

## Biochemical and Physiological Characteristics of Insecticide Resistance in Diamondback Moth

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### Abstract

A pyrethroid-resistant strain of the diamondback moth, *Plutella xylostella* (L.), originally collected in Thailand showed cross-resistance to all the pyrethroids tested. Upon further selection with fenvalerate in the laboratory, the strain exhibited an extremely high LD<sub>50</sub> value, 41 µg/larva, for fenvalerate. In genetic studies of the resistance, the F<sub>1</sub> progeny showed recessive gene(s), while the B<sub>1</sub> and F<sub>2</sub> progenies had a more complex genetic nature. The in vivo dynamics of fenvalerate applied topically showed: (1) reduced cuticular penetration; (2) increased detoxification; and (3) insensitivity at the site of action being responsible for the resistance mechanism. A large portion of the increased detoxification was due to cytochrome P<sub>450</sub>-dependent monooxygenases, judging from a remarkable synergism of fenvalerate toxicity by piperonyl butoxide. The resistance was unusually unstable in the absence of selection pressure. The LD<sub>50</sub> decreased about 50% in every generation. Selection pressure once with fenvalerate or even with malathion, however, increased the LD<sub>50</sub> restoring the fenvalerate resistance. There was no significant difference in the fitness between the resistant strain and its revertant. These results may imply the presence of an unknown factor(s) necessary to maintain the insecticide resistance in diamondback moth, which cannot be explained by the conventional preadaptation theory.

### Introduction

A unique feature of insecticide resistance in the diamondback moth (DBM) (*Plutella xylostella* (L.)) (Lepidoptera: Yponomeutidae) is that the development of resistance can take place quickly. At the same time, the insect can lose resistance fairly quickly if the population is freed from insecticidal pressure. That has been the case with organophosphorus and carbamate resistance as well as with *Bacillus thuringiensis* Berliner resistance.

We also found that pyrethroid resistance in a DBM strain originally collected in Thailand was extremely unstable. The results on mechanisms, genetics, and instability of the resistance in the strain are presented below. We believe an understanding of the mechanism of disappearance of resistance is as important as understanding the mechanism of development of resistance. Both may eventually enable us to develop a method to control insecticide resistance.

### Cross-Resistance Spectrum

The resistant (R) strain used in the present paper was originally collected in Thailand in 1983, and maintained in our laboratory without exposure to insecticides for 6 months prior to the study. The R/S ratio of LC<sub>50</sub> indicated that the R strain was cross-resistant to all the

pyrethroids tested and to DDT, suggesting the involvement of KDR factor which is a common mechanism for pyrethroid and DDT resistance (Osborne and Smallcombe 1983).

The R strain further selected with fenvalerate in the laboratory showed a remarkably high LD<sub>50</sub> for fenvalerate, i.e. 41 µg/larva and the R/S ratio of 8000-fold (Table 1). The strain was resistant to some extent to the other insecticides as well.

Table 1. Resistance factor to several insecticides of the R strain after further selection with fenvalerate in the laboratory.

Compound	LD <sub>50</sub> (µg/larva)		R/S
	S	R	
Fenvalerate	0.005	41	8200
Malathion	0.19	21	110
Carbaryl	31	> 100	> 3.2
Vamidothion	> 100	> 100	-

Source:Maekoshi and Motoyama (1987).

### Mechanisms of Fenvalerate Resistance

As the first step of mechanism studies, the *in vivo* dynamics of topically applied <sup>14</sup>C-fenvalerate to the 4th instar larvae was compared between the R and S strains. The treated larvae were placed in the holding vial, and maintained with a small piece of cabbage leaf at 25°C. Volatile metabolites and CO<sub>2</sub> in the expired air were trapped and measured using a liquid scintillation counter. However, no significant radioactivity was detected from either trap at any time period investigated.

The larvae were washed with acetone to determine the external radioactivity, and then homogenized with methanol to determine the internal radioactivity. The radioactivity in the holding vial was fractionated with chloroform and water, and they were regarded as rub-off and excreta, respectively. Three different doses of fenvalerate, equivalent to LD<sub>50</sub> and LD<sub>90</sub> of the S strain and LD<sub>50</sub> of the R strain, were applied.

A difference between the strains is shown in Fig. 1. The R strain showed a slower rate of cuticular penetration and a smaller amount of radioactivity in the internal extract than the S strain. The R strain also showed a higher rate of excretion than the S strain. Similar results were observed regardless of the doses applied.

The cuticular penetration of fenvalerate was further compared between the R and S strains without the influence of excretion factor. Larvae were fixed on a piece of scotch tape after anesthetization and received <sup>14</sup>C-fenvalerate topically at four different doses. The radioactivity in the internal extract representing the amount of fenvalerate penetrated was later measured.

The results obtained are shown in Fig. 2. The left side of the graph shows that fenvalerate penetrated at a significantly slower rate in the R strain. The graph on the right of Fig. 2 shows results of a similar experiment except that cuticular wax of both strains was removed by washing the larvae with acetone prior to the application of <sup>14</sup>C-fenvalerate. The wax removal almost doubled the penetration rate as can be seen from the different scale of the ordinates, although the R/S difference persisted. The mechanism of the slower penetration therefore lies somewhere beneath the wax layer of the cuticle.

The effect of piperonyl butoxide, a methylenedioxyphenyl compound which inhibits oxidative degradation of insecticides and is used as a synergist, on the cuticular penetration of <sup>14</sup>C-fenvalerate was examined. When a mixture of <sup>14</sup>C-fenvalerate at 1:3 ratio was applied topically, the rate constant of fenvalerate penetration rather decreased in both strains, although the interstrain difference remained at about the same level. An *in vitro* measurement of penetration rate using common cutworm skin and a diffusion cell system also confirmed the conclusion (Maekoshi 1988).

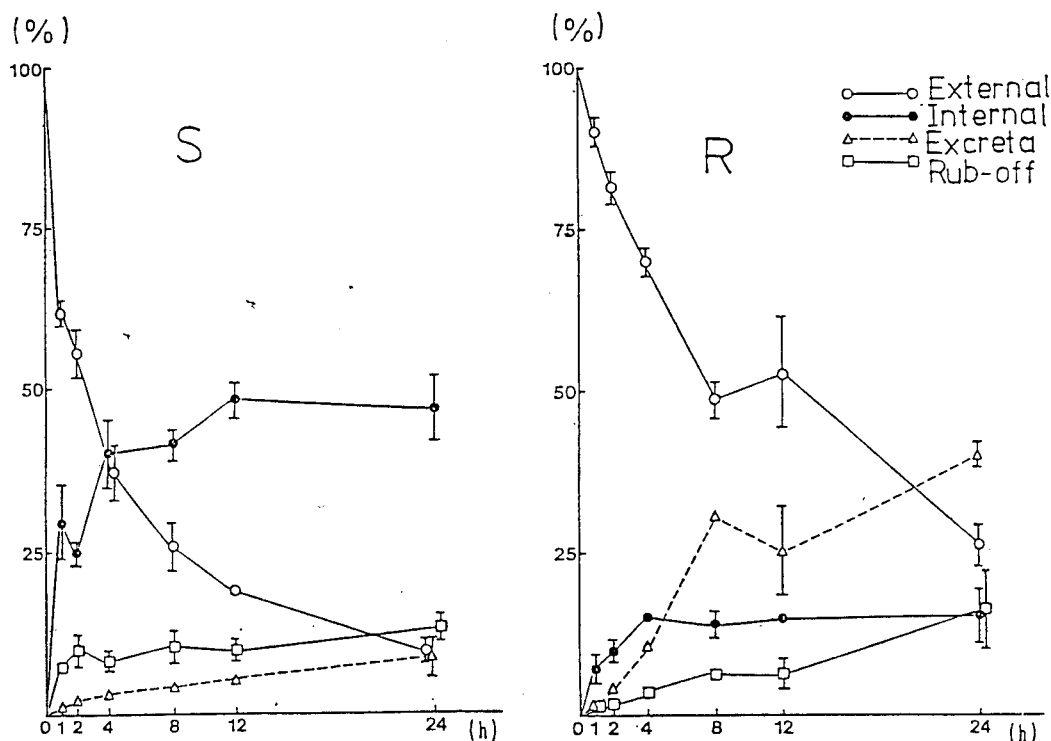


Fig. 1. In vivo dynamics of  $^{14}\text{C}$ -fenvalerate ( $25\ \mu\text{g/larva}$ ) applied topically to the S and R larvae. Source: Maekoshi and Motoyama (1987).

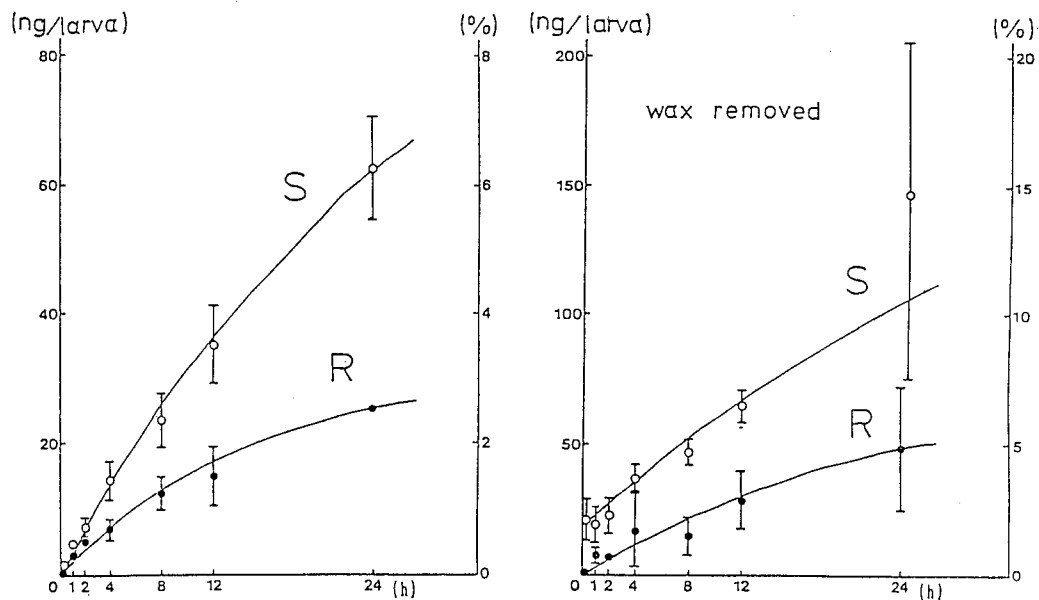


Fig. 2. Cuticular penetration of  $^{14}\text{C}$ -fenvalerate ( $1\ \mu\text{g/larva}$ ) applied topically to the S and R larvae. Cuticular wax was removed prior to the application in the right graph. Source: Maekoshi and Motoyama (1987).

The effect of piperonyl butoxide on fenvalerate toxicity to the R strain was examined. Pretreatment of the R larvae with 2.5  $\mu\text{g}$  of piperonyl butoxide resulted in a 150-fold synergism, decreasing the LD<sub>50</sub> of fenvalerate from 40 to 0.27  $\mu\text{g}/\text{larva}$ . The results suggest that increased metabolism of fenvalerate by the cytochrome P<sub>450</sub>-dependent monooxygenase system is at least in part responsible for the resistance mechanism.

The thin layer radiochromatogram of the internal extract following the application of <sup>14</sup>C-fenvalerate demonstrated the presence of several metabolites in addition to fenvalerate itself. The production of these metabolites was compared between the R and S strains. At 1 and 24 hours after the topical application of <sup>14</sup>C-fenvalerate, the total radioactivity in the internal extract was significantly less in the R strain than in the S strain as a result of less cuticular penetration. The rate of metabolite formation, however, was higher in the R strain than in the S strain, confirming the increased ability of the R strain to degrade fenvalerate.

In conclusion, the extremely high level of fenvalerate resistance in the DBM strain can be attributed to a multiplying effect of three mechanisms: (1) reduction in cuticular penetration; (2) increase in metabolism of which a large portion is mediated by the cytochrome P<sub>450</sub> monooxygenase system; and (3) insensitivity at the site of pyrethroid action which is called KDR factor.

### Genetics of Resistance

Susceptibility to fenvalerate of F<sub>1</sub>, F<sub>2</sub> and B<sub>1</sub> progenies from the S and R strains was determined. The Log Dosage-Probit line of the F<sub>1</sub> progeny derived from either crossing method was located very close to that of the S strain, suggesting the mode of genetics of fenvalerate resistance being essentially recessive (Fig. 3). A slight deviation of the LD-P line toward the right direction might be due to the presence of more than one resistance mechanism with different degrees of dominance.

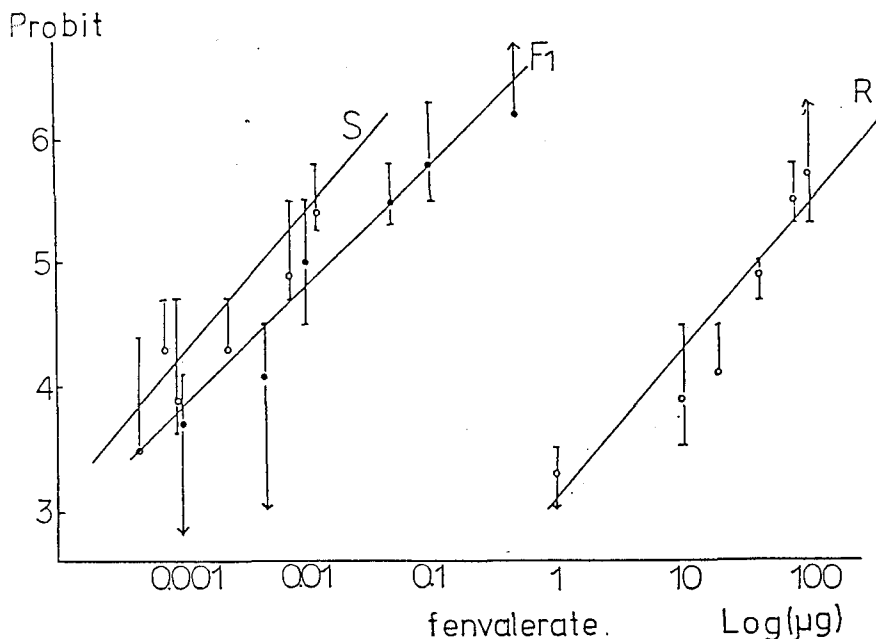


Fig. 3. The LD-P line of the F<sub>1</sub> progeny obtained by crossing S♀ × R♂. Source: Suganuma and Motoyama (1986).

The LD-P lines of the backcross progeny  $B_1$ , and  $F_2$  which is not shown here, suggested the genetics of fenvalerate resistance being of a complex nature (Fig. 4). At least it is not monofactorial. The four results may be due to either the effect of three mechanisms involved or unstable nature of fenvalerate resistance in the DBM as will be discussed later.

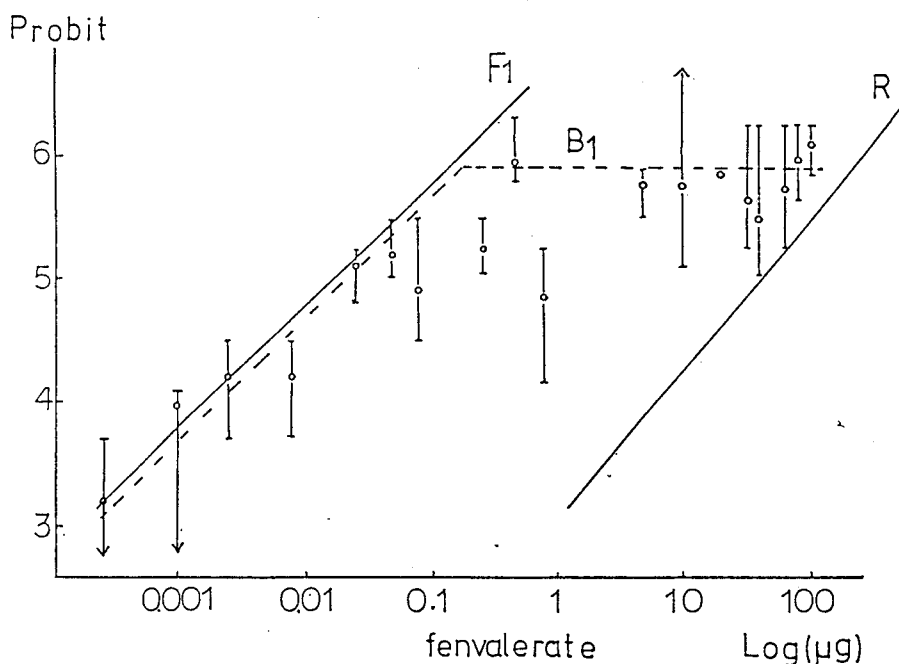


Fig. 4. The LD-P line of the  $B_1$  progeny obtained by crossing  $F_1$  ( $S\bar{\sigma} \times R\delta$ )  $\delta \times R\bar{\sigma}$ . Source: Suganuma and Motoyama (1986).

### Instability of Resistance

The R strain has been kept under successive selection with 20  $\mu\text{g}$  of fenvalerate per larva, which stabilized the  $LD_{50}$  at about the 40  $\mu\text{g}$  level. Judging from (a) the high  $LD_{50}$  value, (b) the sharp slope of the LD-P line, (c) the recessive nature of the genetics, and (d) the stability of the  $LD_{50}$ , the R strain appears to be highly homogeneous. However, Fig. 5 shows what happens with the R strain once the selection pressure is removed. As soon as the pressure was removed, the  $LD_{50}$  dropped from about 40  $\mu\text{g}$  to 20  $\mu\text{g}$ , 10  $\mu\text{g}$ , 5  $\mu\text{g}$ , and then dropped gradually thereafter.

At the 8th generation after the termination of selection, the strain was divided into two groups: one was kept without selection, and the other was selected again with 20  $\mu\text{g}$  of fenvalerate. The selection immediately increased the  $LD_{50}$  to 40  $\mu\text{g}$ , restoring the initial level of resistance, which dropped again without the selection pressure.

At the 11th generation after the termination of selection, a similar attempt was made with 0.1  $\mu\text{g}$  of fenvalerate, a dose insufficient to kill larvae of the strain. The treatment, in contrast, did not restore the resistance at all, indicating that the restoration of resistance observed with 20  $\mu\text{g}$  of fenvalerate was not due to induction, but due to intrinsic selection of resistance mechanisms.

At the 14th generation after the termination of selection, the  $LD_{50}$  became as low as 0.2  $\mu\text{g}/\text{larva}$  (Fig. 5). When the strain at this generation was selected with 50  $\mu\text{g}$  of malathion, which killed more than 50% of the larvae, the  $LD_{50}$  of fenvalerate increased almost three times. On

the other hand, the strain maintained without insecticidal pressure kept losing the resistance spontaneously, eventually reaching the level of the S strain.

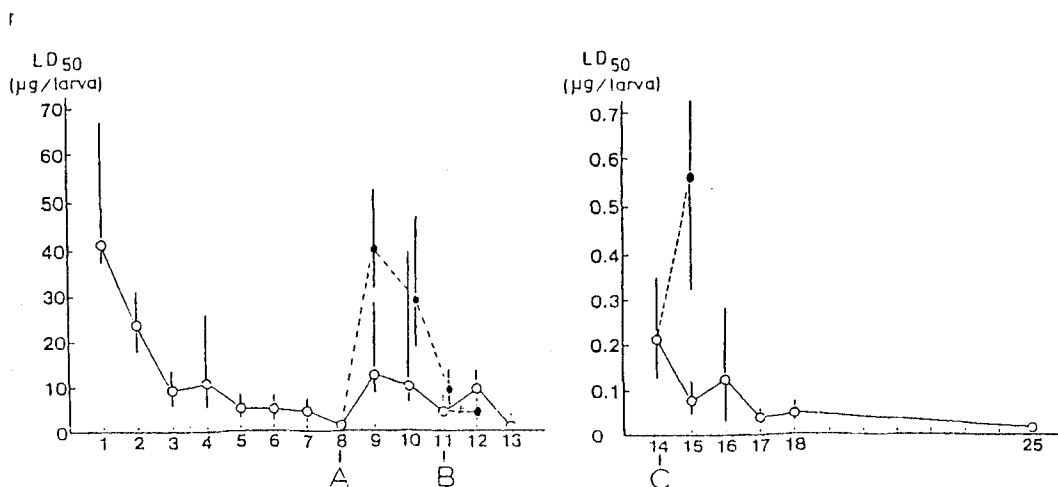


Fig. 5. Change in response to fenvalerate in the R strain following the termination of selection pressure, and the effect of resuming selection with 20 µg (A) or 0.1 µg (B) of fenvalerate or 50 µg (C) of malathion per larva. Source: Maekoshi (1988).

### Ecological Fitness

Disappearance of insecticide resistance in some cases has been attributed to a disadvantage in the ecological fitness of resistant strains (Inoue 1980). In other words, under natural conditions where there is no insecticidal pressure, the reproductive ability of a resistant strain would be inferior to that of a susceptible strain. In order to compare various factors determining the reproductive rate, two substrains were produced from the R strain in the present study. One was maintained under successive selection with 20 µg of fenvalerate, and the other was maintained without selection for 33 generations. The latter unselected R strain showed the same level of LD<sub>50</sub> as the S strain. The two substrains were reared at five different temperatures: 15, 20, 25, 30, and 35°C.

Table 2 contains a summary of data obtained at 25°C, showing no difference between the selected and unselected R strains in the following characteristics: life span of adult female, length of oviposition period, total number of eggs produced per female or per milligram weight of female, percent pupation, percent adult emergence, and length of egg period, larval period, and pupal period. Similarly, no significant difference in all of these parameters was observed between the substrains at 15, 20 and 30°C (data not shown). At 35°C, females of both substrains could lay eggs but none of them reached pupal stage.

The developmental zero and effective accumulative temperature calculated from the data showed no significant disadvantage for reproduction for the selected R strain as compared to the unselected R strain. In other words, there was no difference in the ecological fitness which can account for the spontaneous loss of fenvalerate resistance in the R strain.

### Conclusion

Although the R strain showing 8000-fold resistance to fenvalerate appeared highly homogeneous, a spontaneous loss of resistance still occurred in the absence of insecticidal

Table 2. Biological data of the R strains selected and unselected with fenvalerate.

Characteristics	R strain	
	Selected	Unselected
No. of pairs tested	11	9
Life span of female adult (days)	8.9 ± 3.5	9.9 ± 3.2
Oviposition period (days)	7.4 ± 2.9	7.8 ± 1.9
Fecundity per female	113.6 ± 3.5	115.4 ± 12.1
Fecundity per milligram weight of female	21.3 ± 5.7	25.9 ± 1.7
Pupation (%)	51.7 ± 12.1	56.7 ± 14.6
Adult emergence (%)	49.8 ± 10.8	55.6 ± 14.8
Egg period (days)	3.2 ± 0.6	3.0 ± 0.6
Larval period (days)	9.9 ± 1.8	9.9 ± 1.2
Pupal period (days)	4.8 ± 0.6	4.6 ± 0.6

Insects were reared at 25°C. Source: Maekoshi (1988).

pressure, despite the fact that there was no disadvantage in the ecological fitness. This is apparently outside the context of conventional preadaptation or postadaptation theory for resistance. The phenomenon may be best explained by assuming a possible existence of an unknown mechanism regulating the expression of resistance genes in DBM. The existence of such a mechanism has already been demonstrated with a resistant clone of the green peach aphid, *Myzus persicae*, which has amplified sequences of DNA encoding carboxylesterase production (Field et al. 1988, 1990). Understanding such a mechanism may eventually enable us to turn off the genetic switch to cancel insecticide resistance which has already developed.

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