Possible amplification of insecticide hormoligosis from resistance in the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae)

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Abstract

The possibility that insecticide hormoligosis might be amplified by resistance in the diamondback moth, *Plutella xylostella*, was tested. The fecundity of females treated with sublethal doses (LD_{12.5}, LD₂₅ and LD₅₀) of fenvalerate or methomyl in the non-selected generation increased in comparison with the control group treated with acetone. The number of eggs laid by treated females in the 10th generation of the fenvalerate-selected strain, which had developed about 10,000-fold resistance, was the same or smaller than that of the control. On the other hand, fecundity in the 8th generation of the methomyl-selected strain, which had about 4.9-fold resistance, was slightly higher in the LD_{12.5} and LD₂₅ than that of control. The intensity of insecticide hormoligosis did not depend on the dose of the insecticide although a sublethal dose of the insecticides stimulated reproduction in the susceptible DBM. Furthermore, the hormoligosis was not amplified in concert with the development of resistance in the DBM.

Key words: *Plutella xylostella*, insecticide hormoligosis, insecticide resistance, fenvalerate, methomyl

INTRODUCTION

The diamondback moth (DBM), *Plutella xylostella*, is a major pest of cabbage and other cruciferous crops throughout the world. The DBM is also known to have developed resistance to various kinds of insecticides, including organophosphates, carbamates and pyrethroids.

Resurgence after application of some insecticides has also been found in the DBM (Nemoto et al., 1984; Muckenfuss et al., 1992). According to Ripper (1956), resurgence is attributed to the following processes: (a) reduction of natural enemies along with the pest by pesticide use, (b) favorable influences of pesticide on phytophagous arthropods, and (c) removal of competitive species. The second process, so called physiological resurgence, is induced by two different mechanisms, as follows: (1) tropobiosis (Chaboussou, 1966) and (2) hormoligosis (Townsend and Luckey, 1960). Hormoligosis induced by insecticides was termed "insecticide hormoligosis" (Luckey, 1968). Nemoto (1993)

reported that the application of methomyl to DBM caused a resurgence of the insect population through the development of insecticide resistance, differential susceptibilities between the DBM and its predators, and stimulating its reproductive potential.

In the present study, three hypotheses concerned with insecticide hormoligosis were postulated and evaluated: (1) application of sublethal doses of an insecticide stimulates the reproduction of an insect; (2) intensity of hormoligosis may depend on the dose of the insecticide; and (3) resurgence will, thus, be amplified if the insect develops resistance against the insecticide. The applicability of these hypotheses to the insecticidal hormoligosis of the DBM was tested using a pyrethroid, fenvalerate, and a carbamate, methomyl, as the model insecticides.

MATERIALS AND METHODS

Insect stock culture. DBM were collected from a field at Okayama University in October, 1991.

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About 50 adults were introduced into a polypropylene cage ($15 \times 26 \times 8$ cm) for egg laying for one or two days. Hatched larvae were fed on leaves of commercial cabbage until they pupated in the same cage. Emerged adults were again introduced into the cage and the stock culture was maintained at 25°C under 16L8D. Larvae of the 25th generation after establishment of stock culture were used for the following experiment.

Determination of insecticide susceptibility. Topical application was employed to determine the insecticide susceptibility of the DBM. Various concentrations of technical-grade fenvalerate (95.3% purity) and methomyl (100% purity) were prepared with acetone. The 4th stadium larvae were anesthetized by carbon dioxide and placed on a filter paper. A $0.5 \mu\text{l}$ droplet of insecticide was applied with an Arnold hand Microapplicator (Burkard, U.K.) to the dorsum. Ten larvae treated with insecticide were placed in a plastic cup (6.5 cm in diameter, 4 cm in depth) with filter paper and a piece of cabbage leaf, and maintained at 25°C under 16L8D. Three or four replications of this treatment were done for each concentration of insecticide. Larvae in the control plot were treated with acetone. After 24 h, the numbers of dead or moribund larvae, and surviving larvae were recorded. Data were analyzed by the probit analysis (Abbott, 1925) with a personal computer.

Selection for resistant strain. Two resistant strains of the DBM were established by selection of the stock culture with fenvalerate (FR) and methomyl (MR). In order to carry out the

selection experiment, the LD_{50} values were monitored for certain generations (Table 1). The LD_{50} equivalent doses of the insecticides were applied to about 200 4th instars of every generation. The surviving larvae were reared until adult emergence and the adults were allowed to lay eggs. This procedure was repeated for 10 generations of the FR strain and 8 generations of the MR strain.

Treatment of sublethal dose. Fourth stadium larvae were treated with each insecticide at $\text{LD}_{12.5}$, LD_{25} and LD_{50} equivalent doses in the non-selected generation (FR-0) and the 10th generation (FR-10) of the fenvalerate-selected strain and the non-selected generation (MR-0) and the 8th generation (MR-8) of the methomyl-selected strain. The insects in the control plot were treated with acetone only. Surviving larvae were reared in the same cup, and allowed to pupate. The pupae were weighed on the day after pupation and were then introduced into a 20 ml glass vial covered with gauze until emergence. Upon emergence, a pair of male and female moths were introduced into the plastic cup with filter paper, absorbent cotton with 10% honey solution and a piece of cabbage leaf for egg laying. DBM adults were anesthetized with carbon dioxide for handling. The number of eggs laid were observed daily until the death of the female.

RESULTS

The biotic performances of DBM females before selection (FR-0) were compared between the sublethal dose treatments with fenvalerate and the acetone control (Table 2). Pupal weight was not significantly different between the treatments and the control. The number of eggs laid tended to be larger in the female adults treated with fenvalerate than those of the control. The females treated with $\text{LD}_{12.5}$ laid significantly more eggs than did in the control females ($p < 0.05$, *U*-test). Furthermore, there were significantly more eggs per pupal weight in all treated plots than in the control ($p < 0.05$).

In order to compare the reproductive rate between the treatment and the control, the intrinsic rate of natural increase (r_m) and finite rate of increase in a month (λ) were calculated (Birch, 1948). The values of r_m and λ in the

Table 1. Change in the susceptibility to fenvalerate (FR) and methomyl (MR) in each selected strain of the DBM

Generation	LD_{50} ($\mu\text{g}/\text{larva}$)	Resistance factor ^a
Before selection	0.007	
FR-1	0.006	0.86
FR-4	26.73	3818.57
FR-6	32.51	4644.29
FR-10	73.45	10492.86
Before selection	0.64	
MR-5	3.48	5.44
MR-8	3.12	4.88

^aResistance factor = LD_{50} of each selected generation / LD_{50} of the generation before selection.

Table 2. Comparison of biotic performances of female adults treated with various doses of fenvalerate in the non-selected generation (FR-0)

	Acetone control	LD _{12.5} (2 ng/larva)	LD ₂₅ (4 ng/larva)	LD ₅₀ (7 ng/larva)
No. of pairs	30	20	36	30
Pupal weight (mg) ^a	4.86±0.79	5.01±0.70	4.51±0.72	4.74±0.65
No. of eggs ^a	154.2±47.0	184.0±48.5*	160.9±36.1	168.0±46.8
No. of eggs/pupal weight ^a	31.5±7.1	37.1±9.7*	35.7±5.9**	35.6±9.0*
Intrinsic rate of natural increase per day (r_m)	0.203	0.245 (1.21) ^b	0.238 (1.17)	0.226 (1.11)
Finite rate of increase per month (λ)	440.22	1572.29 (3.57) ^b	1264.47 (2.87)	870.01 (1.98)

^aMean±S.D.^bFigures in parentheses indicate the rate of relative increase as compared with that of the control.** $p<0.01$, * $p<0.05$ (U -test).When value of r_m were calculated, 19, 16, 16 and 17 days as the mean developmental period of immature stages in the control, LD_{12.5}, LD₂₅ and LD₅₀, 1 : 1 as the sex ratio, and 1.0 as survival rate in the immature stages were assumed.

Table 3. Comparison of biotic performances of female adults treated with various doses of fenvalerate in the 10th generation of fenvalerate-selected strain (FR-10)

	Acetone control	LD _{12.5} (5 µg/larva)	LD ₂₅ (10 µg/larva)	LD ₅₀ (75 µg/larva)
No. of pairs	10	7	8	3
Pupal weight (mg) ^a	4.33±0.82	4.89±0.76	4.36±0.54	4.33±0.91
No. of eggs ^a	133.0±34.0	137.4±23.2	92.5±30.9*	116.7±7.0
No. of eggs/pupal weight ^a	31.6±9.7	28.4±4.0	21.3±6.9*	27.6±5.0
Intrinsic rate of natural increase per day (r_m)	0.178	0.178 (1.00) ^b	0.163 (0.92)	0.174 (0.98)
Finite rate of increase per month (λ)	208.43	207.93 (1.00) ^b	133.79 (0.64)	183.47 (0.88)

^aMean±S.D.^bFigures in parentheses indicate the rate of relative increase as compared with that of the control.* $p<0.05$ (U -test).When values of r_m were calculated, 19, 16, 16 and 17 days as the mean developmental period of immature stages in the control, LD_{12.5}, LD₂₅ and LD₅₀, 1 : 1 as the sex ratio, and 1.0 as survival rate in the immature stages were assumed.

treatment of sublethal doses were higher than in the control. The DBM population treated with fenvalerate at LD_{12.5} was estimated to reproduce up to 3.6 times more during one month than the control.

The level of resistance to fenvalerate developed rapidly in the selected strain (FR) up to the 4th generation, as shown in Table 1.

Table 3 compares the biotic performances of DBM females between the treatment with sublethal doses and the acetone control in FR-10. The number of eggs laid by the females in the LD_{12.5} treatment was similar to that in the control. The number of eggs in the LD₂₅ treatment was significantly smaller than in the control ($p<0.05$). The number of eggs per pupal weight was smaller in the LD₂₅ treatments than in the control. The values of r_m and λ at LD_{12.5}

and LD₅₀ were the same as those of the control, but the values at LD₂₅ were lower than in the control. It was noted that the parameter values for reproduction in FR-10 were considerably lower than those in FR-0 (Tables 2 and 3).

The biotic performances of DBM females before selection (MR-0) were compared between the sublethal dose treatments of methomyl and acetone control (Table 4). The number of eggs laid in the treatments with methomyl tended to be larger than in the control. In particular, the females treated with LD_{12.5} and LD₂₅ laid significantly more eggs than in the control ($p<0.05$). In addition, the number of eggs per pupal weight in the LD_{12.5} and LD₂₅ was significantly larger than in the control ($p<0.05$). The values of r_m and λ in the sublethal dose treatments of methomyl were also higher than

Table 4. Comparison of biotic performances of female adults treated with various doses of methomyl in the non-selected generation (MR-0)

	Acetone control	LD _{12.5} (0.2 µg/larva)	LD ₂₅ (0.3 µg/larva)	LD ₅₀ (0.6 µg/larva)
No. of pairs	30	45	34	43
Pupal weight (mg) ^a	4.86±0.79	4.92±0.84	4.91±0.71	4.57±0.88
No. of eggs ^a	154.2±47.0	173.0±43.3*	176.2±45.7*	158.2±46.7
No. of eggs/pupal weight ^a	31.5±7.1	35.3±7.0*	35.7±7.0*	34.5±7.0
Intrinsic rate of natural increase per day (r_m)	0.203	0.217 (1.07) ^b	0.231 (1.14)	0.213 (1.05)
Finite rate of increase per month (λ)	440.22	677.82 (1.54) ^b	1008.9 (2.29)	590.47 (1.34)

^aMean±S.D.^bFigures in parentheses indicate the rate of relative increase as compared with that of the control.* $p < 0.05$ (U -test).

When values of r_m were calculated, 19, 18, 17 and 18 days as the mean developmental period of immature stages in the control, LD_{12.5}, LD₂₅ and LD₅₀, 1 : 1 as the sex ratio, and 1.0 as survival rate in the immature stages were assumed.

Table 5. Comparison of biotic performances of female adults treated with various doses of methomyl in the 8th generation of methomyl-selected strain (MR-8)

	Acetone control	LD _{12.5} (1.0 µg/larva)	LD ₂₅ (2.0 µg/larva)	LD ₅₀ (4.0 µg/larva)
No. of pairs	24	16	26	12
Pupal weight (mg) ^a	4.89±0.36	4.93±0.52	5.19±0.70	4.64±1.06
No. of eggs ^a	150.8±38.3	159.9±37.7	158.7±47.2	130.5±33.3
No. of eggs/pupal weight ^a	30.7±7.0	32.7±8.1	30.6±8.3	28.7±6.6
Intrinsic rate of natural increase per day (r_m)	0.210	0.225 (1.07) ^b	0.212 (1.01)	0.203 (0.97)
Finite rate of increase per month (λ)	538.47	843.59 (1.57) ^b	579.34 (1.08)	437.30 (0.81)

^aMean±S.D.^bFigures in parentheses indicate the rate of relative increase as compared with that of the control.

When values of r_m were calculated, 18, 17, 18 and 18 days as the mean developmental period of immature stages in the control, LD_{12.5}, LD₂₅ and LD₅₀, 1 : 1 as the sex ratio, and 1.0 as survival rate in the immature stages were assumed.

those in the control. The DBM population treated with methomyl at LD₂₅ was estimated to reproduce 2.3 times more per one month than that of the control.

The resistant level of the MR strain against methomyl did not change notably, although the strain was selected for 8 generations (Table 1).

The biotic performances of the DBM females were compared between the sublethal dose treatments of methomyl and the acetone control in the MR-8 (Table 5). The numbers of eggs laid by the females in the LD_{12.5} and LD₂₅ groups were slightly larger than those of the control, although the number in the LD₅₀ group was less than that of the control. The numbers of eggs per pupal weight in the sublethal treatments were similar to those in the control. The values

of r_m and λ in the LD_{12.5} and LD₂₅ were slightly larger than in the control, but the values in the LD₅₀ were lower than in the control. The parameter values for reproduction in the MR-8 were not different from those in the MR-0.

DISCUSSION

Insecticide hormoligosis is known to occur in some insects, e.g., the grain weevil, *Sitophilus granarius* (Kuenen, 1958), the two-spotted spider mite, *Tetranychus urticae* (Dittrich et al., 1974), the western corn rootworm, *Diabrotica virgifera* (Ball and Su, 1979), the citrus red mite, *Panonychus citri* (Jones and Parrella, 1984), and the green peach aphid, *Myzus persicae* (Lowery and Sears, 1986). The phenomenon was especially prominent in the brown plant-

hopper, *Nilaparvata lugens* (Chelliah et al., 1980) and the DBM (Nemoto et al., 1984). These two studies involved topical application of insecticides to larvae or pupae.

In this study, the fecundity of the DBM females in the non-selected populations (FR-0 and MR-0) increased when larvae were treated with fenvalerate or methomyl at sublethal doses. We compared the r_m of females in the sublethal dose treatments of insecticides with that of females in the control as an index of the increased survival rate. The present study corroborated the results by Nemoto et al. (1984). It is apparent that the application of fenvalerate or methomyl at sublethal doses causes insecticide hormoligosis in DBM. As one of the mechanisms of this phenomenon, Roan and Hopkins (1961) mentioned that the excitation of the nervous system by an insecticide initiates a chain of events beginning with the release of neuroendocrine secretions which, in turn, induce responses in target organs. Seifert (1961) demonstrated that two oocytes of female *Panonychus ulmi* matured simultaneously when the females were kept on DDT residues, while only one oocyte matured with the control females. The mechanisms by which insect reproduction is stimulated by a sublethal dose of insecticides have not been clarified.

With regard to the second hypothesis in the present study, it was assumed that the intensity of hormoligosis may depend on the treated doses, if insecticides stimulate the reproductive organs of insects directly. Abd-Elghafar and Appel (1992) reported that fecundity in the females of the German cockroach, *Blattella germanica*, treated with chlorpyrifos increased linearly with the insecticide concentration. However, the fecundity of the DBM females did not increase in concert with the dose increase in either the non-selected or the selected strain in this study, nor was this tendency found in the results of Nemoto et al. (1984). Therefore, the second hypothesis was rejected in the present study. We conclude that the insecticide hormoligosis did not become amplified even when the DBM developed resistance against the insecticides.

The reproductive rate of the fenvalerate-selected strain (FR-10) was reduced under the

sublethal dose treatments. In addition, the reproductive rate of the selected strain (FR-10) was considerably lower than that of the non-selected DBM (FR-0), irrespective of the sublethal dose treatment and the acetone control. The resistant level in FR-10 was about 10,000-fold higher than in the FR-0. Certain physiological mechanisms have been described for the development of fenvalerate resistance in the DBM (Hama et al., 1987; Noppun et al., 1989; Yao et al., 1988; Motoyama et al., 1992). A highly specific condition, such as severe selection at every generation, may affect sensitivity to the sublethal doses of fenvalerate and also DBM viability. The strain selected by methomyl (MR-8) did not reveal a remarkable change, compared with the non-selected strain (MR-0). Although the strain (MR-8) was selected by methomyl for 8 generations, the resistance developed was moderate.

Numerous cases of insecticide hormoligosis have been reported in various combinations of arthropods and pesticides. However, the physiological mechanisms of the insecticide hormoligosis have not been elucidated. Further studies should address this issue.

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REFERENCES

- Abbott, W. S. (1925) A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18: 265-267.
- Abd-Elghafar, S. F. and A. G. Appel (1992) Sublethal effects of insecticides on adult longevity and fecundity of german cockroaches (Dictyoptera: Blattellidae). *J. Econ. Entomol.* 85: 1809-1817.
- Ball, H. J. and P. P. Su (1979) Effect of sublethal dosages of carbofuran and carbaryl on fecundity and longevity of the female western corn rootworm. *J. Econ. Entomol.* 72: 873-876.
- Birch, L. C. (1948) The intrinsic rate of natural increase of the insect population. *J. Anim. Ecol.* 17: 15-26.
- Chaboussou, F. (1966) Nouveaux aspects de la phytiairie et la phytopharmacie. Le phenomene de la trophobie. *Proc. FAO Symp. Integ. Pest. Control., Rome* 1: 33-61.
- Chelliah, S., L. T. Fabellar and E. A. Heinrichs (1980) Effect of sub-lethal doses of three insecticides on the reproductive rate of the brown planthopper, *Nilaparvata lugens*, on rice. *Environ. Entomol.* 9: 778-780.
- Dittrich, V., P. Streibert and P. A. Bathe (1974) An old case reopened: mite stimulation by insecticide residues. *Environ.*

- Entomol. 3: 534-540.
- Hama, H., Y. Kono and Y. Sato (1987) Decreased sensitivity of central nerve to fenvalerate in the pyrethroid-resistant diamondback moth, *Plutella xylostella* Linne (Lepidoptera: Yponomeutidae). *Appl. Entomol. Zool.* 22: 176-180.
- Jones, V.P. and M.P. Parrella (1984) The sublethal effects of selected insecticides on life table parameters of *Panonychus citri* (Acari: Tetranychidae). *Can. Entomol.* 116: 1033-1040.
- Kuenen, D.J. (1958) Influence of sublethal doses of DDT upon the multiplication rate of *Sitophilus granarius* (Coleopt. Curculionidae). *Entomol. Exp. Appl.* 1: 147-152.
- Lowery, D.T. and M.K. Sears (1986) Effect of exposure to the insecticide azinphosmethyl on reproduction of green peach aphid (Homoptera: Aphididae). *J. Econ. Entomol.* 79: 1534-1538.
- Luckey, T.D. (1968) Insecticide hormoligosis. *J. Econ. Entomol.* 61: 7-12.
- Motoyama, N., T. Saganuma and Y. Maekoshi (1992) Biochemical and physiological characteristics of insecticide resistance in diamondback moth. In *Diamondback Moth and Other Crucifer Pests, Proc. of the Second International Workshop* (N.S. Talekar ed.). AVRDC, Tainan, Taiwan, pp. 411-418.
- Muckenfuss, A.E., B.M. Shepard and E.R. Ferrer (1992) Natural mortality of diamondback moth in Coastal South Carolina. In *Diamondback Moth and Other Crucifer Pests, Proc. of the Second International Workshop* (N.S. Talekar ed.). AVRDC, Tainan, Taiwan, pp. 27-36.
- Nemoto, H. (1993) Mechanism of resurgence of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae). *JARQ* 27: 27-32.
- Nemoto, H., K. Kiritani and H. Ono (1984) Enhancement of the intrinsic rate of natural increase induced by the treatment of the diamondback moth (*Plutella xylostella* (L.)) with sublethal concentrations of methomyl. *Jpn. J. Appl. Entomol. Zool.* 28: 150-155 (in Japanese with English summary).
- Noppun, V., T. Saito and T. Miyata (1989) Cuticular penetration of S-fenvalerate in fenvalerate-resistant and susceptible strains of the diamondback moth, *Plutella xylostella* (L.). *Pestic. Biochem. Physiol.* 33: 83-87.
- Ripper, W.E. (1956) Effect of pesticides on balance of arthropod populations. *Ann. Rev. Entomol.* 1: 403-438.
- Roan, C.C. and T.L. Hopkins (1961) Mode of action of insecticides. *Ann. Rev. Entomol.* 6: 333-346.
- Seifert, G. (1961) Der einfluss von DDT auf die eiproduktion von *Metatetranychus ulmi* Koch (Acari, Tetranychidae). *Z. Angew. Zool.* 29: 441-452.
- Townsent, J.F. and T.D. Luckey (1960) Hormoligosis in pharmacology. *J. Am. Med. Assoc.* 173: 44-48.
- Yao, M.C., C.F. Hung and C.N. Sun (1988) Fenvalerate resistance and aldrin epoxidation in larvae of the diamondback moth. *Pestic. Biochem. Physiol.* 30: 272-278.