Prolonged Effect on Pupae and Adults of the Diamondback Moth,
Plutella xylostella (L.) (Lepidoptera: Yponomeutidae)
after Larval Treatment with Chlorfluazuron¹

Adel Ramzy FAHMY and Tadashi MIYATA

*Laboratory of Applied Entomology and Nematology, Faculty of Agriculture,
Nagoya University, Chikusa-ku, Nagoya 464-01, Japan*

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The prolonged effect of larval treatment with chlorfluazuron on pupation and adult emergence in the diamondback moth (DBM), *Plutella xylostella* (L.) was investigated. Two chlorfluazuron resistant and two susceptible strains of DBM were tested. The leaf-dipping technique was adopted and the insecticide was applied to third instar larvae. Larval mortalities were counted one week after application then pupated larvae were observed until adult emergence. Results indicated that chlorfluazuron applied to larvae exhibited a prolonged effect on pupae observed as pupal death or failure of emergence and on adults observed as deformed or dead adults. The failure of emergence was more obvious in the chlorfluazuron resistant strains, which were exposed to higher doses, than in the susceptible strains.

Key words: prolonged effect, chlorfluazuron, diamondback moth, pupation, adult emergence

INTRODUCTION

Since the discovery of the insecticidal activity of benzoylphenyl ureas (BPUs) with the first commercial compound, diflubenzuron, many improvements have been made leading to the development of more potent chitin synthesis inhibitors such as teflubenzuron and chlorfluazuron, which have proved more effective than diflubenzuron on various agricultural pests (RETNAKARAN, 1982; NEUMANN and GUYER, 1987; GAZIT et al., 1989).

During laboratory selection of the diamondback moth (DBM), *Plutella xylostella* (L.) with chlorfluazuron we noticed that treatment of the third instar larvae with a high concentration of chlorfluazuron (more than 150 ppm) has caused about 50% mortality in the pupal stage, and even many of the successfully pupated insects could not emerge to normal adults (FAHMY et al., 1991).

In this study we investigated the prolonged effect of larval treatment with chlorfluazuron on pupation and normal adult emergence in chlorfluazuron susceptible and resistant strains of DBM.

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MATERIALS AND METHODS

Insects. Two chlorfluazuron resistant and two susceptible strains of DBM were used in this study. The resistant strains were derived from the susceptible strains, namely TL and BK strains, by laboratory selection with chlorfluazuron and their resistance ratios were 93 and 124 fold for the TL and BK strains, respectively (FAHMY et al., 1991). The susceptible strains were obtained by laboratory rearing of field collected strains, which were collected at Tup Luang and Bang Khae, Thailand, and had been reared in the laboratory for two years without exposure to any insecticidal pressure.

The insects were reared at 25°C under long day photoperiod (16L-8D). The rearing technique used here was the same as that of FAHMY et al. (1991). Eggs were laid on radish seedlings and larvae were reared on the same seedlings until the second instar. After that larvae were fed on cabbage leaves until pupation.

Insecticide testing technique and statistical methods. Chlorfluazuron 5% EC (obtained from Ishihara Sangyo Kaisha Ltd., Osaka) was used in this study. The leaf dipping technique was adopted as reported previously (FAHMY et al., 1991). Cabbage leaves (5×15 cm) were dipped for 10 s in chlorfluazuron solution containing 200 ppm spreading agent (Linoh®, Nihon Noyaku Co. Ltd., Osaka) then left to air dry. Treated leaves were then introduced into a paper padded plastic cup (500 ml) and 25 third instar larvae were placed on each leaf. For control tests cabbage leaves were dipped in distilled water containing spreading agent only. For each concentration four replicates were made as well as for the controls. Larval mortality was counted one week after application then normal pupae were observed until adult emergence. Results were analyzed by the probit analysis (FINNEY, 1971).

RESULTS

The results indicating the cumulative effect of chlorfluazuron on larvae, pupae and adults of chlorfluazuron susceptible and resistant TL and BK strains of DBM are shown in Tables 1 and 2.

It is clear that chlorfluazuron exerts an extended effect throughout the pupal and adult stages, after being applied to the larvae. This effect was very clear from the LC_{50} values in both resistant and susceptible strains. Concerning the chlorfluazuron susceptible TL strain, the LC_{50} values until pupation, until adult emergence, and until adult emergence+dead and deformed adults were 1.023, 0.785 and 0.614, respectively. Those for the chlorfluazuron resistant TL strain were 168.6, 90.36 and 71.45, respectively (Table 1). Concerning the susceptible BK strain, the LC_{50} values were 0.571, 0.358 and 0.278, respectively. On the other hand, those for the resistant BK strain were 243.2, 63.39 and 49.88, respectively (Table 2).

It was interesting to recognize that there were remarkable differences in the resistance ratio (RR) values (LC_{50} of the resistant strain/ LC_{50} of the susceptible strain) until pupation, until adult emergence and those until adult emergence+dead and deformed adults (Tables 1 and 2).

Comparison of the log LC_{50} values of the two susceptible and the two resistant strains indicated that for both resistant strains the values of log LC_{50} until pupation-log LC_{50} until adult emergence were considerably higher than those of the susceptible

Table 1. Cumulative susceptibility levels to chlorfluazuron at different developmental stages of the chlorfluazuron resistant and susceptible TL strains of DBM^a

Criterion	Susceptible strain		Resistant strain		RR ^b
	LC ₅₀ (ppm) (95% conf. int.)	Slope	LC ₅₀ (ppm) (95% conf. int.)	Slope	
Until pupation	1.023 (0.777–1.367)	0.962	168.6 (148.7–189.7)	2.06	165
Until adult emergence	0.785 (0.579–1.079)	0.920	90.36 (81.15–101.9)	2.37	115
Until adult emergence + dead and deformed adults	0.614 (0.456–0.843)	0.949	71.45 (64.98–79.56)	3.17	116

^a Control mortalities until adult emergence did not exceed 2% in all experiments.

^b RR=resistance ratio (LC₅₀ of the resistant strain/LC₅₀ of the susceptible strain).

Table 2. Cumulative susceptibility levels to chlorfluazuron at different developmental stages of the chlorfluazuron resistant and susceptible BK strains of DBM^a

Criterion	Susceptible strain		Resistant strain		RR ^b
	LC ₅₀ (ppm) (95% conf. int.)	Slope	LC ₅₀ (ppm) (95% conf. int.)	Slope	
Until pupation	0.571 (0.405–0.839)	0.773	243.2 (198.4–290.0)	1.36	426
Until adult emergence	0.358 (0.255–0.528)	0.829	63.39 (55.41–73.88)	1.96	177
Until adult emergence + dead and deformed adults	0.278 (0.199–0.412)	0.867	49.89 (44.07–57.53)	2.40	179

^a Control mortalities until adult emergence did not exceed 2% in all experiments.

^b RR=resistance ratio (LC₅₀ of the resistant strain/LC₅₀ of the susceptible strain).

strains (for the susceptible TL strain 0.114, and for the resistant TL strain 0.272; for the susceptible BK strain 0.203 and for the resistant BK strain 0.584). On the other hand there was no significant differences in the values of log LC₅₀ until adult emergence–log LC₅₀ until adult emergence + dead and deformed adults between susceptible and resistant strains (for the susceptible TL strain 0.107, and for the resistant TL strain 0.102; for the susceptible BK strain 0.110 and for the resistant BK strain 0.104) (Table 3).

DISCUSSION

Chlorfluazuron, a benzoylphenyl urea compound, is mainly effective against lepidopterous insect pests disrupting chitin formation and mainly effective against immature insects with relatively slow but strong action (HASHIZUME, 1988).

In this study we detected that treatment of DBM larvae with chlorfluazuron did not only exert its action as larval deaths but also there was an extended effect on success-

Table 3. Prolonged effect of chlorfluazuron on larvae, pupae and adults of the chlorfluazuron resistant and susceptible TL and BK strains of the DBM

Criterion ^a	Susceptible strains		Resistant strains	
	Log LC ₅₀	Difference	Log LC ₅₀	Difference
TL strain				
A	0.0098	→ 0.114	2.227	→ 0.272
B	-0.105		1.956	
C	-0.212	→ 0.107	1.854	→ 0.102
BK strain				
A	-0.243	→ 0.203	2.386	→ 0.584
B	-0.446		1.802	
C	-0.556	→ 0.110	1.698	→ 0.104

^a A: Until pupation, B: Until adult emergence, C: Until adult emergence+dead and deformed adults.

fully pupated treated larvae recognized as pupal death and failure of emergence and even some adult deaths or deformations.

Although it might be expected that chitin synthesis inhibitors affect insect growth and development, this effect has not been thoroughly investigated in DBM. KOHYAMA (1986) has reported that treatment of DBM larvae at the final instar with teflubenzuron has resulted in inhibited reproduction of the adults derived from treated larvae. On the other hand, there are some reports on the prolonged effects of BPUs on other insect species. ISHAAYA and YABLONSKI (1987) have reported a considerable decrease in adult emergence of *Tribolium castaneum* directly proportional with chlorfluazuron concentrations applied to the 4th larval instar.

Our results agree with those stated by GAZIT et al. (1989) that in assays with chlorfluazuron carried out on the 4th instar larvae of *T. castaneum* the LC₅₀ value until emergence were much lower than that until pupation, and more than 90% pupae-adult intermediates were observed. The same authors have stated that the unchanged level of chlorfluazuron transported into the pupae was almost 40% of the original amount applied to the 5th instar larvae which also explains the high potency of chlorfluazuron during the pupal stage.

The reason of this phenomenon as confirmed strongly by many investigators is due to the characteristic stability of chlorfluazuron to metabolic detoxification during the larval and pupal stages and its long half-life in insect bodies. NEUMANN and GUYER (1983) stated that in *Spodoptera littoralis* larvae the better toxicological performance of chlorfluazuron in comparison to diflubenzuron was correlated with its longer half-life. Also the abnormal adults observed in *T. castaneum* after larval treatment with chlorfluazuron were owed to its stability to metabolic detoxification during larval and pupal stages (ISHAAYA et al., 1987) which also caused a significant decrease in pupation and emergence ratios (ISHAAYA and YABLONSKI, 1987).

Many other reports have stated similar observations in *Heliothis virescens* and *S. littoralis* (GUYER and NEUMANN, 1988); in *T. castaneum* (GAZIT et al., 1989; ISHAAYA, 1990) and in *Spodoptera frugiperda* (ROSS and BROWN, 1982).

An interesting issue for discussion now arises as "Is there any relation between chlorfluazuron resistance level of DBM and the potency of the prolonged action of chlorfluazuron?"

Table 3 shows apparently the differences in the log LC₅₀ values of the chlorfluazuron susceptible and resistant strains. The values of log LC₅₀ until pupation-log LC₅₀ until adult emergence were significantly higher in the resistant strains than in the susceptible strains. On the other hand regarding the values of log LC₅₀ until adult emergence-log LC₅₀ until adult emergence+dead and deformed adults, there was no significant difference between the susceptible and the resistant strains. Also it was observed that the LC₅₀ doses until pupation of both susceptible strains have caused 15% and 28% pupal death or failure, while the LC₅₀ values until pupation of both resistant strains have caused 85% and 90% pupal death or failure in the TL and BK strains, respectively. These observations indicate that at the equitoxic dose (LC₅₀ level) there was clear evidence supporting the hypothesis of stronger prolonged effect with increasing chlorfluazuron resistance level. However, the concentrations used in testing the resistant strains could not be applied to the susceptible strains due to the large difference in their susceptibility levels, so that the relation between prolonged effect and resistance level to chlorfluazuron was not conclusive.

Further more this may reveal that selection pressure applied to the larvae has mainly led to the development of resistance to chlorfluazuron in this developmental stage, which unlikely to take place in the pupal or adult stages.

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