



Pupal diapause of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) mediated by larval host plants: pupal weight is important

Zhudong Liu^{*}, Peiyu Gong, Dianmo Li, Wei Wei

State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beichenxilu 1-5, Chaoyang District, Beijing 100101, China

ARTICLE INFO

Article history:

Received 1 July 2010

Received in revised form 5 August 2010

Accepted 5 August 2010

Keywords:

Helicoverpa armigera
Larval host plants
Diapause incidence
Pupal weight
Adaptive significance

ABSTRACT

Facultative diapause, a strategy that allows insects to initiate additional generations when conditions are favorable or to enter diapause when they are not, has a profound effect on the ecology and evolution of species. Most previous studies have concentrated on the role of photoperiod and temperature in inducing facultative diapause in insects. In contrast, here we studied pupal diapause mediated by larval host plants in the cotton bollworm *Helicoverpa armigera*, and confirmed that pupal weight is a critical factor. Two groups of third instar *H. armigera* larvae, kept at 25 °C with L:D = 8:16 and 20 °C with photoperiod of L:D = 8:16, respectively, were fed on six host plants and on artificial diet (as a control) to determine how larval host plants affect diapause incidence and related traits (such as pupal weight and developmental duration). The data showed larval host plants affected diapause incidence significantly and the effects could be masked by low temperature. Further analysis showed that pupal size, not the length of the sensitive stage, affected the decision to enter diapause. In a further experiment, third-instar to final-stage larvae deprived of artificial diet for 2 days demonstrated a direct relationship between pupal weight and diapause incidence. These results suggest that larval host plants, by affecting pupal size, may influence diapause occurrence in *H. armigera*. This has important adaptive significance for both over-wintering survival and the possibility for completing an additional generation.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

Diapause is an important strategy by which insects avoid unfavorable environmental conditions (Tauber et al., 1986; Danks, 1987). Insects have the potential to gain significant increases in fitness when conditions are favorable or to enter diapause when conditions are not (Roff, 1983; Carriere et al., 1995; Hunter and McNeil, 1997). Therefore, the strategy of facultative diapause is expected to evolve in unpredictable seasonal environments and appears to be common among insect species living in environments that promote multi-voltinism (Tauber and Tauber, 1982; Mousseau and Roff, 1989; Bradford and Roff, 1993; Carriere and Roitberg, 1994; Hunter and McNeil, 1997). Because the strategy of facultative diapause can alter the number of generations produced by a population per year, it has profound ecological and evolutionary implications for insect species (Roff, 1983; Tauber et al., 1986; Carriere et al., 1995; Hunter and McNeil, 1997).

The best-known cues associated with diapause in insects are photoperiod and temperature (de Wilde, 1962; Tauber et al., 1986;

Brodeur and McNeil, 1989; Hodkova and Socha, 1995). Other environmental factors, such as food quality (Hunter and McNeil, 1997; Takafuji and Morishita, 2001; Ito and Saito, 2006), humidity (Wardhaugh, 1986; Lenga et al., 1993) and predation (Kroon et al., 2004) have received limited attention. Food quality has been shown to be a major factor regulating diapause for a few species of insects (Tauber et al., 1986), especially for those that aestivate (Hagen, 1962; Stewart et al., 1967). Recently, several studies on mites showed that food quality affected diapause incidence (Takafuji and Morishita, 2001; Ito and Saito, 2006). Takagi and Miyashita (2008) also showed that swallowtail butterflies had a higher diapause incidence when they fed on tough leaves than when they fed on tender young leaves.

In herbivorous arthropods, diapause incidence often varies among host-plant species (Tauber et al., 1986; Danks, 1987; Koveos and Tyanakakis, 1989; Takafuji and Morishita, 2001; Ito and Saito, 2006), as does the timing of diapause induction. However, how host-plant quality affects diapause incidence is still unknown. In polyphagous insects, the quality of the host plant affects larval growth rate, which may affect the length of the sensitive stage at which the cues causing diapause are sensed (Hunter and McNeil, 1997; Wednell et al., 1997). Insects have been shown to grow faster and have a lower incidence of diapause on good-quality host plants than on poor-quality host

^{*} Corresponding author. Institute of Zoology, Chinese Academy of Sciences, Beichenxilu 1-5, Chaoyang District, Beijing 100101, China. Tel.: +86 10 64807072; fax: +86 10 64807099.

E-mail address: liuzdqyj@yahoo.com (Z. Liu).

plants. Additionally, insects destined to diapause often accumulate additional energy reserves (Storey and Storey, 1991; Sakurai et al., 1992; Hokkanen, 1993; Zvereva, 2002; Liu et al., 2007), suggesting that diapause occurrence is related to body size.

The cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), is one of the most serious insect pests in China, Australia and India. This species has a wide range of cultured and wild host plants, including at least 172 species of host plants from 40 families in Australia (Zalucki et al., 1994) and nearly 200 species from 30 families in China (Xu et al., 1958). Over most of its range in China, this species has four or five generations a year, mainly depending on temperature and what kind of host plant it feeds on; in addition, it over-winters in a facultative pupal diapause in the soil. Previous work showing that winter diapause in *H. armigera* is induced by the short photoperiod and low temperatures of autumn (Li and Xie, 1981; Wu and Guo, 1995) paid little attention to the role of larval host plants; these include cotton, tobacco, kidney bean, corn, tomato, and hot pepper (Lu and Xu, 1998). The different nutritive values of the host plants of *H. armigera* may affect the rate of development of larvae that feed on them and therefore influence population dynamics (Ruan and Wu, 2001; Liu et al., 2004). Moreover, larval host plants are known to affect *H. armigera* overwintering preparedness and population dynamics in *H. armigera* (Liu et al., 2007, 2009). However, the influence of larval host plants on diapause induction and associated life-history traits remains undefined.

Since host-plant quality is intimately linked to the growth and development of insect herbivores (Bernays, 1990), larval host plants of *H. armigera* might be expected to influence diapause incidence. In our study, *H. armigera* larvae were fed on six host plants of varying quality to see how plant species influence the facultative pupal diapause of *H. armigera* in the laboratory. We tested the following predictions: 1) diapause incidence varies among larval host plants, 2) diapause incidence is influenced by the quality of host plants, 3) diapause occurrence is related to pupal weight attained and/or the duration of the sensitive stage for diapause induction.

2. Materials and Methods

2.1. Insects rearing and host plant culture

A laboratory population of *H. armigera* was established by collecting full-grown larvae from cotton plants growing in the suburbs of Changsha, in the province of Hunan. Larvae were reared on an artificial diet with wheat germ as the main component (Wu and Gong, 1997) at 27 °C under a photoperiod of L:D = 16:8 to prevent diapause. Newly hatched neonates were reared in groups until the third instar, after which they were separated into individual glass tubes (2.0 cm diam. × 8.0 cm high) to prevent cannibalism. Mature caterpillars were allowed to pupate in moist soil substrata with a water content of about 7% (soil was heated to temperature 120 °C for 2 h and sifted through a sieve with 36 apertures per cm²). The insects were fed continuously on the artificial diet for 5 generations before testing to reduce the possible influence of the host source.

The following six *H. armigera* host plants, all of which are commonly found in China, were used in this study: cotton (*Gossypium hirsutum* L.), tobacco (*Nicotiana tabacum* L.), kidney bean (*Phaseolus vulgaris* L.), corn (*Zea mays* L.), tomato (*Lycopersicon esculentum* Mill), and hot pepper (*Capsicum frutescens* L.). Previous study has ranked of the suitability of these host plants for supporting *H. armigera* under developmental conditions (L16:D8 and 27 °C) from low to high (Liu et al., 2004) and reported the contribution of larval host plants to the over-wintering biology of this species (Liu et al., 2007, 2009). The seeds used in the

experiments were acquired from the Chinese Academy of Agricultural Sciences. Plants used in the experiments were collected from fields not exposed to pesticides and were fertilized with a controlled release fertilizer and watered as required.

2.2. Diapause induction

Larvae were fed on artificial diet at 27 °C under a photoperiod of L:D = 16:8 until the third instar, then transferred to conditions under which diapause is usually induced; the third to final instars are the sensitive stage for diapause induction (Li and Xie, 1981). Three experiments were conducted: the first two explored the effects of the larval host plants on diapause and the third tested our hypothesis based on the first two experiments, i.e. diapause occurrence is related to pupal size.

2.2.1. Experiment 1: Induction of diapause at 25 °C with L:D = 8:16

Third-instar larvae reared on artificial diet were transferred to the fruits of cotton, tobacco, kidney bean, corn, tomato and hot pepper (artificial diet continued to be used as a control) and kept at 25 °C under a photoperiod of L:D = 8:16 to induce diapause. Four replicates of 20 larvae each were used for each host plant. The fruits were replaced with fresh ones every other day until pupation, and the duration of the last larval instar and the prepupal stages were recorded. Each pupa was sexed and weighed at the second day after pupation. The pupae were checked to see whether they were in diapause at 10-day after pupation according to two visible morphological characteristics cited by Liu et al. (2006).

2.2.2. Experiment 2: Induction of diapause at 20 °C with L:D = 8:16

This experiment was carried out to see how temperature modified the effects of host plants on diapause. Third-instar larvae, reared on artificial diet at 27 °C under a photoperiod of L:D = 16:8, were transferred to the fruits of cotton, tobacco, corn, kidney bean, tomato (artificial diet was used as the control) and kept at 20 °C under a photoperiod of L:D = 8:16. Mature larvae were allowed to pupate in moist soil as described above, and their status was checked 10 days after pupation. Each treatment group contained 150 individuals. Only the diapause incidence and survival to pupation were recorded since nearly all entered diapause under these conditions.

2.2.3. Experiment 3: Induction of diapause on artificial diet

Host plants differ in their architectural quality for insect herbivores (Hunter and Willmer, 1989), which might influence their propensity to enter diapause. Our previous study also showed that diapausing pupae were heavier than nondiapausing pupae and related this to differences among larval host plants (Liu et al., 2007). Based on Experiment 1, we hypothesize that diapause occurrence is related to body size. To test directly for the effect of body size on diapause induction, we fed *H. armigera* larvae on artificial diet (Wu and Gong, 1997) to produce differently sized pupae. By depriving larvae of food, we hoped to see whether there was a relationship between diapause incidence and pupal size. Third-instar larvae, reared on artificial diet at 27 °C under a photoperiod of L:D = 16:8, were transferred to a chamber set at 25 °C and a photoperiod of L:D = 8:16. Larvae were deprived of food for 2 days when they were at each of the third through sixth instars. Larval duration at each instar and at the prepupa stage was recorded. Pupae were sexed and weighed on the second day after pupation and diapause status was determined at 10-day.

2.3. Statistics

Statistical analyses of pupal weight and duration among host plants and diapause status were conducted using the general linear

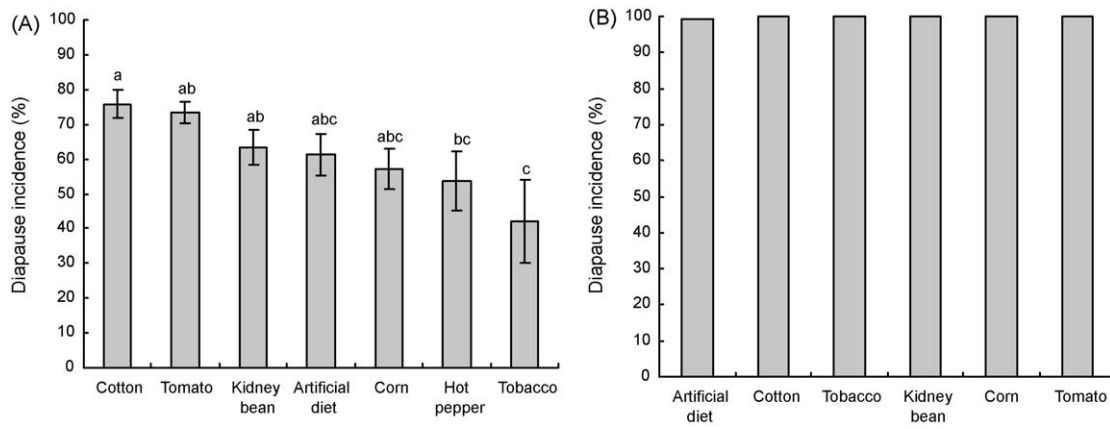


Fig. 1. Diapause incidence of *H. armigera* fed on different larval host plants at 25 °C and 20 °C with LD = 8:16. A, 25 °C; and B, 20 °C. The bar above the column showed S.E., and different letter showed significant differences in diapause incidence due to host plant species at $p < 0.05$ (Scheffe test).

model (GLM) (SPSS, 1999), host plants, diapause status, and sex of insects were the fixed factors. One-way analysis of variance (ANOVA) was performed by SPSS (1999), and means were separated with the Scheffe multiple range test. Diapause incidence after arcsine transformation was carried out using ANOVA with SPSS, in which the means were separated with the Scheffe multiple range test. To compare non-diapausing and diapausing pupae, one-way ANOVA was conducted with a Duncan multiple range test. Survival was tested using the chi-square. Regression analysis was used to determine the relationships among larval host plants, developmental duration, pupal weight, survival and diapause incidence.

3. Results

3.1. Diapause incidence of *H. armigera* reared on larval host plants

At 25 °C under a photoperiod of L:D = 8:16, diapause occurrence was significantly affected by host plants (plant, $F = 3.273$, $df = 6$, $p = 0.004$; Sex, $F = 0.047$, $df = 1$, $p = 0.829$, plant \times sex, $F = 1.095$, $df = 6$, $p = 0.365$), as demonstrated by GLM analysis. Pupal diapause incidence in *H. armigera* mediated by larval host plants varied from 42.3% for those fed on tobacco fruits to 75.9% for those fed on cotton bolls (Fig. 1A, $F = 11.639$, $df = 7, 23$; $p < 0.0001$). However, at 20 °C under L:D = 8:16, no differences were noted among larval

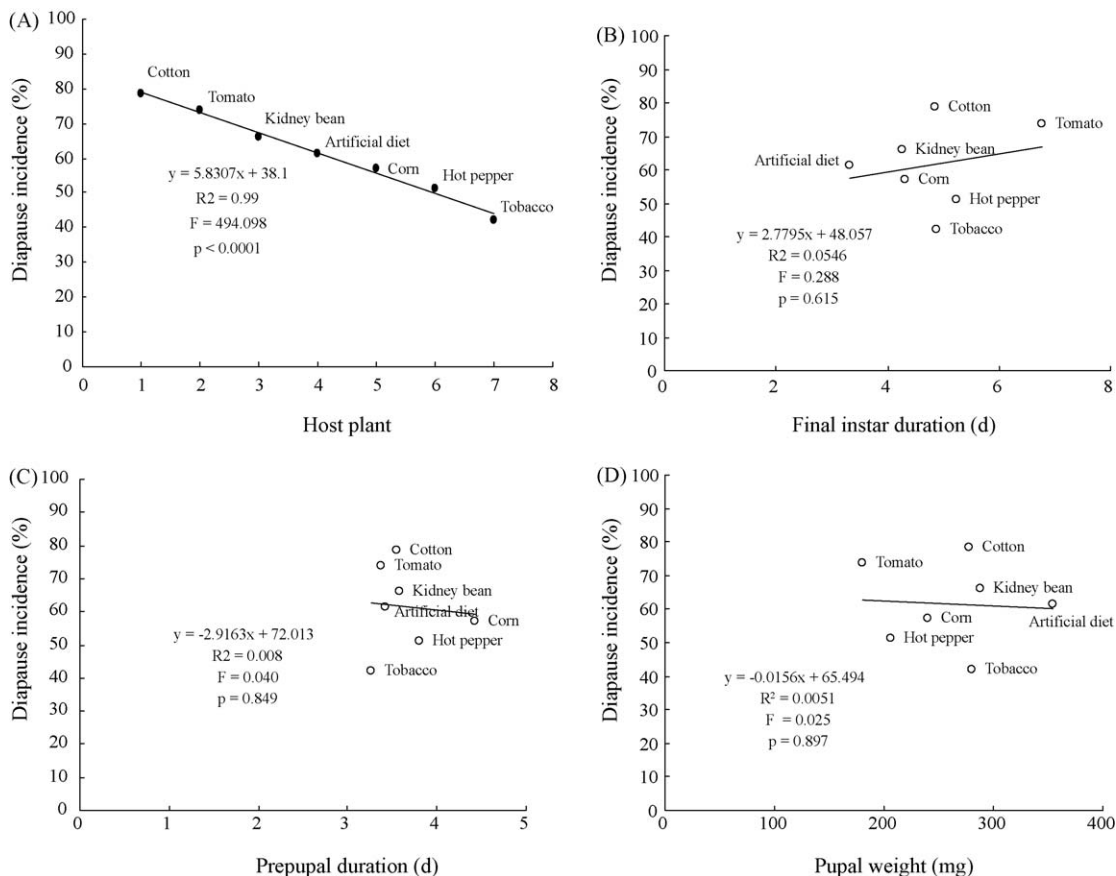


Fig. 2. The relationship of diapause incidence in *H. armigera* with host plants and life-history traits.

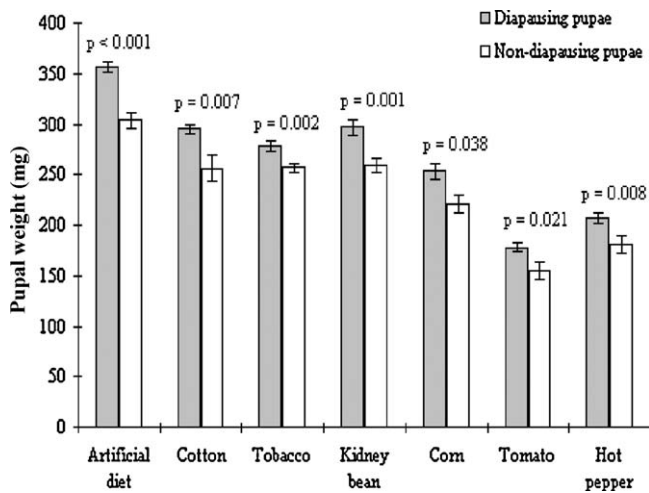


Fig. 3. Pupal weight of diapausing and non-diapausing *H. armigera* for each tested host plants under moderate condition

host plants (Fig. 1B). Further analysis showed that diapause incidence of *H. armigera* fed on host plants at 25 °C was significantly related to host plants (Fig. 2 A).

3.2. Effects of larval host plants and diapause on pupal weight

GLM analysis showed that pupal weight was significantly affected by host plant species and diapause status, respectively (Table 1). Diapausing pupae were significantly heavier than non-diapausing pupae reared at 25 °C and a photoperiod of L:D = 8:16 (Fig. 3). Moreover, pupal weight was significantly related to larval host plants in both diapausing and non-diapausing pupae (Fig. 4 A and B, diapausing pupae: $y = -$

$25.973x + 367.39$, $R^2 = 0.9214$, $p = 0.001$; non-diapausing pupae: $y = -22.795x + 324.39$, $R^2 = 0.9246$, $P = 0.001$).

When the plant acted as a covariate, further GLM analysis showed that developmental duration (both the final larval instar and prepupal stage) significantly influenced pupal weight independently of one another (plant, $F = 0.313$, $df = 1$, $p = 0.576$; sex, $F = 0.180$, $df = 1$, $p = 0.672$; final instar, $F = 22.937$, $df = 7$, $p < 0.001$; prepupa, $F = 2.894$, $df = 5$, $p = 0.014$; final instar \times sex, $F = 0.422$, $df = 5$, $p = 0.833$; prepupa \times sex, $F = 0.113$, $df = 2$, $p = 0.893$; final instar \times prepupa, $F = 1.647$, $df = 12$, $p = 0.078$; final instar \times prepupa \times sex, $F = 1.004$, $df = 7$, $p = 0.429$). Regression analysis showed that pupal weight was significantly correlated with final instar larval duration in both diapausing and non-diapausing pupae (Fig. 4C and D).

3.3. Effects of larval host plants and diapause on developmental duration

Although developmental duration in both the final instar stage and the prepupal stage, was significantly affected by larval host plants (Tables 1 and 2), diapause status did not play a direct role. Further regression analysis showed that the development duration of the final larval instar and prepupal stage was significantly correlated with larval host plants among both diapausing and non-diapausing pupae (Fig. 5 A, B, C, D).

3.4. Diapause incidence in insects reared on artificial diet with 2 d of deprivation

Diapause incidence significantly varied from 14.8 to 56.9% when the larvae were deprived of food for 2 d at the third through sixth instars, respectively (Table 3 chi-square = 23.049, $df = 4$, $p < 0.0001$). The corresponding pupal weight gain varied significantly from 288.4 to 356.5 mg (Table 3 $F = 18.903$; $df = 4$, 260; $p < 0.0001$). Further regression analysis showed that diapause incidence correlated significantly with pupal weight (Fig. 6).

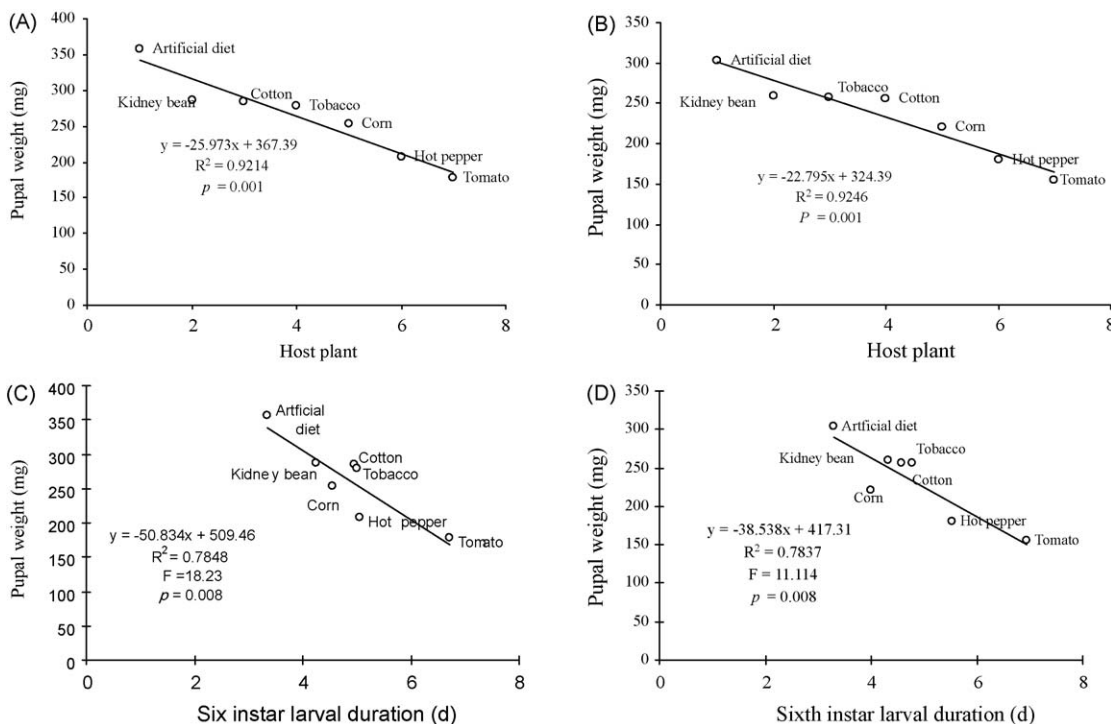


Fig. 4. Regression analysis of pupal weight with host plant species and sixth instar larval duration for both diapausing and non-diapausing pupae reared on different host plants. A and C, Diapausing pupae; B and D, Non-diapausing pupae.

Table 1Analysis of variance partitioning effects (GLM) of host, diapause status and sex on performance of *Helicoverpa armigera*.

Source of variation	Pupal weight ^a			Duration of final stage ^b		Duration of prepupa ^c	
	df	MS	F	MS	F	MS	F
host	6	163521.032	133.393****	66.381	72.481****	4.492	10.760****
diapause status	1	56.034.603	45.711****	0.168	0.183	0.0245	0.059
sex	1	33.214	0.027	1.726	1.885	0.309	0.739
host × diapause	6	1934.201	1.578	1.890	2.063	0.587	1.407
diapause × sex	1	144.815	0.118	1.774	1.904	0.017	0.040
host × sex	6	1480.451	1.208	0.889	0.970	0.521	1.248
host × diapause × sex	6	911.756	0.744	1.994	2.177 [*]	0.237	0.567
error		1225.858		0.916		0.417	

* P≤0.05, **P≤0.01, ***P≤0.001, ****P≤0.0001

^a Error df=330, ^b Error df=368, ^c Error df=373.**Table 2**Post hoc tests for developmental duration of final instar larvae and prepupae with Scheffe test using the GLM model for both destined to diapause and non-diapause larvae of *H. armigera* fed different host plants.

	Duration of final instar larva (d)				Duration of prepupa (d)			
	Diapausing	Non-diapausing	F	p	Diapausing	Non-diapausing	F	p
cotton	4.95 ± 0.21(22)b	4.57 ± 0.43(7)bcd	0.734	0.399	3.50 ± 0.14(22)b	4.00 ± 0.22(7)ab	3.117	0.089
tobacco	5.00 ± 0.17(30)b	4.78 ± 0.15(41)bc	0.976	0.327	3.34 ± 0.11(32)b	3.20 ± 0.07(44)c	1.304	0.257
kidney bean	4.25 ± 0.14(52)b	4.33 ± 0.29(27)bcd	0.087	0.769	3.63 ± 0.10(52)b	3.48 ± 0.17(27)bc	0.716	0.400
corn	4.56 ± 0.32(16)b	4.00 ± 0.25(12)cd	1.884	0.182	4.31 ± 0.19(16)a	4.58 ± 0.15(12)a	1.059	0.313
tomato	6.71 ± 0.13(45)a	6.94 ± 0.28(16)a	0.659	0.420	3.42 ± 0.93(45)b	3.25 ± 0.14(16)bc	0.940	0.336
hot pepper	5.05 ± 0.17(22)b	5.53 ± 0.25(19)b	2.729	0.107	3.82 ± 0.11(22)ab	3.74 ± 0.15(19)bc	0.203	0.655
artificial diet	3.35 ± 0.10(54)c	3.29 ± 0.12(34)d	0.132	0.717	3.54 ± 0.10(54)b	3.24 ± 0.10(34)bc	4.351	0.040
F	58.728	25.706			4.971	10.526		
p	< 0.001	<0.001			< 0.001	<0.001		

Data are shown as mean ± S.E. Different letter in the same column means significant at P < 0.05 (Post hoc tests with Scheffe using the GLM model).

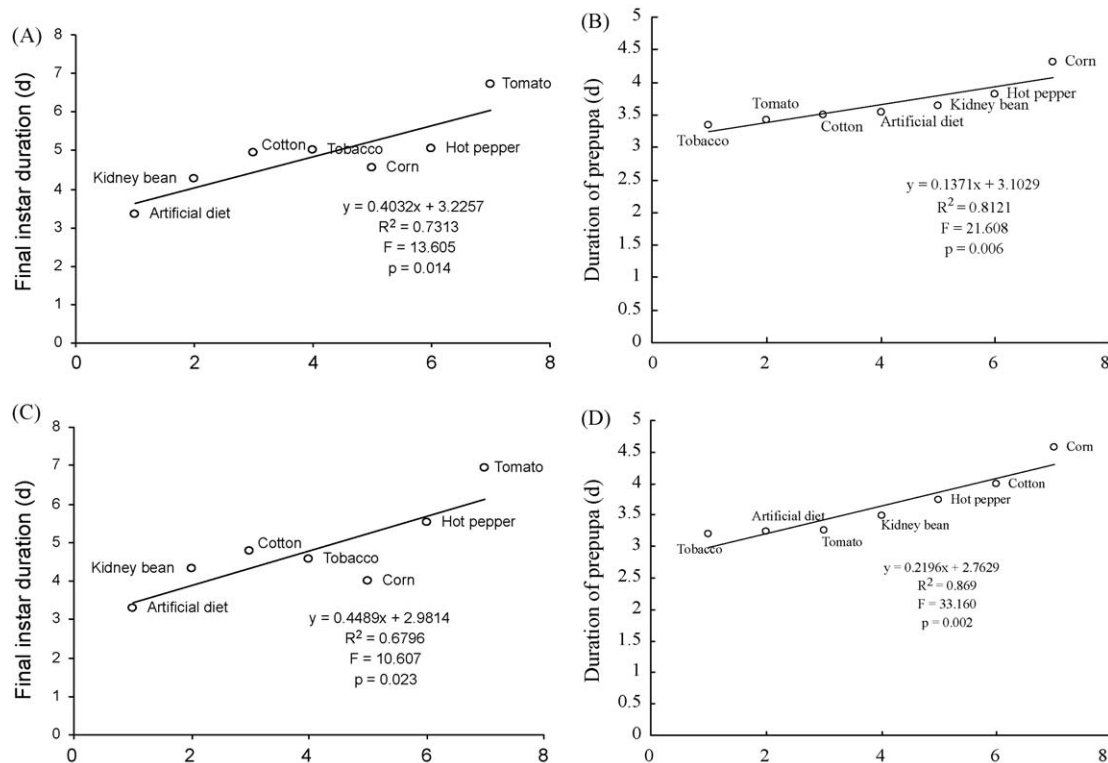
**Fig. 5.** Regression analysis of final instar larval and prepupal duration with host plants. A and B for final instar larval and prepupal duration of diapausing pupae, respectively; C and D for final instar larval and prepupal duration of non-diapausing pupae, respectively.

Table 3Diapause incidence and pupal size of *Helicoverpa armigera* caused by deprivation of artificial diet.

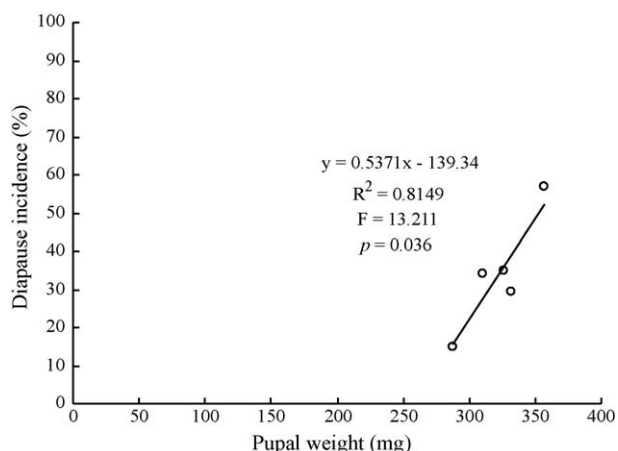
	N	Diapause incidence (%) ^a	Pupal weight	Duration of larval stage	Duration of prepupa
control (without deprivation)	65	29.23	332.11 ± 4.16 b	14.98 ± 0.23 c	5.28 ± 0.16 b
2d deprivation at 3 rd instar stage	58	56.89	356.54 ± 6.65 a	17.27 ± 0.33 b	6.17 ± 0.20 a
2d deprivation at 4 th instar stage	47	34.78	326.43 ± 6.81 ab	17.13 ± 0.32 b	5.93 ± 0.18 ab
2d deprivation at 5 th instar stage	40	34.15	309.99 ± 6.06 bc	19.80 ± 0.32 a	6.76 ± 0.26 a
2d deprivation at 6 th instar stage	53	14.81	288.38 ± 6.25 c	17.46 ± 0.34 b	6.17 ± 0.16 a
F/Chi-square		23.049	18.9003	30.798	7.895
df		4	4, 260	4, 258	4, 258
p		< 0.0001	< 0.0001	< 0.0001	< 0.0001

Data are shown as mean ± S.E. Different letters in the same column mean significant at $p < 0.05$ (Post hoc tests with Scheffe).^a Chi-square test was conducted.

4. Discussion

Our laboratory experiments with host plants suggest that the proportion of *H. armigera* pupae that enter diapause varies among host-plant species at a moderate temperature (25 °C) and may be concealed at lower temperatures (20 °C). Our results showed that larval host plants had a direct effect on diapause induction for *H. armigera*, and this effect is independent of abiotic factors such as temperature and photoperiod. Different host plants contain various nutrient levels, which could affect life-history traits such as developmental duration and pupal weight (Liu et al., 2004). In this study, pupal weight and developmental duration were significantly correlated with host-plant species, and further analysis showed pupal weight rather than duration of sensitive stages for diapause induction was significantly affected by the diapause status, i. e. diapausing pupae of *H. armigera* were heavier than non-diapausing pupae in all treatments, which indicates the important influence of pupal weight on diapause induction. Further deprivation experiments with an artificial diet demonstrated that diapause occurrence in *H. armigera* is a weight issue: individuals must attain sufficient weight if they are to be candidates for diapause.

In the experiment of diapause induction on host plant, we did find diapause incidence was correlated with host plant (Fig. 2A, not pupal weight and duration of *H. armigera* fed on host plant (Fig. 2 B, C, D). Analysis showed pupal weight, which is affected by host plant and insect developmental duration, was a critical factor for diapause occurrence. Further experiments with diet deprivation indicated that pupal weight was critical for diapause induction as shown by linear regression between diapause incidence and pupal weight. These two results are not contradictory since host plant, compared with artificial diet, is a complex system which

**Fig. 6.** Diapause incidence based on pupal weight of *H. armigera* fed artificial diet

contains various nutrients and secondary substances that could affect diapause occurrence.

Since Hunter and McNeil (1997) reported their results on a leafroller, *Choristoneura rosaceana*, research on the effects of host-plant species on diapause has increased (Takafuji and Morishita, 2001; Ito and Saito, 2006). Hunter and McNeil (1997) demonstrated that host-plant species have a direct effect on diapause induction that is independent of abiotic factors. Furthermore, using artificial diet, they confirmed the direct role that nutritional quality plays in the initiation of diapause: diapause incidence in insects that fed on low-quality diet was twice that of insects that fed on high-quality diet under constant day length and temperature. Takagi and Miyashita (2008) also showed that swallowtail butterflies had a higher diapause incidence and grew more slowly when they fed on tough leaves than when they fed on soft leaves. According to the optimal timing of diapause induction (Harrison and Munns, 1984; Taylor, 1986; McNamara, 1994), a high-quality host should support heavy body mass and shorten developmental duration, which in turn should diminish diapause incidence (Hunter and McNeil, 1997; Wedell et al., 1997; Takagi and Miyashita, 2008). Our results confirmed that larval host-plant species affected diapause occurrence, supporting a close relationship between insect life-history traits and host-plants species. However, we also found that diapause occurrence correlated directly with pupal weight and only indirectly with the length of duration of sensitive stage for diapause induction. Only those well-prepared individuals, meaning those with sufficient body size, are candidates for diapause. According to our finding, under warm diapause-inducing conditions such as 25 °C with L:D = 8:16 (as opposed to harsh conditions, namely 20 °C with L:D = 8:16), good-quality host plants would support sufficient body size and cause a higher diapause incidence (as shown in Fig. 2A). This does not confirm the results of Hunter and McNeil (1997), which showed good quality host plants supporting low diapause incidence. The main difference between our study and previous ones is the harshness of the conditions under which diapause was induced (such as 20/25 °C with L:D = 8:16). Our study shows that harsh conditions lead to nearly 100% diapause incidence and conceal the effects of body size.

It is particularly obvious in polyphagous insects that host-plant quality affects larval growth rate, which may in turn further affect the sensitive stage for diapause induction (Hunter and McNeil, 1997; Wednell et al., 1997). Insects that grew faster on good-quality host plants had a lower diapause incidence than those that fed on poor-quality host plants. Possibly diapause occurrence has something to do with the duration of the sensitive stage for diapause induction. For *H. armigera* larvae, the final instar stage is the most sensitive stage for diapause induction (Li and Xie, 1981). In our study, developmental duration correlated significantly with host-plant species, which further correlated with pupal weight. However, the duration of the final instar larval and prepupal stages was not positively correlated with diapause incidence among the

host plants tested, and diapause status did not affect developmental duration (as shown in Table 2).

In this paper, we confirmed that diapausing cotton bollworm pupae weighed more than non-diapausing pupae that had been raised under the same conditions. *H. armigera* need to accumulate energy storage for over-wintering diapause (Liu et al., 2007), just as many species do (Storey and Storey, 1991; Sakurai et al., 1992; Hokkanen, 1993; Zvereva, 2002). Larvae destined to become diapausing pupae accumulate energy before diapause, and the ability to store energy varies according to the larval host plants because of their nutritional quality and, possibly, the amount of their secondary metabolites (Liu et al., 2007). The higher the quality of the larval host plants, the better the insect's preparedness for over-wintering (Liu et al., 2007; 2009). Moreover, there appears to be a trade-off between pupal weight and diapause occurrence in *H. armigera*. As facultative diapause is expected to evolve in unpredictable seasonal environments that promote multi-voltinism (Tauber and Tauber, 1982; Mousseau and Roff, 1989; Bradford and Roff, 1993; Carriere and Roitberg, 1994; Hunter and McNeil, 1997), the ability of enter into it has profound ecological and evolutionary implications for an insect species (Roff, 1983; Tauber et al., 1986; Carriere et al., 1995; Hunter and McNeil, 1997). In theory, a characteristic threshold body weight for each host plant feeding insects can be defined to separate diapause and non-diapause pupae. If body size doesn't reach this threshold, the individual will not enter diapause, which has significant implications: individuals unprepared to over-winter will die during long cold winters (Liu et al., 2007). Facultative diapause can maximize insect fitness by producing additional generations in a favorable year or by causing individuals to enter diapause if conditions are unfavorable (Tauber and Tauber, 1982; Rausher, 1985; Mousseau and Roff, 1989; Carriere, 1994). *H. armigera* appears to have evolved a mechanism for diapause based on pupal weight to maximize its fitness. For insects that have to accumulate energy reserves prior to diapause (reviewed by Hahn and Denlinger, 2007), only those big enough to enter diapause would survive the cold winter because of better energy reserve preparedness.

In summary, host-plant quality influences diapause in *H. armigera* due to differences in pupal size that are attained on specific plants. As review by Hahn and Denlinger (2007), some insects need to accumulate energy reserves prior to diapause and some insect do not, *H. armigera* belongs to the former. Under warm diapause-inducing conditions, insects that have accumulated energy reserves and therefore gained more weight are more prone to enter diapause, and there is a linear regression between diapause incidence and pupal size; moreover, diapause occurrence does not correlate directly with development duration. *H. armigera* has evolved a strategy of determining diapause incidence partially based on pupal body size. Each kind of plant on which *H. armigera* feeds has a threshold pupal weight for the insect to enter diapause, allowing them to adapt to unpredictable environments, i.e. to survive over-wintering and to possibly produce additional generations during the season.

Acknowledgments

We thank Professor David Denlinger for reviewing the manuscript and offering constructive comments and Emily Wheeler for editorial assistance. The work was supported by National Basic Research Program of China (973 Program, No. 2006CB102006) and the National Nature Sciences Fund (No.30070128).

References

- Bernays, E.A., 1990. Insect – plant interactions. Volume II. CRC Press, New York. New York, USA.
- Bradford, M.J., Roff, D.A., 1993. Bet hedging and the diapause strategies of the cricket *Allonemobius fasciatus*. *Ecology* 74, 1129–1135.
- Brodeur, J., McNeil, J.N., 1989. Biotic and abiotic factors involved in diapause induction of the parasitoid, *Aphidius nigripes* (Hymenoptera: Aphidiidae). *Journal of Insect Physiology* 35, 969–974.
- Carriere, Y., Roff, D.A., Deland, J.-P., 1995. Evolution of diapause and insecticide resistance: a test of an optimality model. *Ecology* 76, 1497–1505.
- Carriere, Y., Roitberg, B.D., 1994. Trade-offs in performance on different hosts within a population of a generalist herbivore. *Choristoneura rosaceana* (Lepidoptera: Tortricidae). *Entomologia Experimentalis Applicata* 72, 173–180.
- Danks, H.V., 1987. Insect dormancy: an ecological perspective. Biological Survey of Canada Monograph series 1. Ottawa, Canada.
- de Wilde, J., 1962. Photoperiodism in insects and mites. *Annual Review of Entomology* 7, 1–26.
- Hagen, K.S., 1962. Biology and ecology of predaceous Coccinellidae. *Annual Review Entomology* 7, 289–326.
- Hahn, D.A., Denlinger, D.L., 2007. Meeting the energetic demands of insect diapause: nutrient storage and utilization. *Journal of Insect Physiology* 53, 760–773.
- Harrison Jr., N.G., Munns Jr., W.R., 1984. The timing of copepod diapause as an evolutionary stable strategy. *American Naturalist* 123, 733–751.
- Hodkova, M., Socha, R., 1995. Effect of temperature on photoperiodic response in a selected nondiapause strain of *Pyrrhocoris apterus* (Heteroptera). *Physiological Entomology* 20, 303–308.
- Hokkanen, H.M.T., 1993. Overwintering survival and spring emergence in *Meligethes aeneus*: effects of body weight, crowding, and soil treatment with *Beauveria bassiana*. *Entomologia Experimentalis Applicata* 67, 241–246.
- Hunter, M.D., McNeil, J.N., 1997. Host-plant quality influences diapause and voltinism in a polyphagous insect herbivore. *Ecology* 78, 977–986.
- Hunter, M.D., Willmer, P.G., 1989. The potential for interspecific competition between two abundant defoliators on oak: leaf damage and habitat quality. *Ecological Entomology* 14, 267–277.
- Ito, K., Saito, Y., 2006. Effects of host-plant species on diapause induction of the Kanzawa spider mite, *Tetranychus kanzawai*. *Entomologia Experimentalis Applicata* 121, 177–184.
- Kroon, A., Veenendaal, R., Bruin, J., Efas, M., Sabelis, M.W., 2004. Predation risk affects diapause induction in the spider mite *Tetranychus urticae*. *Experimental and Applied Acarology* 34, 304–317.
- Lenga, A., Glieth, I., Huignard, J., 1993. Interactions between photoperiod, relative humidity and host-plant cues on the reproductive diapause termination in *Bruchidius atrolineatus* (Pic) (Coleoptera: Bruchidae). *Invertebrate Reproduction and Development* 24, 87–96.
- Li, C., Xie, B.Y., 1981. Effects of temperature and photoperiod on diapause of cotton bollworm, *Helicoverpa armigera*. *Entomological Knowledge* 18, 58–61.
- Liu, Z.D., Gong, P.Y., Heckel, D.G., Wei, W., Sun, J.H., Li, D.M., 2009. Effects of larval host plants on overwintering physiological dynamics and survival of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Journal of Insect Physiology* 55, 1–9.
- Liu, Z.D., Gong, P.Y., Wu, K.J., Wei, W., Sun, J.H., Li, D.M., 2007. Effects of larval host plants on over-wintering preparedness and survival of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Journal of Insect Physiology* 53, 1016–1026.
- Liu, Z.D., Gong, P.Y., Wu, K.J., Sun, J.H., Li, D.M., 2006. A true summer diapause induced by high temperature in the cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Journal of Insect Physiology* 52, 1012–1020.
- Liu, Z.D., Li, D.M., Gong, P.Y., Wu, K.J., 2004. Life table studies of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), on different host plants. *Environmental Entomology* 33, 1570–1576.
- Lu, Z.Q., Xu, Y.H., 1998. The consideration with the incessant outbreak of cotton bollworm *Helicoverpa armigera* (Hübner). *Entomological Knowledge* 35, 132–136 (In Chinese with English Abstract).
- McNamara, J.M., 1994. Timing of entry into diapause: optimal allocation to “growth” and “reproduction” in a stochastic environment. *Journal of Theoretical Biology* 168, 201–209.
- Mousseau, T.A., Roff, D.A., 1989. Adaptation to seasonality in a cricket: patterns of phenotypic and genotypic variation in body size and diapause expression along a cline in season length. *Evolution* 43, 1483–1496.
- Rausher, M.D., 1985. Competition, frequency-dependent selection, and diapause in *Battus philenor* butterflies. *Florida Entomologist* 69, 63–78.
- Roff, D.A., 1983. Phenological adaptation in a seasonal environment: a theoretical perspective. In: Brown, V.K., Hodeck, I. (Eds.), *Diapause and life cycle strategies in insects*. Junk, The Hague, The Netherlands, pp. 253–270.
- Ruan, Y.M., Wu, K.J., 2001. Performances of the cotton bollworm, *Helicoverpa armigera* on different food plants. *Acta Entomologica Sinica* 44, 205–212 (In Chinese with English Abstract).
- Sakurai, H., Kawai, T., Takeda, S., 1992. Physiological changes related to diapause of the lady beetle. *Harmonia axyridis* (Coleoptera: Coccinellidae). *Applied Entomology and Zoology* 27, 479–487.
- SPSS. 1999. The Basics: SPSS for Windows 10.0. SPSS Inc. Chicago, IL.
- Stewart, J. W., Whitcomb, W. H., Bell, K. O. 1967. Estivation studies of the convergent lady beetle in Arkansas. *Journal of Economic Entomology* 60, 1730–1735.
- Storey, K.B., Storey, J.M., 1991. Biochemistry of cryoprotectants. In: Lee, Jr., R.E., Denlinger, D.L. (Eds.), *Insects at Low Temperature*. Chapman & Hall, New York, pp. 64–93.
- Takagi, S., Miyashita, Y., 2008. Host plant quality influences diapause induction of *Byasa alcinous* (Lepidoptera: Papilionidae). *Annals of the Entomological Society of America* 101, 392–396.

- Takafuji, A., Morishita, M., 2001. Overwintering ecology of two species of spider mites (Acari: Tetranychidae) on different host plants. *Applied Entomology and Zoology* 36, 169–175.
- Taylor, F., 1986. The fitness functions associated with diapause induction in arthropods I. The effects of age structure. *Theoretical Population Biology* 30, 76–92.
- Tauber, M.J., Tauber, C.A., Masaki, S., 1986. *Seasonal adaptations of insects*. Oxford University Press, New York, NY.
- Tauber, C.A., Tauber, M.J., 1982. Evolution of seasonal adaptations and life history traits in *Chrysopa*: response to diverse selective pressures. In: Dingle, H., Hegmann, J.P. (Eds.), *Evolution and genetics of life histories*. Springer-Verlag, New York, NY, pp. 51–72.
- Wardhaugh, K.G., 1986. Diapause strategies in the Australian plague locust (*Chortoicetes terminifera* Walker). In: Taylor, E., Karban, R. (Eds.), *The evolution of insect life cycles*. Springer-Verlag, New York, NY, pp. 89–104.
- Wedell, N., Nylin, S., Janz, N., 1997. Effects of larval host plant and sex on the propensity to enter diapause in the comma butterfly. *Oikos* 78, 569–575.
- Wu, K.J., Gong, P.Y., 1997. A new and practical artificial diet for the cotton bollworm. *Insect Science* 14, 227–282.
- Wu, K.M., Guo, Y.Y., 1995. Factors for diapause induced in cotton bollworm. *Helicoverpa armigera* (Hübner). *Acta Phytophylacica Sinica* 22, 331–336.
- Xu, M.X., Zhang, G.X., Zhu, H.F., 1958. Research on cotton bollworm. *Acta Oeconomico-Entomologica Sinica* 1, 18–29 (In Chinese).
- Zalucki, M.P., Murray, A.H., Gregg, P.C., Fitt, G.P., Twine, P.H., Jones, C., 1994. Ecology of *Helicoverpa armigera* (Hübner) and *H. punctigera* (Wallengren) in the inland of Australia: larval sampling and host plant relationships during winter and spring. *Australia Journal of Zoology* 42, 329–346.
- Zvereva, E.L., 2002. Effects of host plant quality on overwintering success of the leaf beetle *Chrysomela lapponica* (Coleoptera: Chrysomelidae). *European Journal of Entomology* 99, 189–195.