Pupal diapause of *Helicoverpa armigera*: sensitive stage for photoperiodic induction

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Abstract

*Helicoverpa armigera* exhibits a facultative pupal diapause, which depends on temperature and photoperiod. Pupal diapause is induced at 20°C by short photoperiods and inhibited by long photoperiods during the larval stage. Sensitive stages for photoperiodic induction of pupal diapause were determined by transferring larvae of various instars between long and short photoperiods. When larvae reared at 20°C under a short photoperiod during egg to fourth instar were transferred to a long photoperiod at the early fifth instar, diapause was not induced. However, when larvae were transferred to a long photoperiod in the middle of fifth instar, diapause was induced. On the other hand, when larvae reared under a long photoperiod during egg to fourth instar were transferred to a short photoperiod at the early fifth instar, about 80 to 100% pupae entered diapause. These results show that the early fifth larval instar is the main sensitive stage for pupal diapause determination even though photoperiod has some effect in the third and fourth instars.

Key words: Diapause; *Helicoverpa armigera*; photoperiodic induction; sensitive stage

INTRODUCTION

The cotton bollworm, *Helicoverpa armigera* (Hübner) is one of the most serious pests of crops in Asia, Australia, Africa and Europe (Reed, 1965; Hackett and Gatehouse, 1982; Zalucki et al., 1986; Farrow and Daly, 1987). The species is distributed over most of Japan and attacks many host plants (Yoshimatsu, 1995; Hamamura, 1998). The wide geographic distribution shows that the species can adapt to seasonal changes from tropical regions to cool temperate regions where it overwinters in a diapause stage. Izumi et al. (2005) have reported that this species enters diapause with high cold tolerance to overwinter in temperate regions, as in other insect species (Tauber et al., 1986). Diapause induction in numerous insect species is regulated by environmental cues such as photoperiod and temperature (Danilevsky, 1961; Tauber et al., 1986; Danks, 1987; Tsumuki, 1990). The pupal diapause of *H. armigera* is also regulated by environmental cues during the larval stage (Reed, 1965; Roome, 1979; Nibouche, 1998; Qureshi et al., 1999, 2000; Shimizu and Fujisaki, 2002). When larvae of the Okayama population of this species were reared at 20°C pupal diapause was induced by a short photoperiod (10L : 14D), but not by a long photoperiod (14L : 10D) (Qureshi et al., 1999, 2000). However, the photosensitive larval instar for diapause induction is unknown. In the present study, we identified the photosensitive instars for diapause induction in the Okayama population of *H. armigera*.

MATERIALS AND METHODS

Insects. Larvae were collected initially from cabbage fields in Ushimado (34.6°N; 134.1°E), Okayama Prefecture in September 2001. Insects were reared in the laboratory for several generations. Photosensitive strains in which diapause is induced at 20°C under a short photoperiod (10L : 14D) were selected during two or three generations and used in the present experiment. Eggs were collected daily and the larvae reared on artificial diet by the method of Qureshi et al. (1999) with some modifications. To observe the date of burrowing into soil, about 2 cm of vermiculite was placed in the bottom of the 30 ml of plastic rearing
vial at the beginning of the fifth larval instar.

**Treatments.** Larvae and pupae were transferred between long (14L : 10D) and short (10L : 14D) photoperiods at different larval instars and pupal stages to determine the incidence of diapause in the different treatments. Newly oviposited eggs were held in the photoperiods to which the first instar was exposed. Larvae in individual instars were transferred between the two photoperiods within 24 h after molting. Some late fifth instar larvae were transferred to dark conditions. Fourteen to 32 eggs or larvae (mean 23) were used in the individual treatments.

**Diapause determination.** Diapause was determined by a lack of eye spot movement. Pupae that retained eyespots in the post genal region for more than 15 d were considered to be in diapause (Cullen and Browning, 1978).

**Statistical analysis.** Fisher’s exact test for count data was used to determine the significance of differences in diapause induction rates. Data for each group were compared to data for the initial photoperiod in each experimental series.

**RESULTS**

**Transfers between photoperiods in individual instars**

When larvae were reared from eggs to pupae at 20°C under either a short or a long photoperiod, more than 80% and less than 10% pupae entered diapause, respectively. When larvae exposed to a short photoperiod from egg through first or combined first and second instars were transferred to a long photoperiod, less than 10% of both male and female pupae entered diapause (Fig. 1). However, when fourth and fifth instar larvae were transferred to a long photoperiod, diapause induction rates increased in both male and female pupae.

When larvae reared under a long photoperiod from egg through fourth instar were transferred to a short photoperiod, about 80 to 100% of pupae entered diapause (Fig. 2). The incidence of diapause was comparable to that obtained by exposing larvae to a short photoperiod from the egg until the end of the fifth instar.

**Transfers between photoperiods during the fifth instar**

Larvae were transferred between the two photoperiods at the early (within 1 d after molting), middle (end of feeding, about 5 d after molting) and late (burrowing into vermiculite, about 8 d after molting) fifth instar. When larvae reared from the egg at 20°C under a short photoperiod were transferred to a long photoperiod in the middle or late fifth instar almost all pupae entered diapause (Fig. 3). Furthermore, the late fifth instar larvae transferred to dark conditions also entered diapause. However, when the larvae were transferred to a long photoperiod early in the fifth instar, pupal diapause induction rate was reduced in males to about 50% and in females to about 25%.

When larvae reared from the egg under a long photoperiod were transferred to a short photoperiod in the middle or late fifth instar, diapause was much reduced (about 20% male and 10% female
The diapause of the larvae transferred to dark conditions was also much reduced. However, when the larvae were transferred to a short photoperiod early in the fifth instar, almost all pupae entered diapause, but diapause induction rate of female pupae was reduced slightly.

**DISCUSSION**

Several species with a pupal diapause are most photosensitive to diapause induction in the last two instars before pupation (Danks, 1987). Furthermore, Phillips and Newsom (1966) have suggested in preliminary experiments that both penultimate and last larval instars of the bollworm, *H. zea* (Boddie) are sensitive to diapause induction. However, no experimental data about the photosensitive stages for diapause induction is comprised in their paper. In the present experiment, we identified the photosensitive larval stage for diapause induction or avoidance by transferring larvae between long and short photoperiods. When larvae reared under a short photoperiod were transferred to a long photoperiod at the beginning of the fourth or fifth instar, diapause induction rates increased compared with transfers at the first, second and third instars (Fig. 1). The results show that third and fourth instar larvae have photoperiodic sensitivity. However, diapause induction rates were less than 40%, showing that these instars may not be the main photosensitive stage. Transfers from short to long photoperiods in the fourth and fifth instars inhibited diapause induction and induced some diapause, respectively, but almost all pupae transferred at the middle fifth instar entered diapause (Fig. 3). These results show that the photosensitive stage for diapause determination is the early fifth instar. When larvae reared under a long photoperiod were transferred to a short photoperiod in the early fifth instar, almost all pupae entered diapause (Fig. 2), but when the larvae were transferred in the middle and late fifth instars, almost no larvae entered diapause (Fig. 4). These results confirm that the main photosensitive stage for diapause determination is the early fifth instar. Because late fifth instar larvae burrow into soil in fields, these larvae or pupae may not be affected by photoperiod. Actually diapause induction rate was not affected when late fifth instar larvae were transferred to dark conditions (Figs. 3 and 4).

Pupal diapause is induced by conditions such as photoperiod and temperature during adult and egg stages in *H. punctigera* (Wallengren) (Cullen and Browning, 1978) and *H. zea* (Wellso and Adkisson, 1966). However, the maternal influence can be reversed by the photoperiod during the developmental period in larval and pupal stages (Wellso and Adkisson, 1966). We did not study the maternal influence in this experiment, but we can say that photoperiod during the egg stage did not affect pupal diapause.
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