Rapid recovery of susceptibility under harsh environmental conditions in fenvalerate-resistant strains of the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae)

Xue Dong CHEN, Sachiyo SANADA-MORIMURA, Shin-ichi YANAGI and Fusao NAKASUJI*

Graduate School of Environmental Science, Okayama University; Okayama 700–8530, Japan

(Received 1 March 2006; Accepted 6 July 2006)

Abstract

Resistance to fenvalerate was examined in a laboratory-selected population of the diamondback moth, *Plutella xylostella*, during exposure through 10 generations. Selection results showed that LD₅₀ values rose from 0.003 µg per larva to 31.1 µg per larva and 36.7 µg per larva respectively, in replications 1 and 2 during those 10 generations. Resistant line moth eggs were significantly smaller than those of moths before selection. Therefore, we selected a subpopulation of resistant lines under harsh environmental conditions of low humidity and high temperature, over 10 generations (Harsh line) without insecticide exposure. We selected another subpopulation of resistant lines under optimal environmental conditions of high humidity and normal temperature through all 10 generations (Optimal line). After 10 generations, the LD₅₀ of fenvalerate decreased to 0.03 µg per larva and 0.02 µg per larva under harsh conditions and 0.2 µg per larva and 0.29 µg per larva under optimal conditions. The LD₅₀ values were lower for the Harsh lines than for the Optimal lines in all generations. The egg size of both lines increased gradually through 10 generations. Comparisons of these two lines showed that the egg size increased more rapidly in the Harsh lines than in Optimal lines in early (first-sixth) generations. In subsequent generations, egg sizes were nearly normal in both lines. Comparison of immature survivability of Harsh and Optimal lines reared under those conditions showed that the survivability of Harsh line individuals was significantly lower than for Optimal lines. Comparison of the two lines' survivability showed an opposite result from the comparison when they were reared under equivalent optimal conditions. These results suggest that susceptibility to insecticides might recover more quickly in Harsh lines than in Optimal lines because resistant insects with small eggs had lower survivability than susceptible insects with normal eggs. They were eliminated more rapidly under harsh environmental conditions.

Key words: Resistance; susceptible; selection; optimal environment; harsh environment

INTRODUCTION

The diamondback moth (DBM) *Plutella xylostella* is a major pest of cabbage and other cruciferous crops throughout the world (Talekar and Shelton, 1993). The DBM is also known to develop resistance to various insecticides (Miyata et al., 1986). For example, the resistance levels of DBMs selected using LD₅₀ equivalent doses of fenvalerate (a synthetic pyrethroid) for 10 generations were estimated as 4,500 times (Tsukahara et al., 2003) and 10,000 times (Sota et al., 1998) the levels before selection. On the other hand, susceptibility to insecticide apparently recovers after insecticidal se-

lection for resistant DBMs ceases (Hama, 1989). For pest insect control, it is important to determine the relationship between the development of resistance and recovery of susceptibility to insecticide.

It is generally considered that resistant genes induce antagonistic pleiotropy for reproductive traits in insects (McKenzie and Batterham, 1994; Arnaud et al., 2002); however, it is still unknown how these resistant genes are genetically correlated with the traits in resistant insects. Chen and Nakasuji (2004) showed that the eggs of resistant lines selected by sub-lethal doses of fenvalerate were significantly smaller than those of a non-selected line through eight generations in DBMs. This observa-

* To whom correspondence should be addressed at: E-mail: nakasuji@cc.okayama-u.ac.jp
DOI: 10.1303/aez.2006.641

tion led us to hypothesize that small eggs laid by resistant individuals engender low fitness of offspring without insecticide selection pressure. Consequently, a susceptible population might replace the resistant population. Egg size is considered to be a maternal effect that influences the life history traits of insects. Our previous study (Chen et al., 2006), however, suggested that egg size might affect biotic performance, e.g. survivability of premature insects, but not strongly. This result might be attributable to the fact that rearing conditions of 25°C and high humidity are optimal environmental conditions for DBMs in incubators (Fujiwara et al., 2002). Even if individuals hatched from small eggs are infirm, they can typically survive or at least live longer than under usual conditions.

Fujiwara et al. (2002) showed that small egg size caused by spraying with insecticide often decreases the survival rate of offspring under harsh environmental conditions of low humidity and high temperature. It is probable that DBM individuals experience various harsh environments in the field, e.g. during mid-summer. It is necessary to determine whether environmental conditions affect the dynamics of resistance traits and egg size to elucidate the mechanism of recovery of susceptibility to insecticides in the field.

Fenvalerate-resistant lines were established in our laboratory, and their LD_{50} values were more than 10,000 times those of susceptible DBM lines. Using those resistant lines, we examined the recovery of susceptibility to fenvalerate through 10 generations without insecticide selection under both harsh-rearing (Harsh lines) and optimal-rearing conditions (Optimal lines), and measured their egg size. Offspring of Harsh lines were also reared individually; their biotic performance was measured under optimal conditions for comparison with those of Optimal lines.

MATERIALS AND METHODS

Insect stock culture and resistant selected lines. An insecticide-susceptible strain of DBMs was obtained from a laboratory colony maintained at the Sumitomo Chemical Co., Ltd., Japan in 1998. The strain has been maintained at our laboratory at Okayama University since 1998 without insecticidal selection. Resistant lines of the DBM were established from susceptible lines through se-

lection with a sub-lethal dose of fenvalerate, LD_{50} . To conduct the selection experiment, 200 fourth-instar larvae were tested and their LD_{50} values were monitored each generation using a method described in detail by Chen and Nakasuji (2004). An LD_{50} equivalent dose of the insecticide was applied to 400 fourth-instar larvae of the new generation. The surviving larvae were reared until adult eclosion and then those adults were mated randomly. These selection treatments were continued through 10 generations. Resistance selection was replicated two times as resistant lines 1 and 2. The susceptible line was also maintained at a similar population size and density corresponding to each of the resistant lines without fenvalerate selection, but treated with acetone at every generation. The resistance ratio was calculated by the formula, LD_{50} value of resistant line/ LD_{50} value of susceptible line.

Selection under harsh and optimal environmental conditions. Reportedly, small eggs exhibit lower viability under harsh environmental conditions of lower humidity and high temperature (Fujiwara et al., 2002). Therefore, we investigated the respective dynamics of recovery of susceptibility, egg sizes, and biotic performance of "Harsh lines" and "Optimal lines" that were selected respectively under the following harsh and optimal environmental conditions.

Harsh lines. About 1000 eggs laid by resistant females of a resistant population, resistant line 1, were transferred in plastic cases (15 cm×26 cm×8 cm) and were reared until hatching under low humidity 29% (NaCl saturated solution) and normal temperature 25°C. The hatched larvae were reared under high-temperature conditions at 31°C and optimal humidity of 79% (calcium-acetate-saturated monohydrate solution). After adult eclosion, the adults were mated randomly at 25°C. About 1,000 eggs laid by adults were then selected randomly, transferred to plastic cups and reared until adult eclosion under optimal environmental conditions: humidity of 79% and normal temperature 25°C. This optimal treatment was conducted to bring the small population size under the previous harsh conditions back to a sufficiently large population. These two rearing treatments were conducted alternately through 10 generations so that the harsh condition treatment was conducted in odd generations: first, third, fifth, seventh and ninth genera-

Fig. 1. Selection procedure of "Harsh" and "Optimal" lines reared under harsh and optimal environmental conditions.

tions. The optimal condition treatment was performed in even generations: second, fourth, sixth, eighth and tenth generations (Fig. 1). This rearing group was called the Harsh line.

Optimal lines. Along with the foundation of the Harsh line, about 1,000 other eggs laid by resistant females of resistant line 1 were reared until adult eclosion under optimal conditions, as described above. Adult females were mated randomly with males under optimal conditions and subsequently laid eggs. This rearing treatment was conducted repeatedly through 10 generations. This rearing group was called the Optimal line (Fig. 1).

All individuals were reared under 16L8D. Fourth-instar larvae were also used to measure fenvalerate susceptibility in every generation in order to facilitate comparison of the two lines. The eggs laid by females of Harsh and Optimal lines were also measured on the first day of oviposition in every generation. Hatchability of eggs and survivability of immature stages were measured in odd generations and compared between the two lines. These rearing treatments and measurements were replicated using the other resistant population: Resistant line 2. These two replications of the paired lines, Harsh and Optimal lines, were designated as Replications 1 and 2.

Comparison of biotic performances of Harsh and Optimal lines reared under optimal conditions. To determine whether biotic performance was different between Harsh and Optimal lines

reared under the same optimal conditions, we selected about 200 larvae randomly from each population in Harsh and Optimal lines in the second, fourth, sixth, eighth and tenth generations. Subsequently, we transferred them individually into plastic cups (diameter 6.5 cm, height 4 cm). All were reared under optimal environmental conditions, as described above, until adult eclosion. The adult females were mated randomly with males. Eggs were laid throughout the females' lifetime under the same conditions. Their developmental time during immature stages, pupal weight, survivability of immature stage, adult longevity of females and males and the number and size of eggs were measured (Fig. 1). This treatment was not conducted in sixth and eighth generations in Replications 1 and 2, respectively, because the population size was too small.

Statistical analysis. All egg size and biotic performance data were treated separately. They were divided into odd and even generation groups to facilitate comparison between Harsh and Optimal lines because the Harsh lines were reared under different environmental conditions in odd and even generations. Egg size and biotic performance data, except survival rates and hatchability, were analyzed using three-way mixed model ANOVA (line (fixed effect) × generation (fixed effect) × replication (random effect)). Second- or first-order interactions were removed from the analytical model if they were not significant (Sokal and Rohlf, 1995).

If data of second- or first-order interactions among the three factors were significant, the data were tested by two-way or one-way ANOVA in each generation and replication (Sokal and Rohlf, 1995). Survival rates and hatchability were analyzed using logistic regression analyses (main factors: line, generation and replication). These interactions were conducted according to the procedures described above. Analyses were performed using SPSS ver. 11 (SPSS Inc., Japan).

RESULTS

The LD_{50} values of susceptible lines were about $30 \times 10^{-4} \mu\text{g}$ per larva in the tenth generation (Fig. 2). The LD_{50} values of respective resistant lines increased gradually in the course of selection with fenvalerate (Fig. 2). The values were $31.1 \mu\text{g}$ per larva and $36.7 \mu\text{g}$ per larva, respectively, in the tenth generation in resistant lines 1 and 2 (Fig. 2). Resistance ratios increased up to about 10,358 times and 12,231 times in the tenth generation of resistant lines 1 and 2, respectively. The ratios were nearly equal to those reported by Sota et al. (1998) and Tsukahara et al. (2003).

The recovery speed of susceptibility was different between two different experimental lines reared under harsh and optimal environmental conditions. The LD_{50} values of Harsh lines declined more rapidly than those of Optimal lines through all 10 generations in both Replication lines 1 and 2 (Fig. 3). Finally, the LD_{50} value of Harsh lines declined from $31.1 \mu\text{g}$ per larva and $36.7 \mu\text{g}$ per larva to $0.03 \mu\text{g}$ per larva and $0.02 \mu\text{g}$ per larva, respec-

tively, in Replications 1 and 2 in the tenth generation (Fig. 3). Those of Optimal lines declined to $0.20 \mu\text{g}$ per larva and $0.29 \mu\text{g}$ per larva in Replications 1 and 2, respectively, in the tenth generation (Fig. 3).

Reportedly, resistant females laid smaller eggs (ca. $1.10 \times 10^{-2} \text{ mm}^3$) than susceptible females (ca. $1.30 \times 10^{-2} \text{ mm}^3$) in the tenth generation selected by a sub-lethal dose of fenvalerate (Chen et al., 2006). In our study, the respective egg sizes of resistant lines 1 and 2 decreased to ca. $1.10 \times 10^{-2} \text{ mm}^3$. After fenvalerate selection, the sizes of eggs laid by resistant females increased gradually through 10 generations in both Harsh and Optimal lines in Replications 1 and 2 (Fig. 4). Since the line $\times$ generation ($F=6.39$, $p<0.01$), the generation $\times$ replication ($F=4.07$, $p<0.01$), the line $\times$ replication ($F=3.95$, $p<0.05$) interactions were signifi-

Fig. 2. LD_{50} value of fenvalerate against generation under insecticide selection.

Fig. 3. LD_{50} value of fenvalerate of Harsh and Optimal lines against generation after release from insecticide selection.

Fig. 4. Egg size of Harsh and Optimal lines against generation after release from insecticide selection.

cant in odd generations, the egg sizes were compared for Harsh and Optimal lines in each treatment and generation (Fig. 4). In the first generation, egg sizes were not significantly different between Harsh and Optimal lines in each replication ($F=2.69$ in Replication 1, $F=2.76$ in Replication 2). In the third generation, egg sizes were significantly larger in Harsh lines than in Optimal lines in both Replications 1 ($F=11.17$, $p<0.01$) and 2 ($F=11.17$, $p<0.01$). In the fifth generation, egg size was significantly larger in Harsh lines than in Optimal lines in Replication 2 ($F=21.80$, $p<0.01$) but not in Replication 1. In the seventh and ninth generations, egg sizes were not different between Harsh and Optimal lines in Replications 1 and 2. Meanwhile, the line $\times$ generations ($F=4.55$, $p<0.01$) and generation $\times$ replication ($F=10.26$, $p<0.01$) interactions were shown to be significant in the even generations, and the egg size was compared for Harsh and Optimal lines in each generation (Fig. 4). In the second, fourth and sixth generations, egg sizes were significantly larger in Harsh lines than in Optimal lines in both Replications 1 and 2 ($F=12.69$, $p<0.01$ in the second generation; $F=13.54$, $p<0.01$ in the fourth generation; $F=7.78$, $p<0.01$ in the sixth generation). In summary, the eggs of Harsh lines were significantly larger than those of Optimal lines in early generations (second, third, fourth and sixth) in both Replications 1 and 2 (Fig. 4). In the other generations (first, seventh, eighth, ninth and tenth generation), egg size was

Fig. 5. Comparison of hatchability between Harsh and Optimal lines in odd generations reared under harsh and optimal conditions, respectively.

not significantly different between Harsh and Optimal lines in Replications 1 or 2 (Fig. 4).

Hatchability (Fig. 5) and survival rates (Fig. 6) were significantly lower for Harsh lines than for Optimal lines in all odd generations when Harsh lines were reared under harsh environmental conditions, whereas Optimal lines were reared under optimal conditions (logistic regression analysis: $p<0.001$ in all cases). On the other hand, survivability was significantly higher for Harsh lines than for Optimal lines in all even generations when both Harsh and Optimal lines were reared under identical optimal conditions (logistic regression analysis: $p<0.05$ in all cases) (Fig. 7). Egg sizes of females reared individually in Harsh lines were significantly larger than those of Optimal lines in second

Fig. 6. Comparison of survivability between Harsh and Optimal lines in odd generations reared under harsh and optimal conditions, respectively.

Fig. 7. Comparison of survivability between Harsh and Optimal lines in generations reared under the same optimal conditions.

Fig. 8. Comparison of egg size between Harsh and Optimal lines in even generations reared under the same optimal conditions. Error bars indicate SE.

($F=34.30$, $p<0.01$) and fourth generations in Replication 1 ($F=22.90$, $p<0.01$). Furthermore, those of the Harsh line were larger than those of Optimal lines in the sixth generation although with only slight statistical significance ($F=3.64$, $p=0.058$). In summary, it is likely that the eggs of females reared individually in Harsh lines were also larger than those of Optimal lines in early generations (Fig. 8). The remaining indicators of biotic performance except the pupal weight of females in Replication 1 were not significantly different between Harsh and Optimal lines in both Replications 1 and 2 (Table 1).

DISCUSSION

Our experimental (DBM) strains gained an extremely high resistant rate, more than 10,000-fold, in the tenth generation under fenvalerate selection in both replications. Two major mechanisms of pyrethroid resistance are found in many resistant insects, i.e. increased metabolic detoxification of pyrethroid including fenvalerate, and structural modification of pyrethroid target sites. Cytochrome P450-dependent monooxygenases are well known to be a common mechanism of metabolic-resistant systems in various insect species including Lepidoptera, e.g. the tobacco budworm *Heliothis virescens* and the cotton bollworm *Helicoverpa armigera* (Scott, 1999). It is likely that cytochrome P450s might participate in metabolic detoxification to fenvalerate (Cheng, 1988), although it remains to be seen whether they are one of the main regulation systems of resistance in DBM. Furthermore,

the structurally modified components were observed to be sodium channel proteins of the nervous system, known as the primary target site of pyrethroid insecticide in many resistant insect species, and some of the components are termed knock-down resistant (*kdr*) or super *kdr* mutants which reduce nerve sensitivity (Soderlund and Knipple, 2003). Some point mutations causing amino acid substitutes also existed in the sodium channel protein coding regions in resistant strains of DBM (Schuler et al., 1998; Tsukakara et al., 2003; Kwon et al., 2004). The two mechanisms, i.e. metabolic detoxification and structural modification of target sites, might interact with each other, causing the level of fenvalerate resistance to become enormously high in DBM as well as other pest insects.

Some pest insects recover susceptibility to insecticides without insecticide sprays (Georgiou, 1963; Sawicki et al., 1980; Strong et al., 1990; Lenormand and Raymond, 1998; Sayyed et al., 2000); however, little information is available regarding the recovery of susceptibility of populations under various environmental conditions. In this study, the LD_{50} values of Harsh lines decreased more rapidly than those of Optimal lines through 10 generations (Fig. 3). This result indicates that resistant individuals were eliminated intensively by harsh environmental pressure. Hatchability and survivability of the immature stage were lower for Harsh lines than for Optimal lines under different environmental conditions in odd generations (Figs. 5 and 6). However, survivability was higher in Harsh lines than in Optimal lines under equivalent optimal environmental conditions in even generations (Fig. 7). These results suggest that the low humidity for eggs and high temperature for larvae and pupae strongly select against insects with less survival performance. It is likely that insects repeatedly experience harsh environmental conditions, such as low humidity or high temperature, during the summer season in the field. Resistance gene(s) are known to act as antagonistic pleiotropy for fitness traits in insects (McKenzie and Batterham, 1994; Arnaud et al., 2002). For instance, resistance genes of *Bacillus thuringiensis* (Bt) toxins affect negatively survivability over winter in the southern house mosquito *Culex pipiens* (Chevillon et al., 1997). Cytochrome P450s and glutathione S-transferase metabolic systems were observed to

Table 1. Comparison of the developmental time, pupal weight, longevity of adults and fecundity (mean $\pm$ SE) between Harsh and Optimal lines reared individually at even generations

Experimental traits		Replication 1					Replication 2					F_1	
		Generation 2	Generation 4	Generation 8	Generation 10	F_2	Generation 2	Generation 4	Generation 6	Generation 10	F_2		
Developmental time (d)	Female												
	Harsh	11.03 $\pm$ 0.09	11.52 $\pm$ 0.10	11.64 $\pm$ 0.47	11.39 $\pm$ 0.59	—	11.46 $\pm$ 0.12	11.37 $\pm$ 0.08	11.61 $\pm$ 0.08	11.34 $\pm$ 0.07	—	0.99	
	Optimal	11.61 $\pm$ 0.10	11.44 $\pm$ 0.58	11.68 $\pm$ 0.08	11.17 $\pm$ 0.06	—	11.41 $\pm$ 0.08	11.55 $\pm$ 0.08	11.42 $\pm$ 0.08	11.32 $\pm$ 0.07	—	—	
	Male												
Male	Harsh	11.23 $\pm$ 0.06	11.57 $\pm$ 0.10	11.75 $\pm$ 0.09	11.40 $\pm$ 0.09	4.24	11.54 $\pm$ 0.10	11.26 $\pm$ 0.10	11.54 $\pm$ 0.10	11.55 $\pm$ 0.09	0.38	—	
	Optimal	11.17 $\pm$ 0.10	11.60 $\pm$ 0.09	11.73 $\pm$ 0.09	11.33 $\pm$ 0.08	—	11.42 $\pm$ 0.11	11.48 $\pm$ 0.09	11.56 $\pm$ 0.10	11.33 $\pm$ 0.09	—	—	
Pupal weight (mg)	Female												
	Harsh	6.94 $\pm$ 0.13	6.71 $\pm$ 0.12	6.41 $\pm$ 0.12	6.57 $\pm$ 0.15	6.29*	6.50 $\pm$ 0.15	6.47 $\pm$ 0.10	6.39 $\pm$ 0.14	6.34 $\pm$ 0.12	0.24	—	
	Optimal	6.05 $\pm$ 0.13	6.58 $\pm$ 0.19	6.53 $\pm$ 0.12	6.34 $\pm$ 0.12	—	6.54 $\pm$ 0.14	6.51 $\pm$ 0.11	6.27 $\pm$ 0.10	6.25 $\pm$ 0.16	—	—	
	Male												
Male	Harsh	4.76 $\pm$ 0.11	4.87 $\pm$ 0.09	4.80 $\pm$ 0.12	4.71 $\pm$ 0.09	—	4.68 $\pm$ 0.10	4.88 $\pm$ 0.08	4.73 $\pm$ 0.10	4.56 $\pm$ 0.06	—	0.75	
	Optimal	4.67 $\pm$ 0.13	4.83 $\pm$ 0.15	4.72 $\pm$ 0.14	4.78 $\pm$ 0.07	—	4.95 $\pm$ 0.08	4.55 $\pm$ 0.09	4.45 $\pm$ 0.09	4.83 $\pm$ 0.07	—	—	
Longevity of adults (d)	Female												
	Harsh	6.88 $\pm$ 0.15	6.63 $\pm$ 0.14	6.18 $\pm$ 0.13	6.19 $\pm$ 0.17	—	6.25 $\pm$ 0.23	6.07 $\pm$ 0.16	6.30 $\pm$ 0.29	6.10 $\pm$ 0.23	—	2.05	
	Optimal	6.56 $\pm$ 0.13	6.69 $\pm$ 5.58	6.09 $\pm$ 0.15	6.03 $\pm$ 0.12	—	6.78 $\pm$ 0.23	6.57 $\pm$ 0.22	6.00 $\pm$ 0.29	6.31 $\pm$ 0.27	—	—	
	Male												
Male	Harsh	6.83 $\pm$ 0.21	6.26 $\pm$ 0.15	6.25 $\pm$ 0.18	6.75 $\pm$ 0.13	—	6.50 $\pm$ 0.26	6.50 $\pm$ 0.22	6.50 $\pm$ 0.22	6.25 $\pm$ 0.23	—	0.09	
	Optimal	6.78 $\pm$ 0.12	6.47 $\pm$ 0.16	6.52 $\pm$ 0.23	6.30 $\pm$ 0.10	—	6.50 $\pm$ 0.27	6.57 $\pm$ 0.29	6.30 $\pm$ 0.26	6.68 $\pm$ 0.21	—	—	
Fecundity	Female												
	Harsh	221.7 $\pm$ 18.8	228.4 $\pm$ 54.3	188.8 $\pm$ 5.6	198.4 $\pm$ 10.7	—	201.1 $\pm$ 7.4	211.2 $\pm$ 7.2	208.4 $\pm$ 0.1	198.5 $\pm$ 8.2	—	0.02	
	Optimal	221.2 $\pm$ 36.1	232.8 $\pm$ 7.6	185.6 $\pm$ 10.5	197.2 $\pm$ 8.4	—	203.1 $\pm$ 8.5	209.4 $\pm$ 8.2	221.5 $\pm$ 8.8	199.2 $\pm$ 7.3	—	—	

F_1 : F value of line's effect (Harsh and Optimal) by three-way ANOVA (line $\times$ replication $\times$ generation), F_2 : F value of line's effect (Harsh and Optimal) by two-way ANOVA (line $\times$ generation) in Replications 1 and 2, * indicates $p < 0.05$.

show major xenobiotic detoxification against diflubensuron in the codling moth *Cydia pomonella*, and its resistant genes decreased in the field population after absence of the insecticide, which suggests that resistance has a fitness cost (Boivin et al., 2003). Nerve-insensitive resistance was also associated with a fitness cost in *H. virescens* (McCaffery et al., 1999). Foster et al. (2003) showed that single point mutations conferring *kdr* genes modify the behavior of resistant insects and result in deleterious pleiotropic effects on the insects in the peach-potato aphid *Myzus persicae* and the house fly *Musca domestica*. It was reported, however, that *kdr* genes do not have high fitness costs in the field population of DBM in Korea (Kwon et al., 2004).

The results of this study, suggest that resistant genes are correlated with miniaturization of eggs and induce a major fitness costs under harsh environmental conditions. Although P450-dependant metabolic systems are still unknown to operate antagonistic effects on resistance in DBM, the system has many functional roles, such as development, growth, activity and so on in several insect species (Scott, 1999). It is interesting to investigate the dynamics of resistance genes concerning P450s in DBM resistant population to understand the mechanisms of rapid recovery of susceptibility in the field.

Fitness costs associated with resistant genes in the absence of insecticides are observed for numerous characteristics including life-history traits (Carrière et al., 1994; Hollingsworth et al., 1997; Alyokhin and Ferro, 1999), diapause induction (Carrière et al., 1995), mating ability (Alyokhin and Ferro, 1999), and predator avoidance (Rowland, 1991). Our previous study (Chen and Nakasuji, 2004) showed that eggs were miniaturized by insecticide selection in DBMs. Furthermore, egg size was a maternal inheritable trait (Chen et al., 2006). Small eggs reportedly correlated with low viability of offspring under harsh environmental conditions such as low humidity and high temperature (Fujiwara et al., 2002). In this study, the line $\times$ generation interactions of egg size were shown to be significant in both odd and even generations. The results showed that the rate of size recovery was different between Harsh and Optimal lines. In summary, the egg sizes of Harsh lines increased more rapidly than those of Optimal lines through early selections (second, third, fourth and

sixth generations; Fig. 4). Low humidity and high temperature should select against insects of small egg size. Egg sizes were not considerably different between Harsh and Optimal lines in later generations (fifth, seventh, eighth, ninth and tenth generations; Fig. 4) because the egg sizes of both lines were closer to that of the original susceptible population and also reached a plateau in later generations. Although it remains unclear whether close genetic relationships exist between resistance and egg size (Chen et al., 2006), resistant individuals hatched from small eggs are eliminated quickly from resistant populations under harsh environmental conditions. Therefore, susceptibility to insecticides recovered more quickly in Harsh lines than in Optimal lines in early generations free from insecticide pressure.

Miniaturization of eggs was repeatedly observed in DBM resistant populations in several studies (Fujiwara et al., 2002; Chen and Nakasuji, 2004; Chen et al., 2006) as in our study. The DBM individuals used in these studies fundamentally originated from an insecticide-susceptible population maintained at Sumitomo Chemical Co. Ltd., although the stock cultures were established at different times and insecticide selections were conducted independently among these studies. Thus, to determine whether antagonistic pleiotropy is a general phenomenon in DBM around the world, it is necessary to investigate the relationship between resistance level and egg size in DBM originating from some other populations.

The remaining indicators of biotic performance (developmental time, pupal weight, fecundity and adult longevity), aside from the pupal weight of females in Replication 1, were not different for Harsh and Optimal lines (Table 1). Little difference in these biological parameters might be attributable to the fact that insects were reared under optimal conditions where they were able to develop sufficiently. We should compare traits between Harsh and Optimal lines when insects of both lines are reared under harsh environmental conditions opposite to those applied in this experimental treatment.

It is probable that great resistance to insecticide in the insect population is generally associated with reduced fitness, particularly under harsh environmental conditions. This study suggested that the survival cost of resistance increases under harsh environmental conditions in the absence of insecti-

cides (Crow, 1957). This knowledge of resistance costs would be useful for designing management strategies against resistance.

ACKNOWLEDGEMENTS

We thank Professor H. Tsumuki, Dr. T. Miyatake, Dr. K. Matsuura and colleagues at Okayama University for their assistance with this work. Thanks are also extended to Sumitomo Chemical Co., Ltd. for providing technical-grade fenvalerate. This work was supported partly by Grants-in-Aid for Scientific Research (Kakenhi) (14360029, 14606001).

REFERENCES

- Alyokhin, A. V. and D. N. Ferro (1999) Relative fitness of colorado potato beetle (Coleoptera: Chrysomelidae) resistant and susceptible to the *Bacillus thuringiensis* Cry 3A toxin. *J. Econ. Entomol.* 92: 510–515.
- Arnaud, L., Y. Brostaux, L. K. Assié, C. Gaspar and E. Haubruge (2002) Increased fecundity of malathion-specific resistant beetles in absence of insecticide pressure. *Heredity* 89: 425–429.
- Boivin, T., J. C. Bouvier, T. Chadoeuf, D. Beslay and B. Sauphanor (2003) Constrains on adaptive mutations in the codling moth *Cydia pomonella* (L.): measuring fitness trade-offs and natural selection. *Heredity* 90: 107–113.
- Carrière, Y., J. P. Deland, D. A. Roff and C. Vincent (1994) Life-history costs associated with the evolution of insecticide resistance. *Proc. R. Soc. Lond. B* 258: 35–40.
- Carrière, Y., D. A. Roff and J. P. Deland (1995) The joint evolution of diapause and insecticide resistance: a test of an optimality model. *Ecology* 76: 1497–1505.
- Chen, X. D. and F. Nakasuji (2004) Diminished egg size in fenvalerate resistant strains of the diamondback moth *Plutella xylostella* (Lepidoptera: Yponomeutidae). *Appl. Entomol. Zool.* 39: 335–341.
- Chen, X. D. and S. Sanada-Morimura, S. Yanagi and F. Nakasuji (2006) Genetic relationships between development of insecticide resistance and reduction of egg size as a negative effect on fitness in the diamondback moth *Plutella xylostella* (Lepidoptera: Yponomeutidae). *Appl. Entomol. Zool.* 41: 479–486.
- Cheng, E. Y. (1988) Problems of control of insecticide resistant *Plutella xylostella*. *Pestic. Sci.* 23: 177–188.
- Chevillon, C., D. Bourguet, F. Rousset, N. Pasteur and M. Raymond (1997) Pleiotropy of adaptive changes in populations: comparisons among insecticide resistance genes in *Culex pipiens*. *Genet. Res.* 70: 195–204.
- Crow, J. F. (1957) Genetics of insect resistance to chemicals. *Annu. Rev. Entomol.* 2: 227–246.
- Foster, S. P., S. Young, M. S. Williamson, I. Duce, I. Denholm and G. J. Devine (2003) Analogous pleiotropic effects of insecticide resistance genotype in peach-potato aphids and houseflies. *Heredity* 91: 98–106.
- Fujiwara, Y., T. Takahashi, T. Yoshioka and F. Nakasuji (2002) Changes in egg size of the diamondback moth *Plutella xylostella* (Lepidoptera: Yponomeutidae) treated with fenvalerate at sublethal doses and viability of the eggs. *Appl. Entomol. Zool.* 37: 103–109.
- Georghiou, G. P. (1963) Comparative of susceptibility to insecticides of two green peach aphid populations, collected 16 years apart. *J. Econ. Entomol.* 53: 655–657.
- Hama, H. (1989) Characteristics of development of pyrethroid resistance in the diamondback moth, *Plutella xylostella*. *Bull. Chugoku Agric. Exp. Sta.* 4: 119–134 (in Japanese with English summary).
- Hollingsworth, R. G., B. E. Tabashnik, M. W. Johnson, R. H. Messing and D. E. Ullman (1997) Relationship between susceptibility to insecticides and fecundity across populations of cotton aphid (Homoptera: Aphididae). *J. Econ. Entomol.* 90: 55–58.
- Kwon, D. H., B. R. Choi, H. M. Park, S. H. Lee, T. Miyata, J. Marshall and S. H. Lee (2004) Knockdown resistance allele frequency in field population of *Plutella xylostella* in Korea. *Pestic. Biochem. Phys.* 80: 21–30.
- Lenormand, T. and M. Raymond (1998) Resistance management: the stable zone strategy. *Proc. R. Soc. Lond. B* 265: 1985–1990.
- McCaffery, A. R., D. J. Head, T. Jiangul, A. A. Dubbeldam, R. Vadakanthara, V. R. Subramaniam and A. Callaghan (1999) Nerve insensitivity resistance to pyrethroids in heliothine Lepidoptera. *Pestic. Sci.* 51: 315–320.
- McKenzie, J. A. and P. Batterham (1994) The genetic, molecular and phenotypic consequences of selection for insecticide resistance. *Trends Ecol. Evol.* 9: 166–169.
- Miyata, T., T. Saito and V. Noppun (1986) Studied on the mechanism of diamondback moth resistance to insecticide. In *Diamondback Moth Management* (N. S. Talekar and T. D. Griggs eds). Asian Vegetable Research and Development Center, Shanhua, Taiwan, pp. 347–357.
- Rowland, M. (1991) Behaviour and fitness of HCH/dieldrin resistant and susceptible female *Anopheles gambiae* and *An. stephensi* mosquitoes in the absence of insecticides. *Med. Vet. Entomol.* 5: 193–206.
- Sawicki, R. M., A. L. Devonshire, R. W. Payne and S. M. Petzing (1980) Stability of insecticide resistance in the peach potato aphid, *Myzus persicae* (Sulzer). *Pestic. Sci.* 11: 33–42.
- Sayyed, A. H., J. Ferre and D. J. Wright (2000) Mode of inheritance and stability of resistance to *Bacillus thuringiensis* var. *kurstaki* in a diamondback moth (*Plutella xylostella*) population from Malaysia. *Pest Manag. Sci.* 56: 743–748.
- Schuler, T. H., D. Martinez-Torres, A. J. Thompson, I. Denholm, A. L. Devonshire, I. R. Duce and M. S. Williamson (1998) Toxicological, electrophysiological and molecular characterization of knockdown resistance to pyrethroid insecticide in the diamondback moth, *Plutella xylostella* (L.). *Pestic. Biochem. Phys.* 59: 169–182.
- Scott, J. G. (1999) Cytochromes P450 and insecticide resistance. *Insect Biochem. Mol. Biol.* 29: 757–777.
- Soderlund, D. M. and D. C. Knipple (2003) The molecular biology of knockdown resistance to pyrethroid insecticides. *Insect Biochem. Mol. Biol.* 33: 563–577.
- Sokal, R. R. and F. J. Rohlf (1995) *Biometry*. 3rd ed. W. H. Freeman and company, New York. 887 pp.
- Sota, N., N. Motoyama, K. Fujisaki and F. Nakasuji (1998) Possible amplification of insecticide hormoligosis from

- resistance in the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae). *Appl. Entomol. Zool.* 33: 435–440.
- Strong, C. A., P. G. Koehler and R. S. Patterson (1990) Insecticide resistance decline and selection in laboratory-reared German cockroaches (Dictyotera: Blattellidae). *J. Econ. Entomol.* 90: 183–187.
- Talekar, N. S. and A. M. Shelton (1993) Biology, ecology and management of the diamondback moth. *Annu. Rev. Entomol.* 38: 275–301.
- Tsukahara, Y., S. Sonoda, Y. Fujiwara, F. Nakasuji and H. Tsumuki (2003) Molecular analysis of the *para*-sodium channel gene in the pyrethroid-resistant diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae). *Appl. Entomol. Zool.* 38: 23–29.