The Effects of Exogenous 20-Hydroxyecdysone on the Feeding, Development, and Reproduction of Plutella xylostella (Lepidoptera: Plutellidae)

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

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**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 LD$_{50}$ 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-hidroxiecdisona 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 ingerido. No se ha reportado, los efectos específicos del exógeno de 20E sobre *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), una 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 repelir 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 ingerido 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 DL$_{50}$, resultantes del primero-cuarto estadio fueron 0.331, 0.345, 0.439 y 0.252 mg /L, respectivamente. Las hembras adultos 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 ± 4.64%, 8.70 ± 2.91%, 15.86 ± 3.40%, 20.61 ± 2.49% y 28.95 ± 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 armigera Hübner (Noctuidae), Locusta spp. (Acrididae), Spodoptera littoralis Boisdvil (Noctuidae), Lacanobia oleae (L.) (Noctuidae), Acherontia atropos migratoria (L.) (Sphingidae), and Manduca sexta (L.) (Sphingidae), are believed to be 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) (Arnauld & Slama 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 1993), 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 (Bowens 1989). Early findings showed that experimentally increased 20E titers in wild-type Drosophila virilis Surtervent (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 (Lamiaceae: 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-HYDROXYECDSYSONE (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, 16:8 h L:D, and 60% RH. The percentage of hatch, percentage of adults that emerged within 12 h were collected and maintained in an incubator for 5 d at 25 °C. 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.
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: \( R = 0.915, F = 20.562, P = 0.008 \); 2nd instar: \( R = 0.926, F = 24.093, P = 0.008 \); 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 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-
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–48h: \( R = 0.902, F = 17.369, P = 0.014 \)).

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
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               |

Poloplas software was used to analyze the data. $L_1$, $L_2$, $L_3$, and $L_4$ in the first column represent 1st, 2nd, 3rd, and 4th instar, respectively, of *Plutella xylostella*.

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

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![Fig. 6. The morphological changes in diamondback moth larvae and a pupae caused by ingestion of exogenous dietary 20-hydroxyecdysone.](image-url)
Sun et al.: Effects of exogenous 20-hydroxyecdysone on *Plutella xylostella*

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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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, α = 0.05).
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