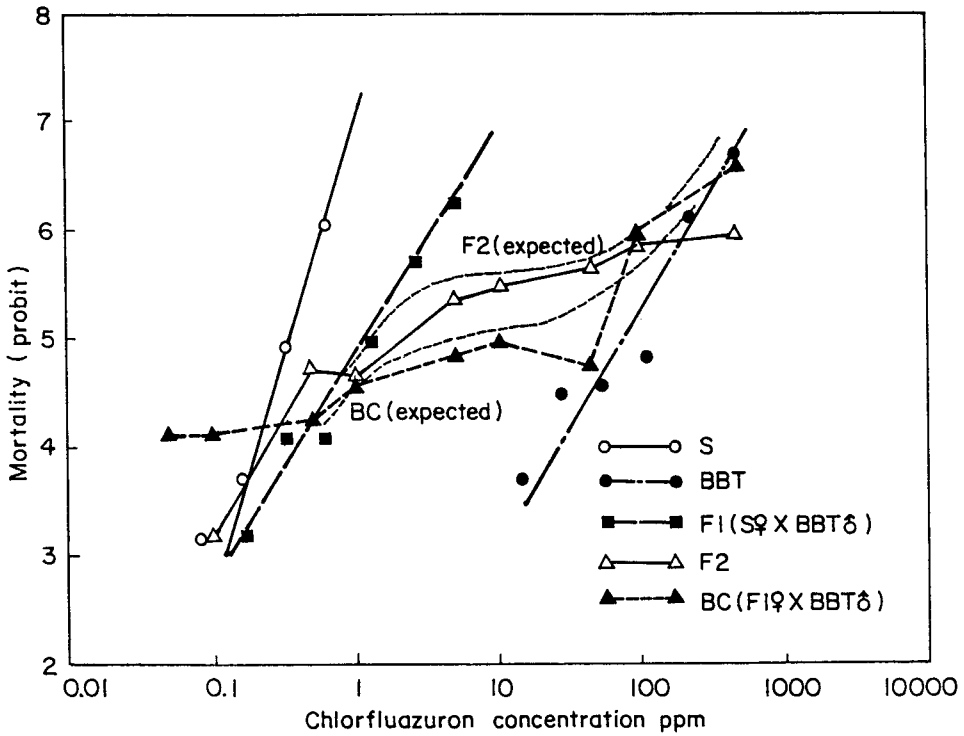


Fig. 1. Dose-mortality regression lines for chlorfluazuron against susceptible (S), resistant (BBT) strain and crosses progeny of the DBM.

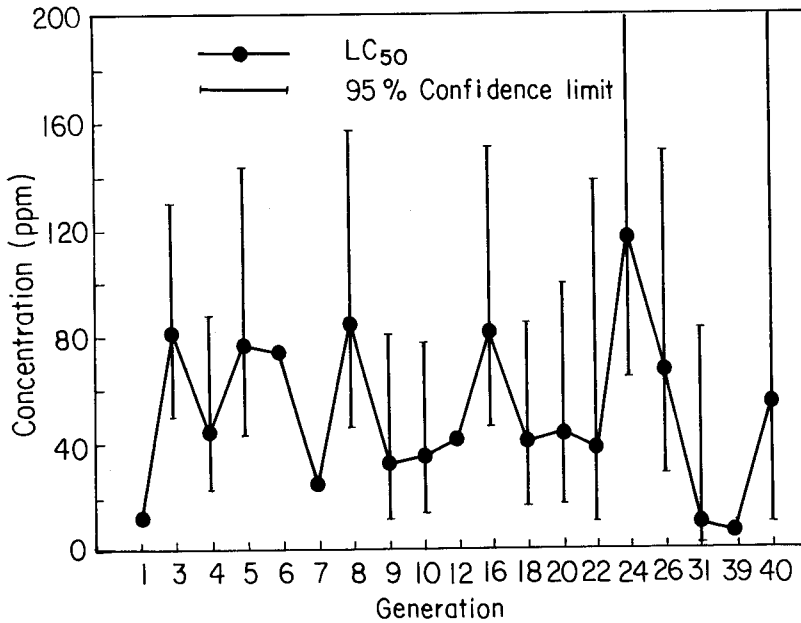


Fig. 2. Stability of resistance to chlorfluazuron of the resistant (BBT) strain of DBM upon noninsecticide pressure.

strain had a resistance ratio of 24.3 to chlorfluazuron, indicating a lowering trend of susceptibility to other IGRs as well. The resistance level in the MZB strain was below that of the BBT and BK strains. The MZB strain, however, showed a similar level of lowered susceptibility to other IGRs (Table 3).

Thus, although the IGRs are considered to be highly effective insecticides, as seen in the MZB strain, the resistance to these chemicals is an unavoidable problem.

Our study has given us new insight into IGR resistance. Although the IGR resistance was completely recessive it was supposed to develop in the parallel with the increasing insecticide pressure. As there are currently few promising replacements, some insecticides have to be used urgently to curb resistance. Based upon this information, a proper method of DBM control is urgently needed. From our test results, some conventional insecticides were found to have useful activities and rotational use of these insecticides would help avoid or delay the IGR resistance.

It may also be necessary to search for some mixture in the application of synergism. More study is needed to clarify the mechanism of DBM resistance to IGRs.

Pimprikar and Georghiou (1979, 1982) pointed out that the resistance mechanism of diflubenzuron in the housefly is collectively associated with the reduced chitin synthesis, effect of reduced cuticular penetration, increased metabolism and the rapid excretion of the chemical. In the case of DBM, the time shift for chitin synthesis is inducing the resistance, as reported by Kurihara et al. (1990). Also Perng et al. (1988) reported detoxification by microsomal oxidation, suggesting that several factors play a part in the resistance.

Table 3. Susceptibility to several chitin synthesis inhibitors of the larvae of susceptible (S) and low level-resistant (MZB) strains of DBM.

Insecticide	LC ₅₀ ppm	MZB-strain (95% CL) ^a	Slope	RR ^b	S-strain ^c LC ₅₀ ppm
chlorfluazuron 5%EC	2.43	(1.33-4.43)	1.67	24.3	0.1
diflubenzuron 23.5% WP	2285	(ND ^d)	1.34	9.2	248
hexaflumron 5% EC	2.53	(1.70-4.06)	1.87	16.9	0.15
PH 70-23 25%L	9.53	(2.10-44.3)	1.35	24.4	0.39
NK-081 5%EC	0.49	(0.17-1.38)	1.01	16.9	0.029
NI-18 2.5% EC	1.06	(0.56-2.29)	2.97	6.6	0.16

^a95% CL: 95% Confidence Limit. ^bRR: Resistance Ratio, (LC₅₀ of resistant strains/LC₅₀ of susceptible strain). ^cLC₅₀ of S-strain quoted from another source. ^dND: Not determined.

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