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Effects of a Nonsteroidal Ecdysteroid Agonist on Survival and Development of the Diamondback Moth, *Plutella xylostella*

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ABSTRACT

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Effects of a nonsteroidal ecdysteroid agonist, *viz.*, tebufenozide (RH5992), on survival and development of the diamondback moth, *Plutella xylostella*, were investigated by feeding larvae on treated kale leaves. This compound was effective to cause larval mortality only when treating various instars with concentrations over 400 ppm. Although almost 100% cumulative mortality was obtained by treating 1st through 3rd instars with 400 ppm, the 4th instar was slightly lowered down to 87.5% with 400 ppm but maintained 97.5% with 800 ppm. The LC_{50} values were 96.3, 95.3, 105.8 ppm for 1st, 2nd and 3rd instars, respectively. The LT_{50} of 4th instar ranged from 3.4 to 1.7 days at 100 to 800 ppm while it ranged from 2.3 to 12.3 days for the other instars. The leaf consumption per larva was lower in treatments with 100 ppm or above than the untreated control. The pupal weight, the percentage of emergence, adult longevity and fecundity were affected when newly hatched larvae were continuously reared on the leaves treated with different concentrations of RH5992.

(Key words: *Plutella xylostella*, nonsteroidal ecdysteroid agonist, tebufenozide)

INTRODUCTION

The diamondback moth (DBM), *Plutella xylostella* (Lepidoptera: Yponomeutidae) , is a key pest of crucifers in Taiwan. Chemical insecticides have been applied heavily to control DBM, resulting in development of resistance to most of the conventional chemicals[4,5,7,12]. In addition, these insecticides act similarily toward both target and nontarget organisms, therefore they can be harmful to the benificial insects, wildlife, or man. It is thus necessary to develop non-toxic compounds to reduce the use of harzardous chemicals.

By contrast, insect growth regulators (IGRs) have gained attention as a new type of insecticide because of their novel mode of action. Juvenile hormone and 20-hydroxyecdysone are two important hormones in regulating insect metamorphosis. The former inhibits progress of an insect toward the adult form, while the later works as the driving force for molting. The juvenile hormone agonists have been intensively studied and developed to be new insecticides such as methoprene and kinoprene[1,11]. Although ecdysteroids can be extracted from plant and animal sources, they cannot be synthesized economically because of their complex structures, and limitation of penetration into insect cuticle by hydrophilic nature. The eliminating ability of ecdysone by insects may limit the natural ecdysteroids to be developed as insecticides[16]. To solve these problems, it is indispensable to synthesize a relatively simple molecule which can mimic ecdysone and is with appropriate chemical properties, e.g., transportable and acceptable metabolic stability.

A diacylhydrazine ecdysteroid agonist, *viz*., tebufenozide [RH5992: 3,5-dimithal benzoic acid 1-(1,1-dimethylethyl)-2-(4-ethylbenzoyl) hydrazide] is a nonsteroidal mimic of 20-hydroxyecdysone and interferes with the normal molting process in lepidopterous larvae. It displays novel mode of action by acting specifically on the ecdysteroid receptor of the epidermal cells and is potent for crop protection[19]. Recently Blackford and Dinan[2] reported that RH5992 was highly lethal to two lepidopterans, *Acherontia atropos* and *Lacanobia oleracea*, while adding to the diet at 1 ppm. Although the previous studies on the effects of RH5992 on several lepidopteran insects, to our knowledge, its effects on diamondback moth have not yet been studied. This paper reports effects of RH5992 on larval survival, food consumption and adult emergence of *P. xylostella* .

MATERIALS AND METHODS

Insects and chemicals

The diamondback moth, *P. xylostella*, used in this experiment was obtained from a colony maintained on rape seedlings by the method of Koshihara and Yamada[9]. The colony was collected initially from cabbage fields in Taichung area and has been reared in the laboratory at 25±1℃ and a photoperiod of 12:12 (L:D) hr since 1991.

A 10% wettable powder (WP) formulation of RH5992 was provided by Rohm and Haas Company, Philadelphia, PA, USA. The RH5992 WP was dissolved in solution of 0.1% Totalwett (BASF, Germany) as a surfactant to give concentrations of 50, 100, 200, 400, and 800 ppm as test solutions while Totalwett at 0.1% was used as the

control solution.

Determination of toxicity to DBM larvae

Potted Chinese kale plants were dipped into different concentrations of the test solutions or the control solution. The treated plants were maintained in the laboratory at 25 ±1℃ and a photoperiod of 12:12 (L:D) hr. Ten larvae of various stages tested were transferred from the stock colony to a 5-cm petri dish, then a piece of leaf disk (ca. 4 cm^2) clipped from treated plants was fed to the larvae. The petri dishes containing the larvae and leaf disks were sealed with PE wrap. Each treatment consisted of four replications (4 x 10 larvae). Leaf disks clipped from previously treated plants were fed to larvae and were replaced daily. The number of dead larvae and their stage were recorded while changing the leaf disks. Tests were terminated when all larvae pupated or died.

To examine the statistical differences in developmental growth rates (DGR) of the treatments in each larval stage, the method of Zebitz[21] was applied for determining the relative developmental instar (rDI) of the test populations on each day of observation. The formula is $rDIt = \Sigma[(ni \times W)/NI]$, where ni is the number of a particular developmental instar living on day (t) of observation, NI is the total number of all instars living on the day of observation, and W is the weighted factor, e.g., first instar=1, ..., fourth instar=4, and pupa=5.

Leaf consumption was monitored daily. The area of each leaf disk consumed was determined by drawing on 1-mm grid paper over the remained leaf on a light template. To test topical toxicity, 10 third-instar larvae were confined in a 5-cm petri dish lined with filter paper. The petri dish was placed on a tilted plate, and the test or control solution in a beaker was sprayed to the larvae by pressing the spray gun 3 times. After spraying, the larvae along with the filter paper were moved to a sheet of paper and rapidly dried with an electric fan. The treated larvae were then transferred to new petri dishes containing untreated leaf disks. The leaf disks were replaced daily. Percent pupation and adult emergence were recorded. This experiment was replicated 5 times.

Measurement of RH5992 effects on DBM adult emergence and fecundity

Cupped rape seedlings grown in an ice cream cup (7.5 x 4.5 cm) sufficiently for feeding the experimental larvae during the whole larval stage were dipped into different concentrations of test or control solution, and then kept in the laboratory at 25±1℃ with 12:12 (L:D) hr photoperiod. Newly hatched larvae were introduced into the treated rape seedlings. Four replicates (ca. 300 larvae per replicate) were conducted for each treatment. The resulting pupae, 2 days after pupation, were weighted and then individually placed in glass tubes (1.5 x 7 cm) allowing them to emerge. The number of moths emerged was counted.

Five pairs of the moths emerged within 24 hr were placed in an ice cream cup allowing them to mate and lay eggs in the cup. The cup was covered with a piece of nylon screen. On the screen top, a wetted cotton pad was placed to provide water for the moths. The moths were transferred to new oviposition units at 2-day intervals. The number of dead moths was recorded daily. After all moths died, the total eggs laid inside

the cup and on the screen top were counted, and the number of unhatched eggs was also recorded. At least 6 replicates were carried out for each treatment.

Determination of toxicity to DBM egg

Chinese kale leaves with stalk standing on moistened vermiculite in ice cream cups were placed in the oviposition cage for 4 hr to collect eggs. Upon removal of the leaves from the cage, the numbers of egg on the leaves were counted and divided into groups with about 100 eggs for each replication. Four replications were conducted for each treatment. After grouping, the leaves with eggs were dipped into test or control solution. The leaf stalks were wrapped with moistened cotton pledgets and placed in 9-cm petri dishes covered with PE wrap. The cotton pledgets were moistened daily. The number of unhatched eggs was recorded within 5 days.

Data analysis

Data were analyzed by analysis of variance (ANOVA), and the means were separated by the Fisher protected least significant difference (LSD)[17].

RESULTS

The effects of different concentrations of RH5992 on developmental growth rate (DGR)of diamondback moth are shown in Fig. 1. The rDI (relative development instar) values of all treatments on the day when any rDI value reached 5 (pupal stage) were subjected to statistical analysis by LSD. When treatments were started from 1st instar, all larvae in the controls pupated at 8th day after treatment. There was no significant difference in DGR among various concentrations on day 8 (Fig. 1A). The larvae of various treated groups reached pupal stage except those treated with 800 ppm died before 4th instar.

All of the survived larvae reached the pupal stage 8 days after treatment when the 2nd instar larvae were subjected to RH5992 treatments at 50ppm (Fig. 1B). Comparision of DGRs by different concentrations on day 8 did not show significantly different among the treatments of control, 50ppm, and 100 ppm. The rDI values of these three groups were higher than those of the 200 ppm treatment, while treatment with 400 ppm was the lowest. All larvae died before 3rd and 4th instars by 800 and 400 ppm, respectively, while some of the larvae pupated successfully by the rest concentrations.

For the treatments of the 3rd instar, the DGRs on day 7 in 50, 100 and 200 ppm treatments and the controls were not significantly different, but lower at 400 ppm (Fig. 1C). The larvae did not reach pupal stage after treating with 400 and 800 ppm. The DGRs resulting from all treatments were not significantly different when the treatment was started from 4th instar (Fig. 1D). Most larvae did not pupate by 800 ppm.

RH5992 caused 96.7, 100 and 100% cumulative mortality for the treatments with 400 ppm started from 1st, 2nd and 3rd instars, respectively (Fig. 2, A-C). However, when the treatments were started from 4th instar, the cumulative mortality was 87.5% and 97.5% by 400 and 800 ppm, respectively (Fig. 2D). The LC_{50} values was 96.3, 95.3, 105.8 ppm for 1st, 2nd and 3rd instar, respectively (Table 1). Table 2 shows that decrease in LT_{50}

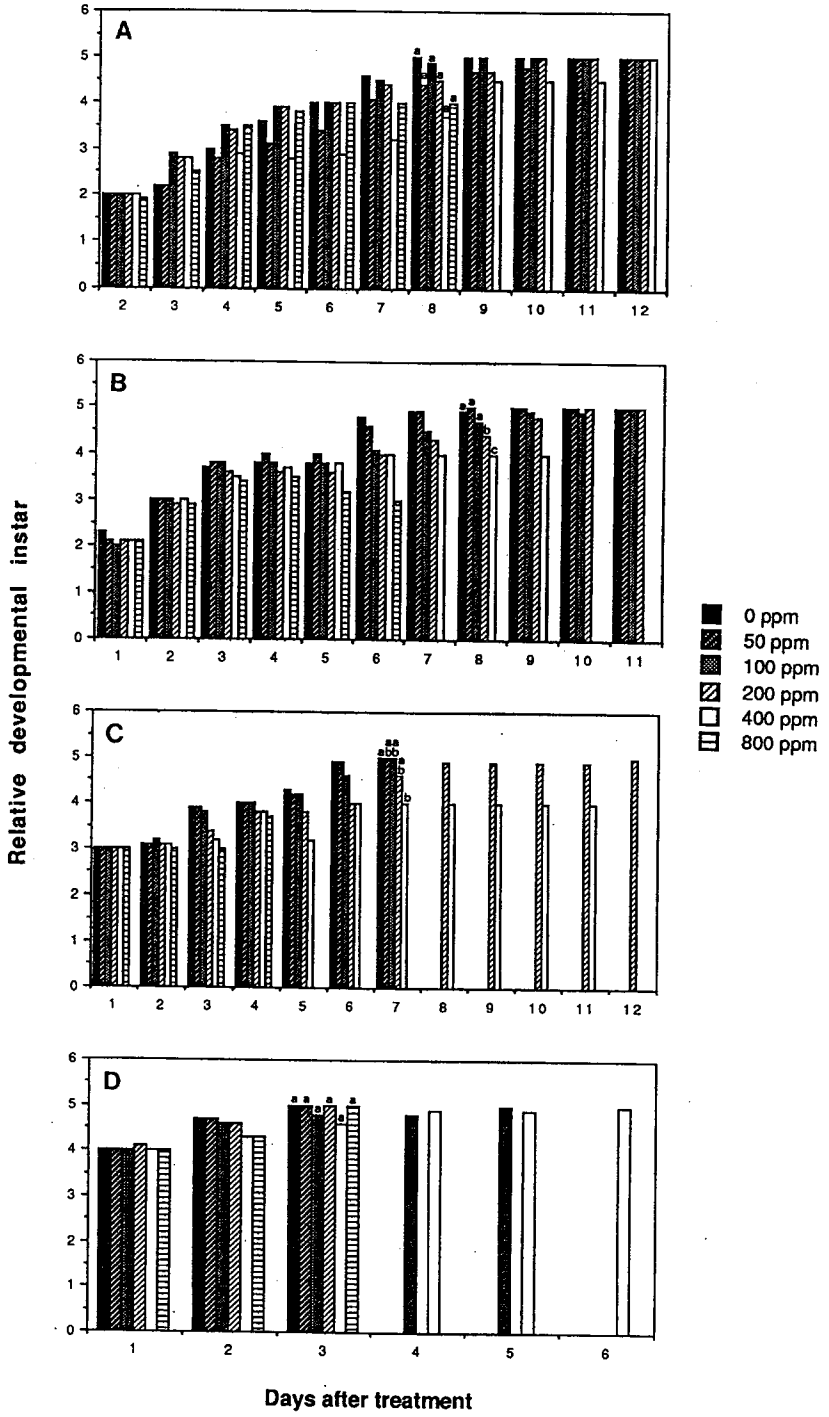

Fig. 1. Development of *P. xylostella* reared on Chinese kale leaves treated with RH5992. Experiments were started from 1st (A), 2nd (B), 3rd (C), and 4th (D) instars. RDIs with the same letter are not significantly different ($P > 0.05$; Fisher protected LSD [SAS Institute 1989]).

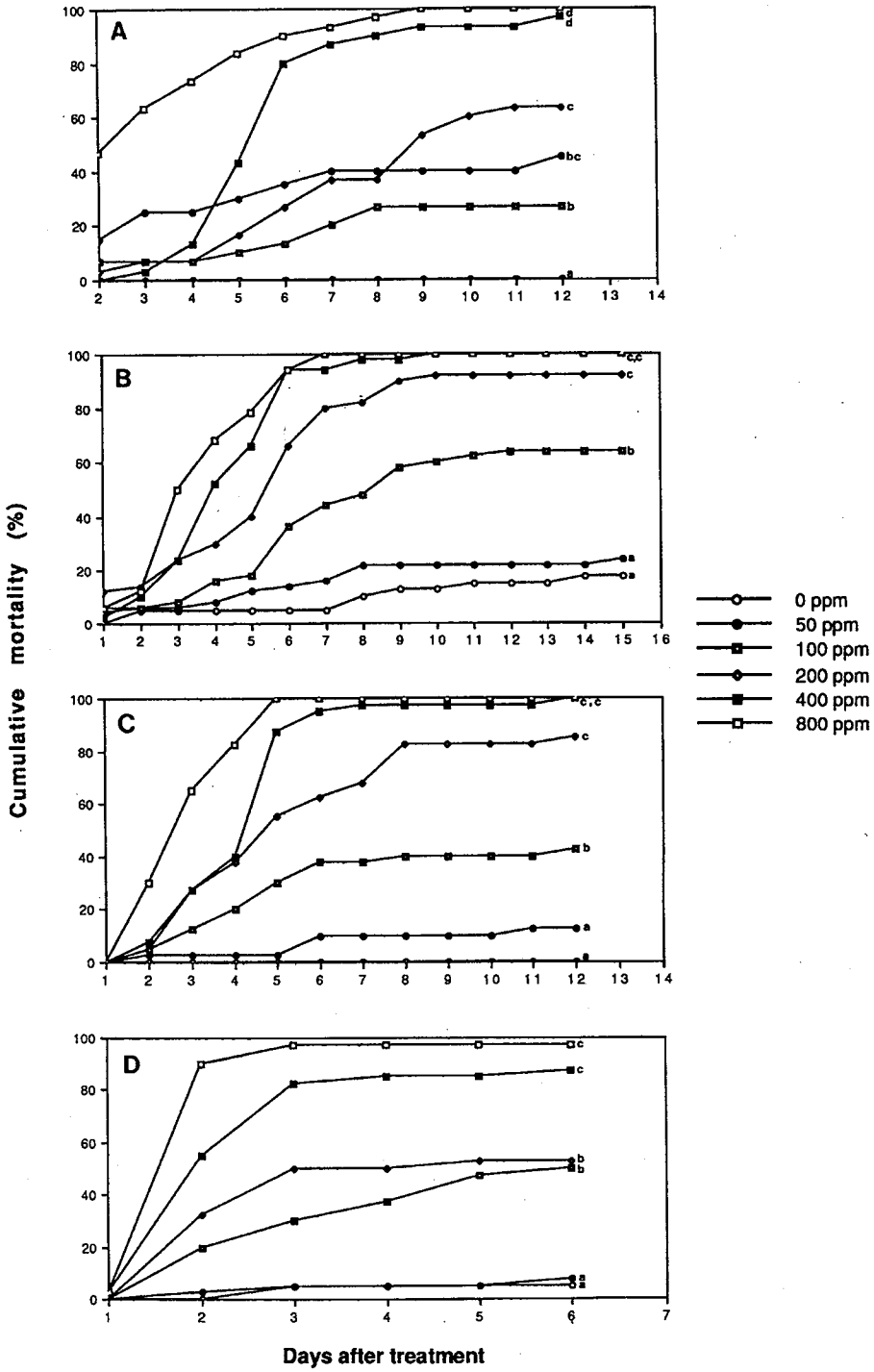

Fig. 2. Cumulative mortality of *P . xylostella* reared on Chinese kale leaves treated with RH5992. Experiments were started from 1st (A), 2nd (B), 3rd (C), and 4th (D) instars. Cumulative mortalities with the same letter are not significantly different ($P > 0.05$; Fisher protected LSD [SAS Institute 1989]).

Table 1. LC_{50} for larvae of *P. xylostella* reared on Chinese kale leaves treated with 5992

Larval instar	LC_{50} (ppm)	LC_{50} 95% confidence limit		Slope + SE
		upper	lower	
1st	96.3	121.1	72.7	2.58±0.40
2nd	95.3	111.7	77.9	4.40 ±0.72
3rd	105.8	123.3	90.1	3.91 ±0.51

Table 2. LT_{50} for larvae of *P. xylostella* reared on Chinese kale leaves treated with different concentrations of RH5992

Larval instar	LT_{50} (day) Concentration (ppm)			
	100	200	400	800
1st	-	9.1	5.2	2.3
2nd	9.3	8.0	4.4	3.2
3rd	12.3	5.0	3.7	2.6
4th	3.4	3.0	2.9	1.7

for 4th instar was ranged from 3.4 to 1.7 days by 100 to 800 ppm while it was ranged from 2.3 to 12.3 days for the other three instars.

When the larvae were continuously fed on RH5992-treated leaves, the amounts of mean leaf areas consumed were significantly decreased by 200 ppm or higher (Table 3). Figure 1A and 3A reveal that while the treatments were started from 1st instar, the peak of consumption occurred at rDI 4 in treatment with 50, 100, or 200 ppm as well as in untreated controls, but food uptake declined afterwards and the survived larvae pupated. However, the feeding activity peak occurred at rDI 3.2 on day 8 by 400 ppm rather than rDI 4.0 by lower concentrations because the survivals pupated or died on day 9. At 800 ppm, leaf consumption peak occurred at rDI 3.5, then the larvae became sluggish and finally died. The amount of leaf consumption for all treatments was greatest in the controls and least at 800 ppm. The mean consumption per larva during the larval stage decreased as raising the concentrations (Table 3). In the test with 3rd instar larvae, the most leaf consumption occurred when rDI ranged from 3.8 to 4.0 in treatments with 50, 100, 200 and 400 ppm as well as untreated controls (Fig. 1C and 3B). The average maximum amount of leaf consumption per larva was highest in the control followed by treatments with 50, 100, 200, and 400 ppm, and lowest with 800 ppm. At 800 ppm, the maximum consumption occurred on day 1; thereafter, the feeding activity was somewhat arrested without any larva survived at 4 days after

Table 3. Mean leaf areas of RH5992-treated Chinese kale consumed by larvae of *P. xylostella* during larval stage

Concentration (ppm)	Mean leaf area consumed(mm^2/larva/day)[1)] Larval instar		
	1st	3rd	4th
0	27.5 a	30.2 a	58.6 a
50	19.1 ab	23.4ab	41.3 a
100	10.5 abc	17.0 bc	17.5 b
200	7.9 bcd	8.0 cd	18.0 b
400	5.8 cd	3.2 d	12.3 b
800	3.2 d	2.2 d	15.6 b

[1)] Means within a column followed by the same letter are not significantly different ($P > 0.05$; Fisher protected LSD [SAS Institute 1989]).

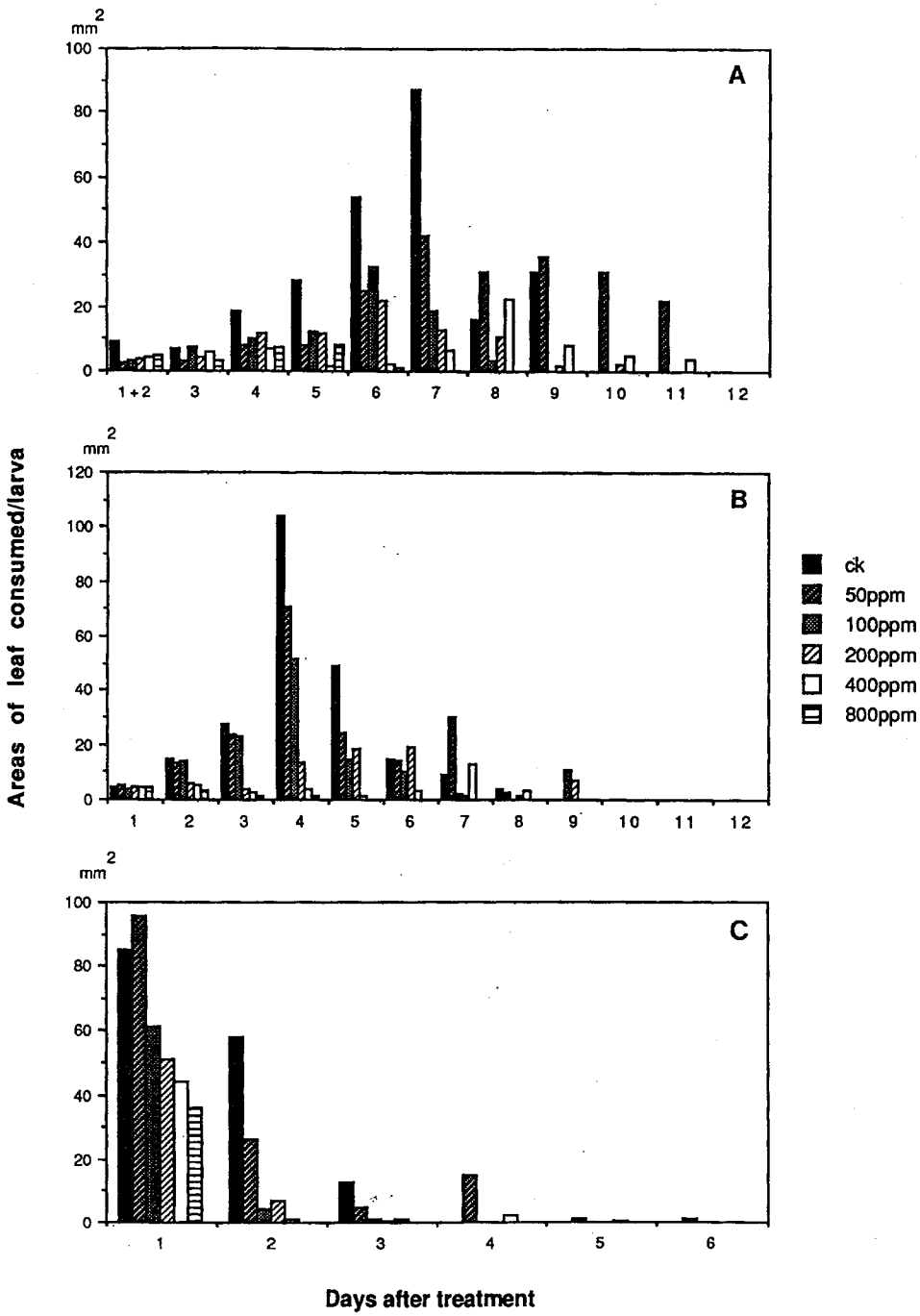

Fig. 3. Leaf areas of Chinese kale consumed by larvae of *P. xylostella* during larval stage. Experiments were started from 1st (A), 3rd (B), and 4th (C) instars.

Table 4. Percent pupation and adult emergence of *P. xylostella* sprayed with different concentrations of RH5992

	Concentration (ppm)[1)]					
	0	50	100	200	400	800
% pupation	86.7 a	83.3 a	80.0 a	73.3 a	90.0 a	66.7 a
% emergence	85.0 a	87.0 a	100.0 a	100.0 a	73.3 a	87.0 a

[1)] Means within a row followed by the same letter are not significantly different ($P > 0.05$; Fisher protected LSD [SAS Institute 1989]).

Table 5. Effects of different concentrations of RH5992 on 1st instar larvae of *P. xylostella* fed on treated rape seedlings[1)]

Concentration (ppm)	Pupal weight (mg)	Percent emergence (%)	Adult longevity (days)	Fecundity (eggs/5 ♀)	Percent hatchability (%)
50	5.1 a	83.8 a	4.0 a	136.2 a	84.6 a
100	4.6 b	74.2 b	3.7 a	81.1 ab	85.7 a
200	4.3 c	56.9 c	3.1 b	47.0 b	86.6 a
C K	5.8 d	97.2 d	4.6 c	248.1 c	83.9 a

[1)] Means within a column followed by the same letter are not significantly different ($P > 0.05$; Fisher protected LSD [SAS Institute 1989]).

Table 6. Percent hatchability of *P. xylostella*as treating the eggs with different concentrations of RH5992

Concentration (ppm)	Percent hatchability[1)] (%)
50	97.0 a
100	95.6 a
200	94.9 a
400	96.3 a
800	95.3 a
C K	89.9 a

[1)]Means within a column followed by the same letter are not significantly different ($P > 0.05$; Fisher protected LSD [SAS Institute 1989]).

treatment. For the treatment started with 4th instar, the maximal consumption occurred on day 1 in all treatments (Fig. 3C), and mean consumption per larva during larval stage was not significantly different between the control and 50 ppm but lower at the concentrations above 100 ppm (Table 3). Table 4 shows that the larvae pupated and emerged normally after directly spraying with RH5992 on the 3rd instar larvae.

The pupal weight, percent emergence, adult longevity and fecundity were affected when newly hatched larvae were continuously reared on different concentrations of RH5992 (Table 5). On the contrary, the percent hatchability was not affected either by feeding the newly hatched larvae with treated rape seedlings or by treating the eggs directly with RH5992 (Table 5 and 6).

DISCUSSION

The ecdysteroid agonist, RH5992, inhibited development of diamondback moth

at the concentrations above 400 ppm, and the larvae treated with this compound did not reach pupal stage. However, the DGRs were not obviously reduced. Prabhaker *et al.*[15] fed larvae of the cabbage looper and beet armyworm with the diets containing neem seed extracts, resulting in developmental retardation in all larval stages. The neem seed extract contains azadirachtin which works similarly to insect ecdysteroids[13] and apparently acts as a moulting inhibitor[10]. But effect of prolonging larval development was not pronounced in our experiments with RH5992.

Application of RH5992 to larval stage causes significant mortality at concentrations above 100 ppm. The larvae did not die immediately when exposed to this compound. They usually survived for several days, but became sluggish and reduced feeding activity. Eventually the larvae died from starvation or unsuccessful moulting. However, the larvae survived longer with the lower concentrations. Hsu[8] stated that a related compound, RH5849, was able to affect all larval instars of Lepidoptera by inducing premature molting. The intoxicated larvae begin molting normally but cannot shed their old cuticles, forming "double head capsules". In our experiments of RH5992 on DBM, some larvae died from the insecticidal effects, and some died during molting due to that the old cuticle was separated from the new one but could not be completely shed. However, "double head" larvae were not observed.

We found that this compound also reduce the food consumption by DBM larvae. Hsu[8] reported that at concentrations below neurotoxic levels, RH5849 functioned as a feeding deterrent. However, the leaf consumption in this study was not significantly reduced when the larvae were treated with different concentrations of RH5992 on the first day, indicating that this compound does not repel DBM larvae. The reduction in feeding activity may be due to the toxic effects.

Ohtaki *et al.*[14] stated that phytoecdysones dissolved in organic solvents could not function as molting hormone when topically applied to larval abdomens in *Sarcophaga peregrina*. The same was reported by Robbins *et al.*[16] in other insects. On the contrary, Sato *et al.*[18] observed the ecdysone activity of phytoecdysones that were applied to some lepidopterous larvae by means of dipping and topical applications. RH5849 could cause 100% mortality in armyworm 48 hr after soil application at 8 ppm, revealing that this compound is effective topically[8]. But our results showed that RH5992 is not penetratable through the DBM cuticle to cause any mortality.

The low LT_{50} values in older instars can be explained by the fact that the older instars consumed more leaf areas than the younger ones. Since 20-hydroxyecdysone is degraded rapidly in insects[6], the young larvae may take longer to accumulate the critical titer of ecdysteroid mimics to be effective in this case.

Reduction in pupal weight and adult longevity when treated newly hatched larvae with RH5992 may be due to feeding inhibition by this compound. Reduction in fecundity may result from the smaller size in adults or malnutrition caused by treating larvae with RH5992. However percent hatchability was not affected. The ecdysteroids presented in the embryos and eggs can

regulate embryonic molts[3]. Our results did not show suppression of hatchability by RH5992. This may be ascribed to biological degradation of this compound or the limitation of its penetration through the egg shell.

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摘　要

侯豐男[1]、鄭玲蘭[1]、陳健忠[2] 1998 非類固醇脫皮素拮抗物對小菜蛾存活及發育之影響　植保會刊 40：49-61. ([1]國立中興大學昆蟲學系；[2]台灣省農業試驗所應用動物系)

本試驗以芥藍菜葉片浸漬不同濃度的tebufenozide (RH5992，一種非類固醇類脫皮素拮抗物)餵食小菜蛾幼蟲，以探討此化合物對不同齡期幼蟲的影響。試驗結果顯示，當處理濃度高於400ppm，可引起各齡期幼蟲死亡。第1至第3齡幼蟲以400ppm藥劑處理之葉片連續餵食，其最終累積死亡率達100%，但同樣濃度連續餵食4齡幼蟲，其累積死亡率爲87.5%，提高濃度至800ppm累積死亡率可達97.5%。RH5992對小菜蛾1、2和3齡幼蟲的半數致死濃度(LC_{50})分別爲96.3、95.3和105.8ppm。當濃度爲100至800ppm時，其對小菜蛾4齡幼蟲之半數致死時間(LT_{50})介於1.7-3.4天，而對其它齡期之幼蟲，則爲2.3-12.3天。葉片經濃度100ppm或更高之劑量處理供幼蟲取食，會降低其取食量。初孵化之幼蟲即以RH5992處理之葉片連續餵食，發育完成後之蛹重、羽化率、成蟲壽命和生殖力都會受影響。

(關鍵詞：小菜蛾、非類固醇類脫皮素拮抗物、tebufenozide)