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The effects of exogenous 20-hydroxyecdysone on the feeding, development, and reproduction of *Plutella xylostella* (Lepidoptera: Plutellidae)

Li Juan Sun^{1,2}, Yong Jie Liu^{1,*}, and Chang Peng Shen²

Abstract

Exogenous 20-hydroxyecdysone (20E) exerts a range of detrimental effects on the development and survival of many insect species, and different species show varying susceptibilities to ingested 20E. The specific effects of exogenous 20E on *Plutella xylostella* (L.) (diamondback moth; Lepidoptera: Plutellidae), a severe pest of cruciferous crops, has not been reported systematically. Here, we studied the effects of exogenous 20E on feeding, development, and survival of *P. xylostella* larvae and on fecundity and longevity of adults by using a leaf dip assay. We found that food consumption and the duration of development of larvae that survived to the next instar decreased with the increasing concentrations of dietary 20E. Ingested 20E exerted adverse effects on the development of larvae by decreasing their weight, and led to death mainly by inducing abnormal molting. The lethal effect of 20E on larvae was also determined by a residual film method, which showed LD₅₀ values of 1st to 4th instars were 0.331, 0.345, 0.439, and 0.252 mg/mL, respectively. Female adults laid reduced numbers of eggs on leaves treated with 20E. There was a negative correlation between the concentration of 20E on the leaf surface and the number of eggs deposited on the leaves ($P < 0.05$). After 5 d, the average fecundity of adult females was reduced and correlated with the concentration of 20E in the diet ($P < 0.05$). The longevity of male adults was significantly shortened after ingesting diet containing 0.50 mg/mL 20E. Thus, ingestion of exogenous 20E exerted adverse effects on feeding, development, and reproduction of *P. xylostella*, and 20E residues on leaves of host plant had significant repellent effects on oviposition by females.

Key Words: dietary 20E; diamondback moth; feeding; development; reproduction

Resumen

El exógeno de 20-hidroxiccdisona exógena (20E) ejerce una serie de efectos perjudiciales sobre el desarrollo y la sobrevivencia de muchas especies de insectos, y diferentes especies muestran diferentes susceptibilidades al 20E ingerida. No se ha reportado, los efectos específicos del exógeno de 20E sobre *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), la polilla del dorso diamante de la col (PDC), una plaga grave de los cultivos de crucíferas. Los efectos del exógeno de 20E sobre la alimentación, el desarrollo y la sobrevivencia de las larvas de PDC, además de repelar la oviposición en las hojas tratadas, la fecundidad y la longevidad de los adultos fueron estudiados en parte mediante el uso de un ensayo de inmersión foliar. Se encontró que el consumo de alimentos y la duración del desarrollo de las larvas que podrían sobrevivir al siguiente estadio disminuyó con el aumento de las concentraciones de la dieta 20E. El 20E ingerida ejerce efectos adversos sobre el desarrollo de las larvas de PDC por la disminución de su peso, y condujo a su muerte, principalmente mediante la inducción de una muda anormal. Se determinó la letalidad del 20E por medio de larvas colocadas en las hojas del rábano que habían sido sumergidas en varias concentraciones de 20E en agua y luego secadas al aire. Los valores de la DL₅₀ resultantes del primero-cuarto estadio fueron 0.331, 0.345, 0.439 y 0.252 mg /l, respectivamente. Las hembras adultas de PDC pusieron un número reducido de huevos en las hojas con un residuo de 20E sobre la superficie. Hubo una correlación muy significativa entre la concentración del 20E en la superficie de las hojas y el número de huevos depositados sobre las hojas. La fecundidad de las hembras adultas se redujo en $5.50 \pm 4.64\%$, $8.70 \pm 2.91\%$, $15.86 \pm 3.40\%$, $20.61 \pm 2.49\%$ y $28.95 \pm 1.58\%$ después de la ingestión de 0.0303, 0.0625, 0.0125, 0.25 y 0.50 mg/L de 20E por 5 días, respectivamente. Se redujo la longevidad de los adultos machos significativamente después de la ingestión de 0.50 mg/l de 20E. Por lo tanto, la ingestión de 20E exógena ejerce efectos adversos sobre la alimentación, el desarrollo y la reproducción de PDC, y 20E tuvo un efecto sorprendentemente repelente sobre la oviposición en las hojas con residuos de 20E.

Palabras Clave: exógeno del 20E; polilla del dorso diamante de la col; alimentación; desarrollo; reproducción

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Since the discovery of 20-hydroxyecdysone (20E) in the leaves of *Podocarpus nakai* Hayata (Pinales: Podocarpaceae) in 1966 (Nakanishi et al. 1966), its effects as a toxin and antifeedant for various invertebrate species have been studied (Dinan 1998). Ingested 20E exerts a range of detrimental effects on the development and survival of many insect species. Polyphagous species, including *Helicoverpa virescens* F. (Noctuidae), *Helicoverpa armigera* Hübner (Noctuidae), *Locusta* spp. (Acrididae), *Spodoptera littoralis* Boisduval (Noctuidae), *Lacanobia olereaceae* (L.) (Noctuidae), *Acherontia atropos migratoria* (L.) (Sphingidae), and *Manduca sexta* (L.) (Sphingidae), are believed most resistant to high concentrations of phytoecdysteroids (Dinan 1998). They were tolerant to dietary ecdysteroids showing no apparent ill-effects when fed 400 ppm or more 20E in their diet (reviewed by Dinan 1998). In comparison, some oligophagous insects seem more susceptible to dietary ecdysteroids, such as *Pectinophora gossypiella* (Saunders) (Gelechiidae) (Kubo et al. 1981, 1983), *Bombyx mori* (L.) (Bombycidae) (Kubo et al. 1983), and *Acrolepiopsis assectella* (Zeller) (Acrolepiidae) (Arnault & Sláma 1986). However, some polyphagous species are susceptible to exogenous 20E, including *Spodoptera frugiperda* J. E. Smith (Noctuidae) (Kubo et al. 1981), *Agrius convolvuli* (L.) (Sphingidae) (Tanaka & Naya 1995), and *Lymantria dispar* (L.) (Erebidae) (Yu et al. 2012). Therefore, insects exhibit a range of degrees of susceptibility to 20E.

As one of the most important gonadotropic hormones in adult insects, 20E plays a critical role in the immediate control of oogenesis (Bownes 1989). Early findings showed that experimentally increased 20E titers in wild-type *Drosophila virilis* Sturtevant (Drosophilidae) females drastically reduced their fecundity (Rauschenbach et al. 2005). Such effect was also found in *S. littoralis* (Ufimtsev et al. 2006).

Plutella xylostella (L.) (diamondback moth; Lepidoptera: Plutellidae) seriously damages cruciferous crops (Talekar & Shelton 1993) and is considered oligophagous (Wu 1993). The 20E extracted from *Ajuga nipponensis* Makino (Lamiales: Lamiaceae) exerts an antifeedant effect on *P. xylostella* larvae (Huang et al. 2008). Zeng et al. (2001) found that ingestion of 0.1 mg/mL of 20E adversely affected survival and pupation of *P. xylostella* larvae. 20E has also been reported to improve the pathogenicity of *Isaria fumosorosea* against *P. xylostella* larvae when used as a mixture in the laboratory and field (Xu et al. 2011). These reports all indicate the potential of 20E as a control agent against *P. xylostella*. Examination of the effects of 20E on *P. xylostella* is useful for the development of 20E analog insecticides. Here, we report the effects of exogenous 20E on food consumption, development, and survival of larvae, as well as the reproduction (including oviposition and fecundity) and longevity of adults of *P. xylostella*.

Materials and Methods

INSECTS AND 20-HYDROXYECDYSONE (20E)

Larvae of a laboratory strain of *P. xylostella* were obtained from the research and development center of Hailier Pharmaceutical Group in Qingdao City, Shandong, China, and were reared in an insectary for more than 10 generations before the bioassays. Insects were maintained at 25 °C and 16:8 h L:D on radish (*Raphanus sativus* L.; Brassicales: Brassicaceae) seedlings. We purchased 20E (high purity grade) from Sigma-Aldrich. Five concentrations of 20E in water (0.031, 0.063, 0.125, 0.250, and 0.500 mg/mL) with 0.01% Tween-20 added were prepared to examine the effect on feeding, development, and survival of larvae. Four concentrations of 20E in water (0.025, 0.050, 0.100, and 0.200 mg/mL) with Tween-20 added were prepared for to examine the repellent effect of 20E to oviposition of females. Five concentrations of 20E in 10% honey (0.031, 0.063, 0.125, 0.250, and 0.500 mg/mL) were prepared to determine the effect of 20E on fecundity and longevity of adults.

BIOASSAY OF THE EFFECT OF EXOGENOUS 20E ON LARVAE

The Effect of Exogenous 20E on Food Consumption. Radish leaves were washed with distilled water and then immersed for 5 s in each 20E solution, and the control leaves were immersed in 0.01% Tween-20 in water. These leaves were air dried at room temperature, and 1.0 cm diameter leaf discs were punched from them. Leaf discs were used to feed newly molted 1st to 4th instars. Single larvae were reared in 4.5 cm diameter Petri dishes at 25 °C, 16:8 h L:D, and 60% RH with 60 to 80 larvae per treatment. The leaf discs were photographed with an Ucmos10000 Digital Imaging System (Beijing Top View Technology Co., Ltd., Beijing, China) at 24 h after treatment and after the larva stopped feeding at the end of the instar before molting. For each treatment, food consumption of 15 males and 15 females randomly selected from those surviving to the next developmental stage was determined using TopView™ (version 3.2). The missing leaf area relative to the starting area of the leaf disc was determined. After treatment, the larvae were continuously reared with 20E-free radish seedlings in order to determine the gender.

The Effect of Exogenous 20E on Larval Development. The radish leaves were treated with 20E solution in water by the same methods described above. The control leaves were treated with 0.01% Tween-20 in water. These leaves were fed to newly molted 1st to 4th instars. The development duration of each instar (20–30 larvae per treatment, 4 replicates), as well as the weight gain of 60 to 70 individuals of 4th instars were determined using a precision electronic autobalance (BS 110S, Sartorius, Germany). After larvae stopped feeding at the end of the instar, just before molting, the treated larvae were carefully moved into a new Petri dish and reared with 20E-free radish seedlings until pupation to determine the weight of the pupae.

The Lethal Effect of Exogenous 20E on Larvae. The radish leaves were treated with 20E solution by the same methods described above. The control leaves were treated with 0.01% Tween-20 in water. These leaves were fed to 1st to 4th instar larvae. Mortality of 1st to 3rd instars was determined at 48 h after treatment, and mortality of 4th instars was determined at 72 h after treatment. Twenty larvae were used in each treatment, and each experiment was repeated 3 times.

BIOASSAY OF THE EFFECT OF EXOGENOUS 20E ON ADULTS

The Repellent Effect of Exogenous 20E on Oviposition of Female Adults. Water-cultured radish seedlings with their growing point removed were placed in a cage (0.5 × 0.5 × 0.5 m) under a LED light after having been immersed in the 20E solution for 5 s, and air dried. A control seedling treated with 0.01% Tween-20 in water was surrounded by seedlings treated with 20E (Fig. 1). The experiment was carried out at 25 °C and 16:8 h L:D. Twenty pairs of adults emerging within 12 hours of each other were paired and released into each cage (12 replicates). The eggs deposited on the radish seedlings were counted at 48 h after the release of the adults.

The Effect of Exogenous 20E on Fecundity and Longevity of Adults. Healthy pupae that pupated within 12 h and had similar weights (6.0–7.0 mg for the females; 4.5–5.5 mg for the males) were selected from the laboratory population. Adults that emerged within 12 h were paired (1 female with 1 male in a plastic cup of 300 mL), and were fed 20E in a honey solution for 5 d after emergence. Thereafter, the diet was replaced by 10% honey solution free of 20E. The fecundity and longevity of the treated adults were determined. Eggs deposited by each female were collected and maintained in an incubator for 5 d at 25 °C, 16:8 h L:D, and 60% RH. The percentage of hatch, percentage of non-embryonated eggs, and duration of the egg stage were then determined. Ten to 15 pairs were used in each treatment, and each treatment was replicated 3 times.

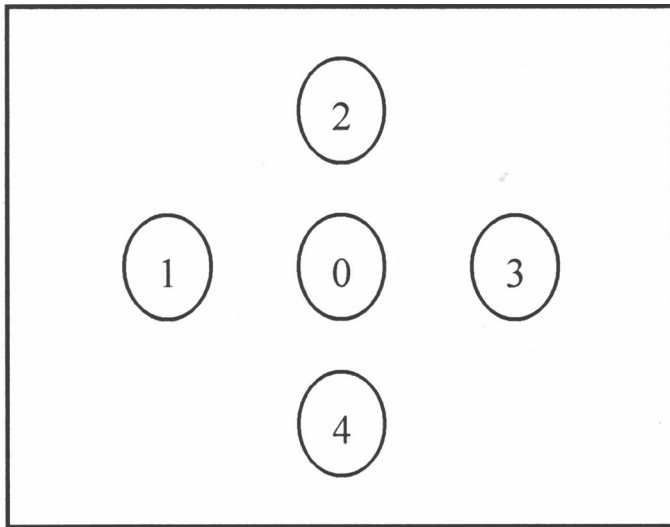


Fig. 1. The arrangement of 20-hydroxyecdysone-treated radish seedlings in a cage. Digit 0 represents the control; digits 1–4 represent the radish seedlings treated with 0.050, 0.100, 0.200, and 0.400 mg/mL of 20E solutions in water, respectively.

DATA ANALYSES

The correlation between concentration of 20E used for treatment and food consumption, duration of each instar, weight gain of larvae, weight of pupae, fecundity of females, number of eggs deposited on the treated leaves, as well as the development of eggs deposited by the treated parent adults were analyzed by linear regression analysis using the SPSS 11.5 software package. The differences in means of longevity of adults were separated by Tukey's multiple comparison test using the SPSS 11.5 software package, and differences were judged to be statistically significant at $P \leq 0.05$. Toxicity data were analyzed by Poloplus 1.0 software.

Results

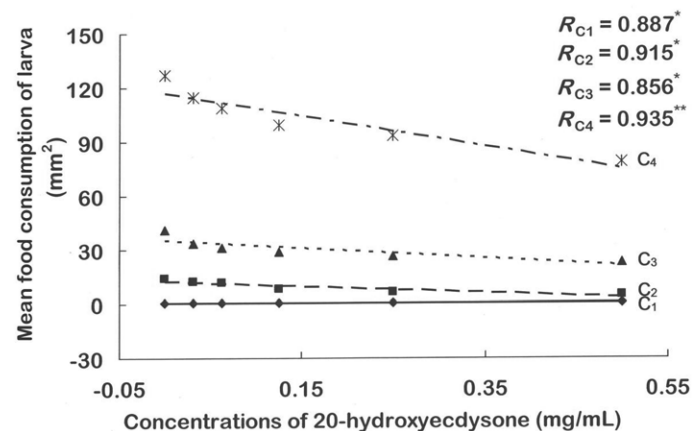
THE EFFECT OF EXOGENOUS 20E ON LARVAE

The Effect of Exogenous 20E on Food Consumption by Larvae. The average food consumption of larvae was determined at 24 h after treatment and after the larva stopped feeding at the end of the instar before molting (Fig. 2). For each instar, the food consumption correlated with the concentrations of 20E used for treatment. The 20E in the diet impeded the feeding of larvae (24 h: $R = 0.887$, $F = 14.791$, $P = 0.018$ for the 1st instar; $R = 0.915$, $F = 20.562$, $P = 0.008$ for the 2nd instar; $R = 0.856$, $F = 10.960$, $P = 0.03$ for the 3rd instar; $R = 0.935$, $F = 27.967$, $P = 0.006$ for the 4th instar; total food consumption during the instar: $R = 0.887$, $F = 14.819$, $P = 0.018$ for the 1st instar; $R = 0.926$, $F = 24.093$, $P = 0.008$ for the 2nd instar, $R = 0.878$, $F = 13.483$, $P = 0.021$ for the 3rd instar; $R = 0.969$, $F = 61.014$, $P = 0.001$ for the 4th instar).

The Effect of Exogenous 20E on the Duration of Development. Figure 3 shows the correlation between duration of each instar and concentration of 20E in the diet. The development duration of each instar decreased with the increasing of concentrations of 20E in the diet. The negative correlation was the most obvious for the 3rd instars (1st instar: $R = 0.659$, $F = 3.070$, $P = 0.155$; 2nd instar: $R = 0.716$, $F = 4.207$, $P = 0.110$; 3rd instar: $R = 0.957$, $F = 43.994$, $P < 0.003$; 4th instar: $R = 0.720$, $F = 4.295$, $P < 0.107$).

The Effect of Exogenous 20E on the Weight Gain of 4th Instars. To determine the effects of ingested exogenous 20E on weight gain of

24 H



Total Food Consumption during the Instar

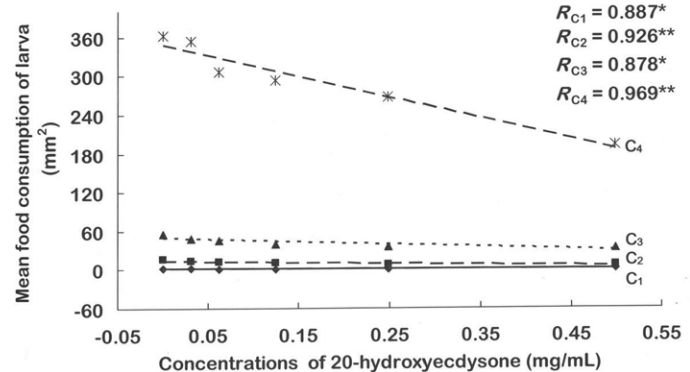


Fig. 2. The correlation between the concentration of 20-hydroxyecdysone in diet and mean food consumption by diamondback moth larvae for each instar. C_1 , C_2 , C_3 , and C_4 represent mean food consumption of 1st, 2nd, 3rd, and 4th instars, respectively. R represents the coefficient; the symbols * and ** next to the coefficient indicate that the correlation between X and Y was significant at $P < 0.05$ and $P < 0.01$, respectively.

larvae, leaves treated with different concentrations of 20E were fed to larvae of the final (4th) instar. We found that there was obvious negative correlation between the weight gain of larvae and the concentra-

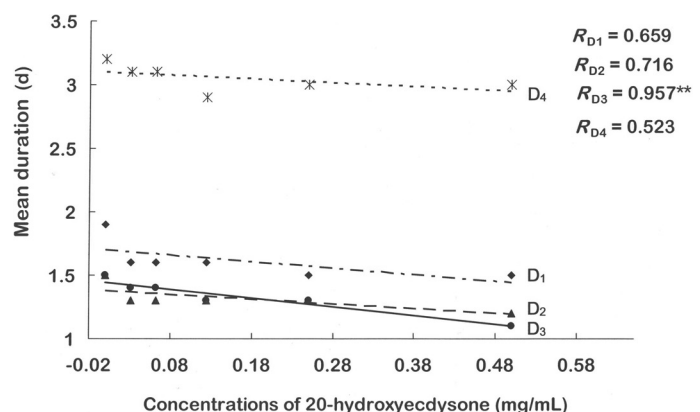


Fig. 3. The correlation between the concentration of 20-hydroxyecdysone in diet and mean duration of each instar of diamondback moth larvae. D_1 , D_2 , D_3 , and D_4 represent mean duration of 1st, 2nd, 3rd, and 4th instars, respectively. R represents the coefficient; the symbol ** next to the coefficient indicates that the correlation between X and Y was significant at $P < 0.01$.

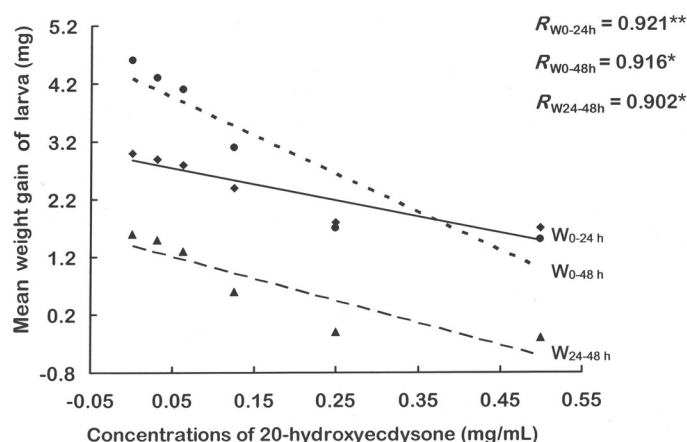


Fig. 4. The correlation between the concentration of 20-hydroxyecdysone in diet and mean weight gain in 4th instars of diamondback moth. W_{0-24h} , W_{0-48h} and W_{24-48h} represent mean weight gain at 24 h, at 48 h, and from 24 h to 48 h after treatment, respectively. R represents the coefficient; the symbols * and ** next to the coefficient indicate that the correlation between X and Y was significant at $P < 0.05$ and $P < 0.01$, respectively.

tions of ingested 20E. Ingesting diet with a high concentration of 20E (0.250 and 0.500 mg/mL) reduced the weight of larvae after treatment for 24 h (Fig. 4; 24 h: $R = 0.921$, $F = 22.398$, $P = 0.09$; 48 h: $R = 0.916$, $F = 20.858$, $P = 0.01$; 24 h–48 h: $R = 0.902$, $F = 17.369$, $P = 0.014$).

The Effect of Exogenous 20E on the Weight of Pupae. The average weight of pupae that developed from the treated 1st and 2nd instars did not correlate with concentrations of 20E used for the treatments (1st instar: $R = 0.394$, $F = 0.737$, $P = 0.439$; 2nd instar: $R = 0.574$, $F = 1.962$, $P = 0.234$). By contrast, for the 3rd and 4th instars, the average weight of pupae decreased with the increasing of concentrations of

20E (Fig. 5; 3rd instar: $R = 0.977$, $F = 82.554$, $P = 0.001$; 4th instar: $R = 0.970$, $F = 64.712$, $P = 0.001$).

Lethal Effect of Exogenous 20E on Larvae. The lethal effect of exogenous 20E on larvae is shown in Table 1. The 4th instars were most sensitive to 20E with a LD_{50} of 0.331 mg/mL, whereas the 3rd instars were more tolerant of exogenous 20E than the other instars. Ingested 20E resulted in a range of defects (Fig. 6), including decreased feeding in larva (i.e., some larvae refused to feed), exosmosis of ecdysial fluid (Fig. 6a), failure to shed the head capsule (Fig. 6b) or exuvium (Fig. 6c), bulging of the hindgut (Fig. 6d), metamorphosis into a deformed pupa (Fig. 6e), or occurrence of a supernumerary instar (Fig. 6a).

THE EFFECT OF EXOGENOUS 20E ON ADULTS

Repellent Effect of Exogenous 20E on Oviposition. We found that female adults avoided laying eggs on radish leaves coated with 20E (Fig. 7). There was a significant negative correlation between the concentration of 20E and the mean number of eggs deposited on the treated seedlings ($R = 0.985$, $F = 95.794$, $P = 0.002$).

Effect of Ingested Exogenous 20E on Fecundity. Adult females fed 10% honey solution containing 20E also showed reduced fecundity (Table 2). The fecundity decreased with increasing 20E in the diet (Fig. 7). ($R = 0.948$, $F = 35.337$, $P = 0.004$).

Effect of Parents Ingesting Exogenous 20E on the Viability and Development of Eggs. Adults ingesting 20E did not exert obvious adverse effect on their progeny, except for a slight delay in the development of eggs ($R = 0.882$, $F = 14.080$, $P = 0.020$) (Table 2). The mean percentage of hatch ($R = 0.683$, $F = 3.493$, $P = 0.05$) and non-embryonated eggs ($R = 0.585$, $F = 2.081$, $P = 0.223$) did not have a correlative relationship with the concentrations of 20E ingested by the parents.

Effect of Ingested Exogenous 20E on the Longevity of Adults. Ingesting diet with 0.5 mg/mL 20E resulted a significant reduction in

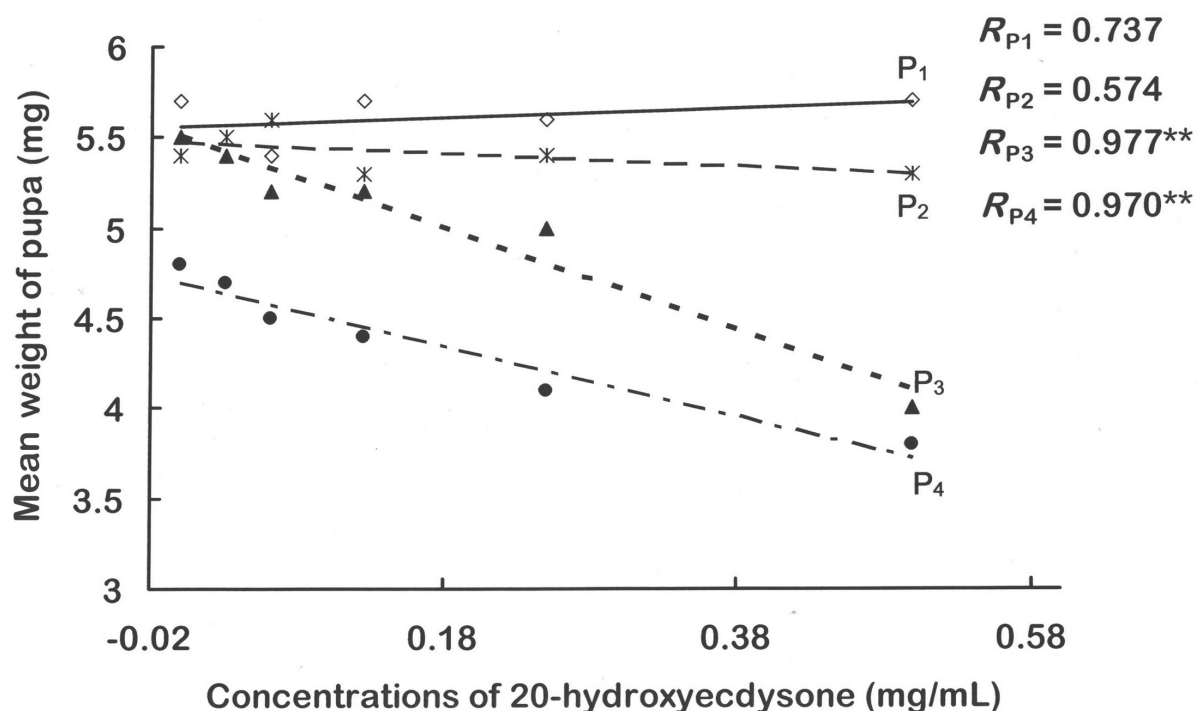


Fig. 5. The correlation between the concentration of 20-hydroxyecdysone in diet and mean weight of diamondback moth pupae. P_1 , P_2 , P_3 , and P_4 represent mean weight of pupae developed from the treated 1st, 2nd, 3rd, and 4th instars, respectively. R represents the coefficient; the symbol ** next to the coefficient indicates that the correlation between X and Y was significant at $P < 0.01$.

Table 1. The lethal effect of exogenous dietary 20-hydroxyecdysone on diamondback moth larvae.

Instar	LD ₅₀ (mg/mL)	Virulence curve	Confidence interval (95%)
L ₁	0.331	y = 1.474x + 0.008	0.236–0.560
L ₂	0.345	y = 1.381x + 0.029	0.260–0.522
L ₃	0.439	y = 1.237x + 0.067	0.304–0.824
L ₄	0.252	y = 1.600x + 0.050	0.195–0.347

Poloplus software was used to analyze the data. L₁, L₂, L₃, and L₄ in the first column represent 1st, 2nd, 3rd, and 4th instar, respectively, of *Plutella xylostella*.

male longevity (Fig. 8) ($F = 8.688$, $df = 5$, $P < 0.05$) but not female longevity. Diet with low concentrations of 20E had no obvious effect on the longevity of adults.

Discussion

It has been reported that 20E is only effective when applied at the final stages of insect development (Francisco & Josep 1993). However, we found that 20E was lethal to all larval instars of the diamondback

moth. Because of the leaf-mining habit of 1st instars, the actual effect of ingested exogenous 20E may be lower than that indicated by these experimental values.

Our data indicate that ingesting exogenous 20E reduces food consumption in each instar. We speculate that the reduction in food consumption was not only caused by an antifeedant effect of 20E, but also by the premature molting that resulted from the ingestion of excessive 20E.

Ingesting 20E caused decreased feeding in larvae, exosmosis of ecdysial fluid, failure of removal of the old head capsule or exuvium, morphogenesis into a deformed pupa, bulging of the hindgut, and supernumerary instars. These toxic symptoms are similar to those caused by tebufenozide, a type of edysteroid analogue (Smagghe et al. 1996; Retnakarn et al. 1997; Dhadialla et al. 1998). The potential resistance of the diamondback moth to tebufenozide has been shown by Cao & Han (2006), and a detailed study of the effect of 20E in this species will provide insight into its resistance to edysteroid analog insecticides, such as tebufenozide.

Ingesting high concentrations 20E greatly reduced the weight of 4th instars. It was reported that animal size and nutritional status were

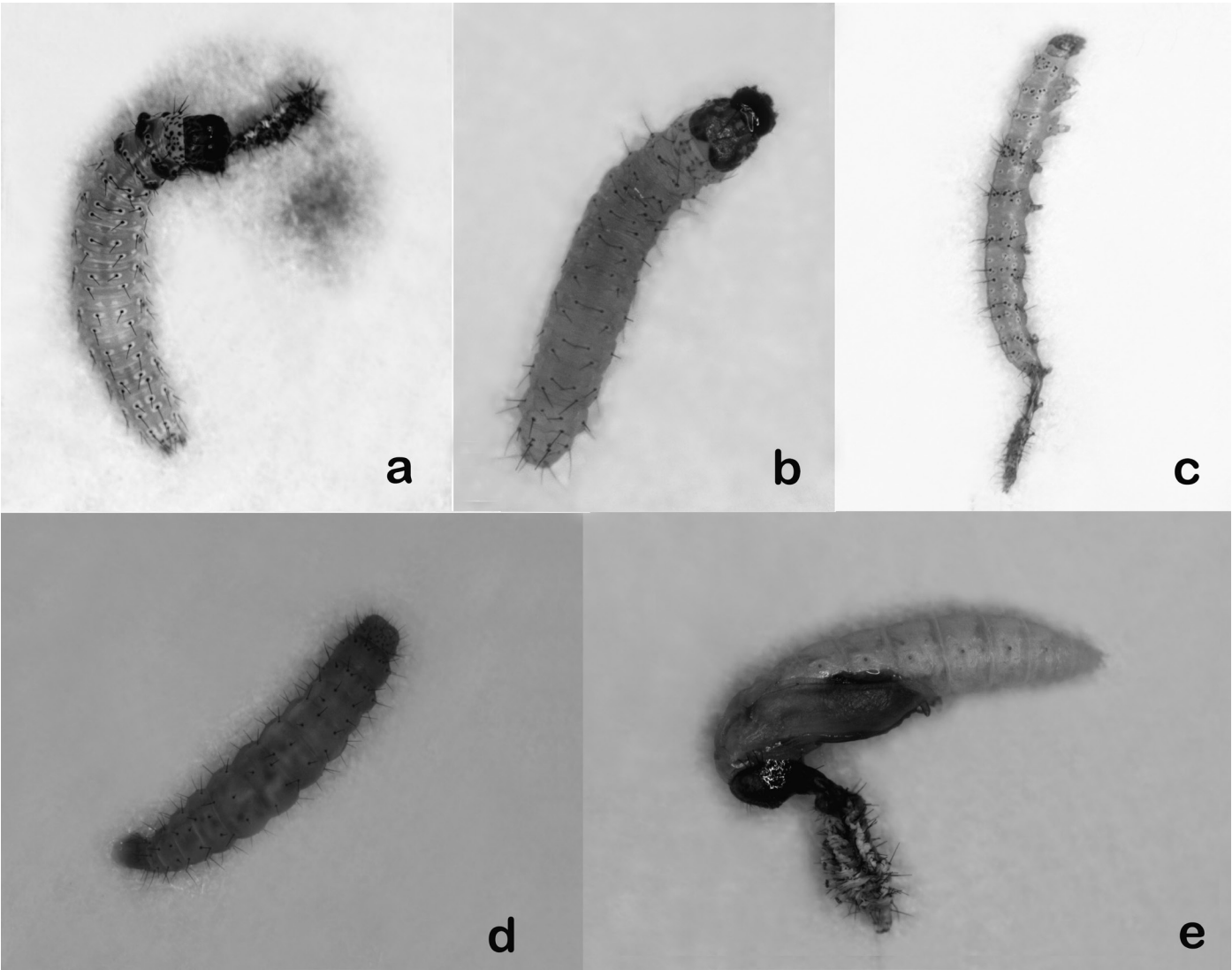
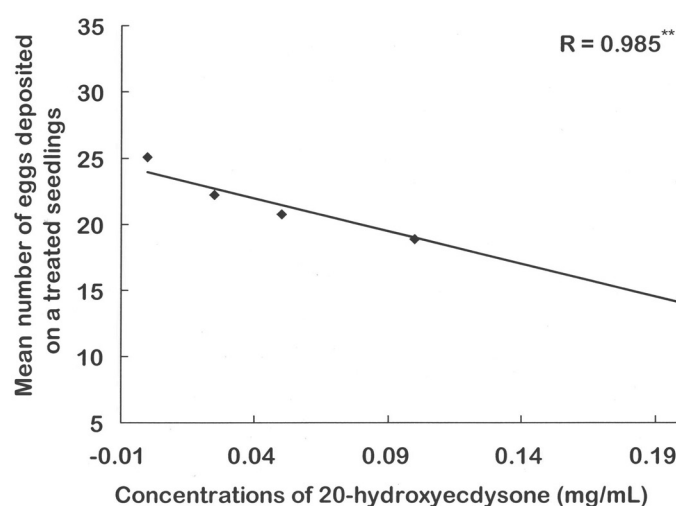
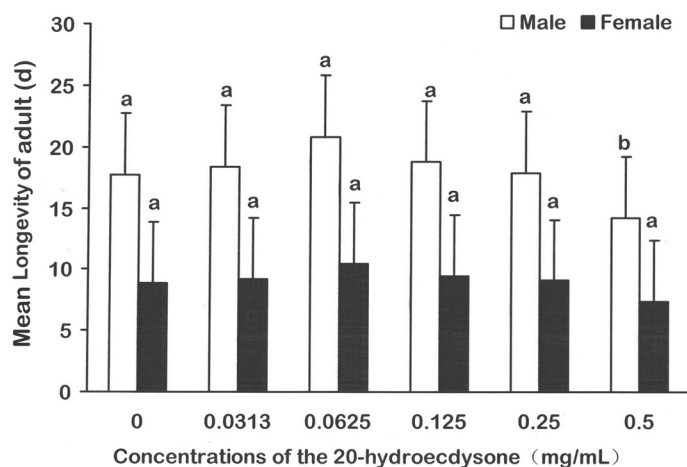


Fig. 6. The morphological changes in diamondback moth larvae and a pupae caused by ingestion of exogenous dietary 20-hydroxyecdysone. a, Exosmosis of ecdysial fluid and an additional molt (15 ×); b, failure to shed the head capsule (30 ×); c, failure to shed the exuvium (20×); d, bulging of the hindgut (10 ×); and e, deformed pupa (10 ×).

Table 2. The correlation between the concentration of 20-hydroxyecdysone and mean ($\pm$ SD) fecundity of females of diamondback moth as well as the development of eggs deposited by the treated parents.

Concentration of 20E (mg/mL)	Average fecundity	Hatch (%)	Non-embryonated eggs (%)	Duration of egg stage (d)
0	181.2 $\pm$ 12.9	94.40 $\pm$ 6.88	3.79 $\pm$ 3.57	2.1 $\pm$ 0.1
0.031	170.8 $\pm$ 4.7	94.81 $\pm$ 0.11	2.40 $\pm$ 1.16	2.1 $\pm$ 0.2
0.063	165.2 $\pm$ 6.3	88.30 $\pm$ 5.97	9.25 $\pm$ 4.58	2.1 $\pm$ 0.1
0.125	152.2 $\pm$ 5.0	88.87 $\pm$ 4.99	9.43 $\pm$ 5.11	2.1 $\pm$ 0.2
0.250	143.6 $\pm$ 5.6	90.62 $\pm$ 0.78	7.01 $\pm$ 0.55	2.1 $\pm$ 0.1
0.500	128.6 $\pm$ 6.4	86.97 $\pm$ 4.70	9.74 $\pm$ 5.93	2.2 $\pm$ 0.1
<i>R</i>	0.948**	0.683	0.585	0.882*
<i>F</i>	35.337	3.493	2.081	14.08
<i>P</i>	0.004	0.135	0.223	0.020

SPSS 11.5 soft package was used to analyze the linear regression of concentrations of 20-hydroxyecdysone used for treatment and the experimental data. *R* represents the coefficient; the symbols * and ** next to the coefficient indicate that the correlation between the concentrations of 20-hydroxyecdysone and each group of experimental data was significant at $P < 0.05$ and $P < 0.01$, respectively.

**Fig. 7.** The correlation between the concentration of 20-hydroxyecdysone used for treatment and the mean number of eggs deposited on the treated seedlings. *R* represents the correlation coefficient; the symbol ** next to the coefficient indicates that the correlation between X and Y was significant at $P < 0.01$.**Fig. 8.** The longevity of adults fed on diet with exogenous dietary 20-hydroxyecdysone. The different lowercase letters above bars indicate statistically significant differences between mean longevity of adults fed on diet with different concentrations of 20-hydroxyecdysone (Tukey test, $\alpha = 0.05$).

monitored by the larval fat body by integrating 20E signaling with the insulin signaling pathway (Nichole et al. 2010). Ingesting excessive 20E may disturb the integrated signal pathway and thus lead to abnormal biosynthesis or metabolism. The detailed mechanism is an interesting topic for further study.

Females laid fewer eggs on 20E treated leaves. Such oviposition repellency was also found in European grapevine moth, *Lobesia botrana* (Denis & Schiffermüller) (Tortricidae) (Delphine et al. 2006), and the European corn borer, *Ostrinia nubilalis* (Hübner) (Crambidae) (Delphine et al. 2007). Phytoecdysteroids are thought to be detected by the female European grapevine moth and the female European corn borer through taste sensilla located on the tarsi of their thoracic legs.

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