Photoperiodic clock of diapause termination in *Pseudopidorus fasciata* (Lepidoptera: Zygaenidae)

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Abstract. Photoperiodic control of diapause termination was systematically investigated in *Pseudopidorus fasciata*. In 24 h light-dark cycles, the rate of diapause termination in this species depended on photoperiod. The critical night length (CNL) for diapause termination was 10 h, 0.5 h shorter than that for diapause induction. Night-interruption experiments with T = 24 showed that diapause was effectively terminated when the scotophases separated by light pulse were shorter than the critical night length (10 h); no developing individuals were found if the duration of the pre-interruption scotophase or the post-interruption scotophase exceeded the CNL. A 15-min light pulse was sufficient to reverse the effect of long night when it was placed 8 h after lights-off. Resonance experiments with a constant photophase of 12 h or 16 h and various scotophases of 4–80 h showed an hourglass-type photoperiodic response, where no rhythmicity was found. In another resonance experiment with constant scotophase of 8 h and various photophases of 4–72 h, all individuals developed into cocoons. In the Bünsow experiment, the response curve showed two apparent peaks for diapause termination, one being 8 h after lights-off, and another 8 h before lights-on. However, there was no periodic rhythmicity, which again indicates an hourglass principle. The results lead to the conclusion that the same photoperiodic clock mechanism (a long-night measuring hourglass) is involved in both diapause induction and termination.

INTRODUCTION

Of all the physical factors that change seasonally, photoperiod is the most regular and reliable cue to seasons. Insects have evolved numerous ways to utilize photoperiod as a diapause-regulating cue (Tauber et al., 1986). So far, photoperiodic control of diapause induction has been examined in a large number of insect species. On the other hand, termination of diapause by photoperiod was reported only in about forty species (see Beck, 1980; Saunders, 1982; Koveos et al., 1993; Claret & Arpagaus, 1994).

If an insect relies upon photoperiodic information for terminating its diapause, one might logically assume that the clock system underlying termination of diapause is the same mechanism as the one involved in induction of diapause. Thus, the methodology used for analyzing photoperiodic induction of diapause is also relevant for the study of diapause termination. Brunnarius and Dumortier (1984) were the first to apply the same technique in an experiment on diapause termination in *Pieris brassicae*. They found that in both diapause induction and termination, it was actually the scotophase length that was being measured. The same conclusion was also found in *Tetranychus urticae* (Koveos et al., 1993).

Induction of larval diapause, an overwintering dormancy, is a photoperiodically controlled event in the life cycle of the lepidopteran, *Pseudopidorus fasciata* (Xue & Kallernborn, 1998). Photoperiodic clock for diapause induction was systematically investigated by Wei et al. (2001) using various light-dark (LD) cycles, night-interruption experiments, resonance experiments and

Bünsow protocols. In the present study, these methods were used to assess the photoperiodic time measurement for diapause termination in *P. fasciata*.

MATERIAL AND METHODS

The population of *P. fasciata* used in this study was collected in the suburbs of Nanchang (28°46′N, 116°50′E), Jiangxi Province, P. R. China. Full-grown larvae prior to cocooning were collected in the field. The larvae were allowed to form cocoons and to emerge under natural conditions. Eggs used in all experiments were obtained from females reared in the insectarium. When larvae newly hatched, they were transferred to a diapause-inducing regime (12L : 12D and 25°C) and kept there for 15 days. Under this condition all individuals enter a larval diapause. Then, all diapausing larvae were transferred to various LD cycles to test termination of diapause. The criterion of diapause termination was the forming of a cocoon; and the duration of diapause included the period of post-diapause larval development. The larvae were fed with fresh foliage of Chinese sweetleaf, *Symplocos chinensis* (a deciduous shrub). At least 50 larvae were used for each treatment unless otherwise noted. Rearing conditions have been described elsewhere (Wei et al., 2001).

RESULTS

Photoperiodic response curves under 24-h light-dark cycles

Diapausing larvae were maintained at a daily mean temperature of 26.7°C in an insectarium and a constant temperature of 26 ± 1°C in the incubators (LRH-250-G) with the photoperiods ranging between 2L : 22D and 22L : 2D as well as continuous light (LL) and continuous darkness (DD). Fig.1 shows that diapause termination in

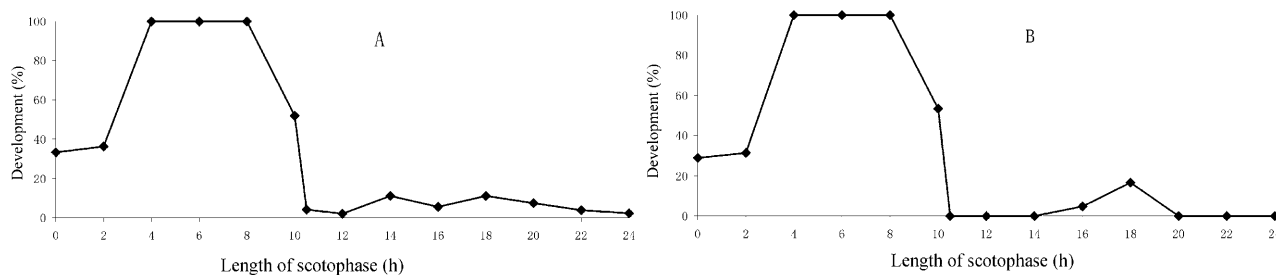* Corresponding author. Fax: 0086-791-3814107; e-mail: fangsen@public.nc.jx.cn

Fig. 1 Photoperiodic response curves for the termination of diapause in *P. fasciata* under 24 h light-dark cycles at a daily mean temperature of 26.7°C (A) and a constant temperature of 26°C (B).

this species is controlled by photoperiod. Night lengths of 4 to 8 h (i.e. from 20L : 4D to 16L : 8D) terminated diapause. However, a low level of diapause termination occurred in LL and a scotophase of 2 h (33.3% and 36.3% in Fig. 1A; 29.0% and 31.4% in Fig. 1B). Night lengths of more than 10 h maintained larvae in diapause. The critical night length (CNL) was 10 h.

Night-interruption in 24-h photoperiod

The night of a 9L : 15D (a most effective diapause-maintaining photoperiod) was systematically illuminated by a single 1-h pulse at 26°C and the percentage of diapause termination was observed (Fig. 2). The result showed that the light pulses placed 7–10 h after the onset of darkness caused a complete reversal of the response. Both the lengths of pre-interruption scotophase and post-interruption scotophase were shorter than the CNL. When the light pulses were placed 2–4 h and 12–14 h in the darkness, very few individuals developed. Here, the durations of the pre-interruption or post-interruption scotophases exceeded the critical day length. Fig. 2 also shows that the light pulses commencing 5–6 h after the onset of darkness induced development in much smaller percentage of individuals (26.1% and 62.9%) than did pulses commencing 10–11 h (43.1% and 100%), although the

lengths of their scotophases are equal (10 h and 9 h). This may suggest that the late phase in the dark period is more photosensitive than the early phase.

Minimal light requirement for night interruption

To find the shortest night interruption required to terminate diapause, light pulses ranging from 5 to 60 min were given during 8 h in the scotophase of LD 9:15 (the most sensitive part to light in diapause induction). An illumination of 15 min was sufficient to reverse the effect of long night, inducing development in 93% individuals (Fig. 3).

Resonance experiments (or Nanda-Hamner experiments)

In the first resonance experiment, the scotophase was serially extended from 4 to 80 h with an interval of 4 h. The unchanged photophase was 12 h (Fig. 4A) or 16 h (Fig. 4B-C). The results showed that photoperiodic responses depended on whether the night lengths exceeded the CNL or not. Development occurred at LD 12:4, 12:8, 12:10, 16:4, 16:8 and 16:10. Night lengths longer than 10 h did not result in the termination of diapause except few individuals in some treatments. No rhythmicity was found in the photoperiodic response.

When a constant scotophase of 8 h was combined with various photophases of 4–72 h, all light regimes provoked
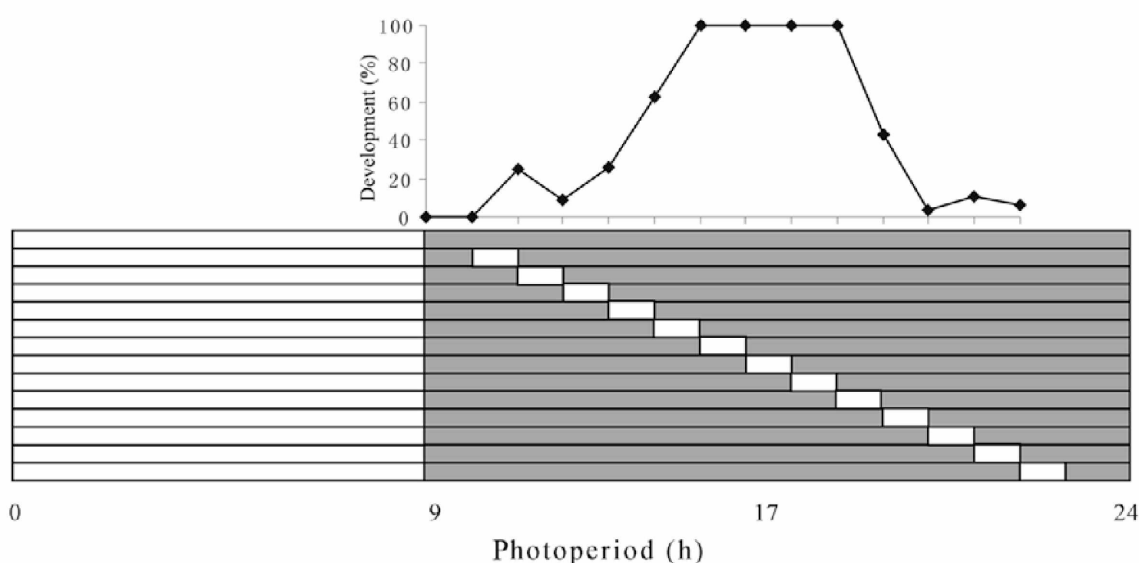

Fig. 2 Night interruption for diapause termination of *P. fasciata* in a 9L : 15D, 26°C regime. The scotophase was systematically scanned by 1-h light pulse.

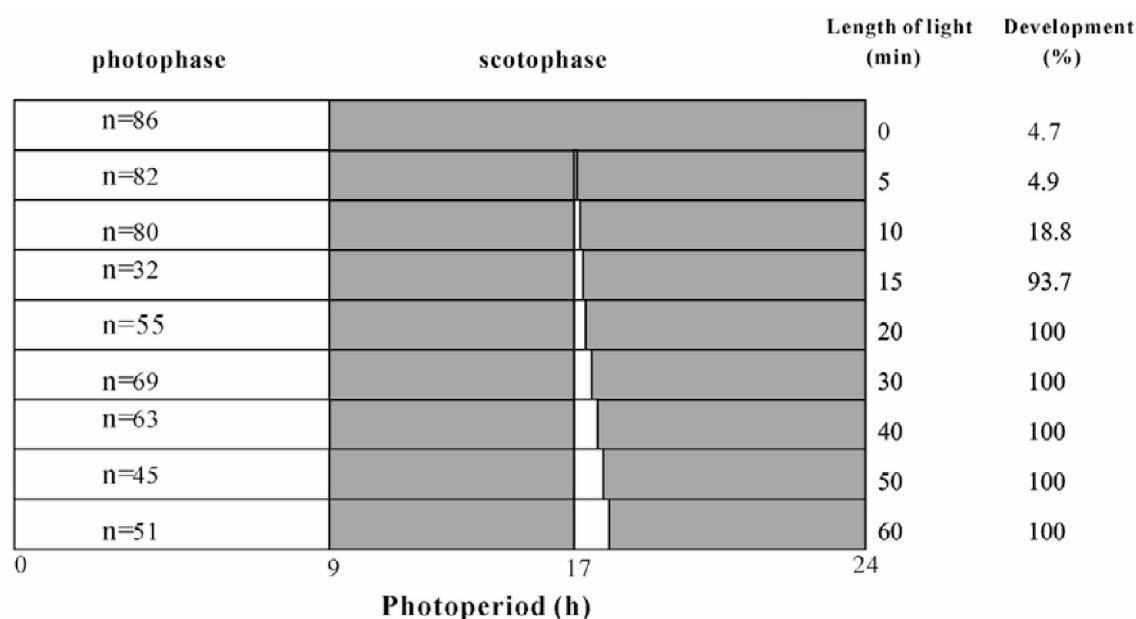| photophase	scotophase	Length of light (min)	Development (%)
n=86		0	4.7
n=82		5	4.9
n=80		10	18.8
n=32		15	93.7
n=55		20	100
n=69		30	100
n=63		40	100
n=45		50	100
n=51		60	100

Photoperiod (h)

Fig. 3 Length requirement of the night interruption to terminate diapause of *P. fasciata*. A photoperiod of 9L : 15D was interrupted by a light pulse of different length commencing 8h after the onset of darkness at 26 °C.

termination of diapause, although the process of diapause termination in the short photophases of 4 h and 8 h took longer than that in the other photophases (Fig. 5). The required mean short nights to terminate diapause at this resonance experiment varies from 27 to 51 days at 26°C (Fig. 6). The result indicates that a period of 8 h of darkness had been measured and that measurement can take place regardless of the period of the light cycle.

These results are consistent with what is expected from an hourglass principle.

Bünsow experiments

In the Bünsow experiments, a constant photophase of 12h was combined with a scotophase of 36, 48 or 60 h, which were scanned by 1-h light pulses at 4-h intervals. The experiments were performed at a daily mean temperature of 27.5°C in an insectarium (Fig. 7). The results showed that only the light pulse placed 8 h after the onset of darkness terminated diapause most effectively. The light pulse placed 8 h before dawn produced a relatively small peak of diapause termination, whereas others had little effect on diapause termination.

DISCUSSION

As compared with the photoperiodic response curve for diapause induction in *P. fasciata* (Wei et al., 2001), the response curve for diapause termination is just a reversal within the photoperiodic regimes ranging between 20L : 4D and DD at 26°C. Namely, short days induced diapause and long days terminated diapause. However, some differences occurred. Very long photophases of 22 h and LL induced 31.4% and 29.0% diapause termination, respectively (Fig. 1), whereas they completely prevented diapause induction. The same kind
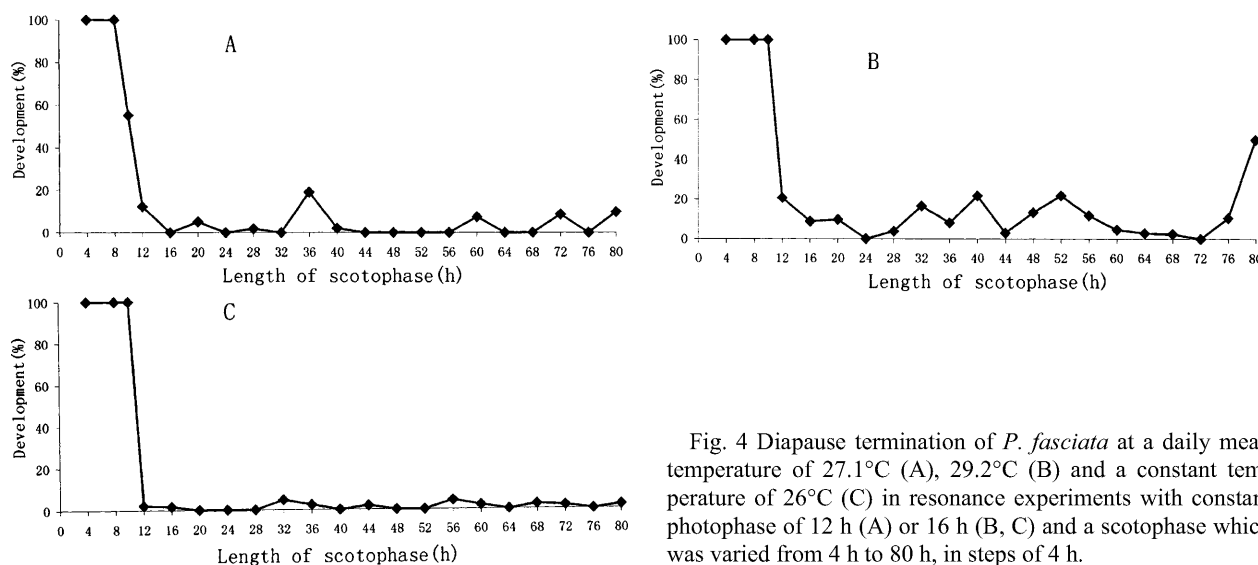

Fig. 4 Diapause termination of *P. fasciata* at a daily mean temperature of 27.1°C (A), 29.2°C (B) and a constant temperature of 26°C (C) in resonance experiments with constant photophase of 12 h (A) or 16 h (B, C) and a scotophase which was varied from 4 h to 80 h, in steps of 4 h.
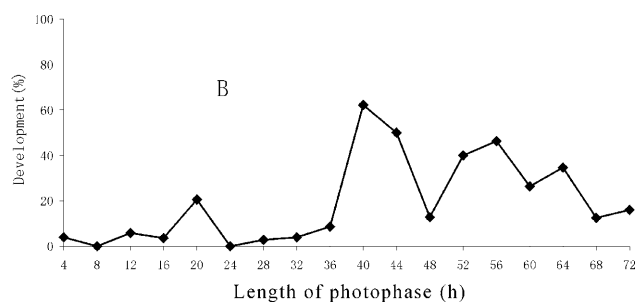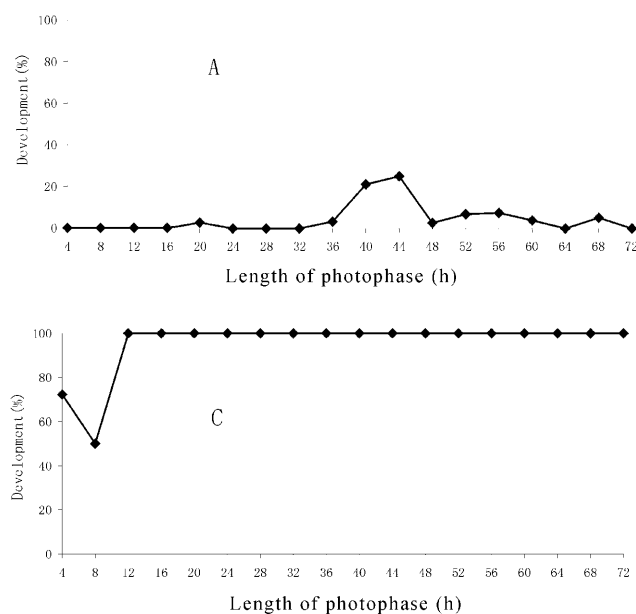
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Fig. 5 Diapause termination of *P. fasciata* in a resonance experiment with a constant scotophase of 8 h and a photophase which was varied from 4h to 72h, in steps of 4h. The number of larvae that had terminated diapause, expressed as the percentage of total larvae present, was determined on the 25th day (A), 28th day (B) and 51st day (C) after diapausing larvae were transferred to the resonance experiments.

of opposite response was found in *Pieris brassicae* (Brunnarius & Dumortier, 1984). The curves were also slightly different in the critical night length, shorter in diapause termination by about 0.5 h (10.5 h for diapause induction). However, the critical night length for both diapause induction and termination appears to be the same under natural conditions. According to field observations for many years, diapause rate of 50% is reached in early September at day length of about 13 h 32 min (including twilight) (Xue & Kallenborn, 1998), and termination of hibernation occurred around April 10 at day length of about 13 h 31 min. The differences between laboratory and field conditions may be caused by temperature conditions, because diapause termination of overwintering larvae in the field experienced a very long period of low winter temperatures.

Comparisons of the critical daylengths for diapause induction and termination have been presented for a number of insects. A longer critical daylength for diapause termination than for diapause induction was found for *Lygus hesperus* (Beards & Strong, 1966), *Pyrrhocoris apterus* (Saunders, 1983), *P. brassicae* (Brunnarius & Dumortier, 1984), *Diatraea grandiosella* (Takeda, 1985), and *Ostrinia nubilalis* (Skopik & Takeda, 1986).
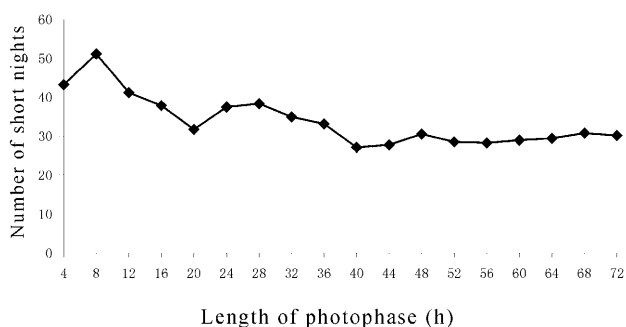

Fig. 6 Average number of short nights required for 100% diapause termination in resonance experiments with 8h of darkness per cycle and various periods of light at 26 °C.

The effect of night interruptions on diapause inhibition has been investigated for a number of insect species (see Saunders, 1982; Koveos et al., 1993), but night interruption experiments on diapause termination have been done only in a few species. The first study was carried out by Hayes et al. (1970) on the oak silkworm *Antheraea pernyi* and the codling moth *Laspeyresia pomonella*. However, no clear effects of light pulses were observed. In a further study, Hayes et al. (1974) applied the same technique to four lepidopteran species under natural light and temperature conditions. They also did not provide any reliable evidence of the role of a light pulse in diapause termination in these species. Afterward, the effect of night interruption on diapause termination was demonstrated in five species. Brunnarius and Dumortier (1984) found one point of long day effect for diapause termination in *P. brassicae*, but two points (A and B) in diapause induction. Skopik et al. (1986) found bimodal peaks (A and B) of long day effect in experiments on diapause termination in *O. nubilalis*, but one broad "trough" in experiments on diapause induction. Takeda (1985) and Koveos et al. (1993) found that the response curve to light pulses in diapause termination was a mirror image of that in diapause induction in the south-western corn borer *D. grandiosella* and the spider mite *Tetranychus urticae*, respectively. Claret & Arpagaus (1994) found two points of maximal long-day effect for diapause termination in *Pimpla instigator*. The feature of response to night interruption in *P. fasciata* is similar to that in *D. grandiosella* and *T. urticae*. A light pulse of 1-h at 9L : 15D averted diapause most effectively when it was placed 7–10 h after lights-off (Wei et al., 2001), because the scotophases separated by these light pulses did not exceed CNL of 10.5 h. The same result was obtained in diapause termination, in which light pulses placed 7–10 h after lights-off completely broke diapause (Fig. 2). The results obtained from night interruption experiments strongly suggest that the same clock mechanism is involved in diapause induction and termination in *P. fasciata*. It confirms that the
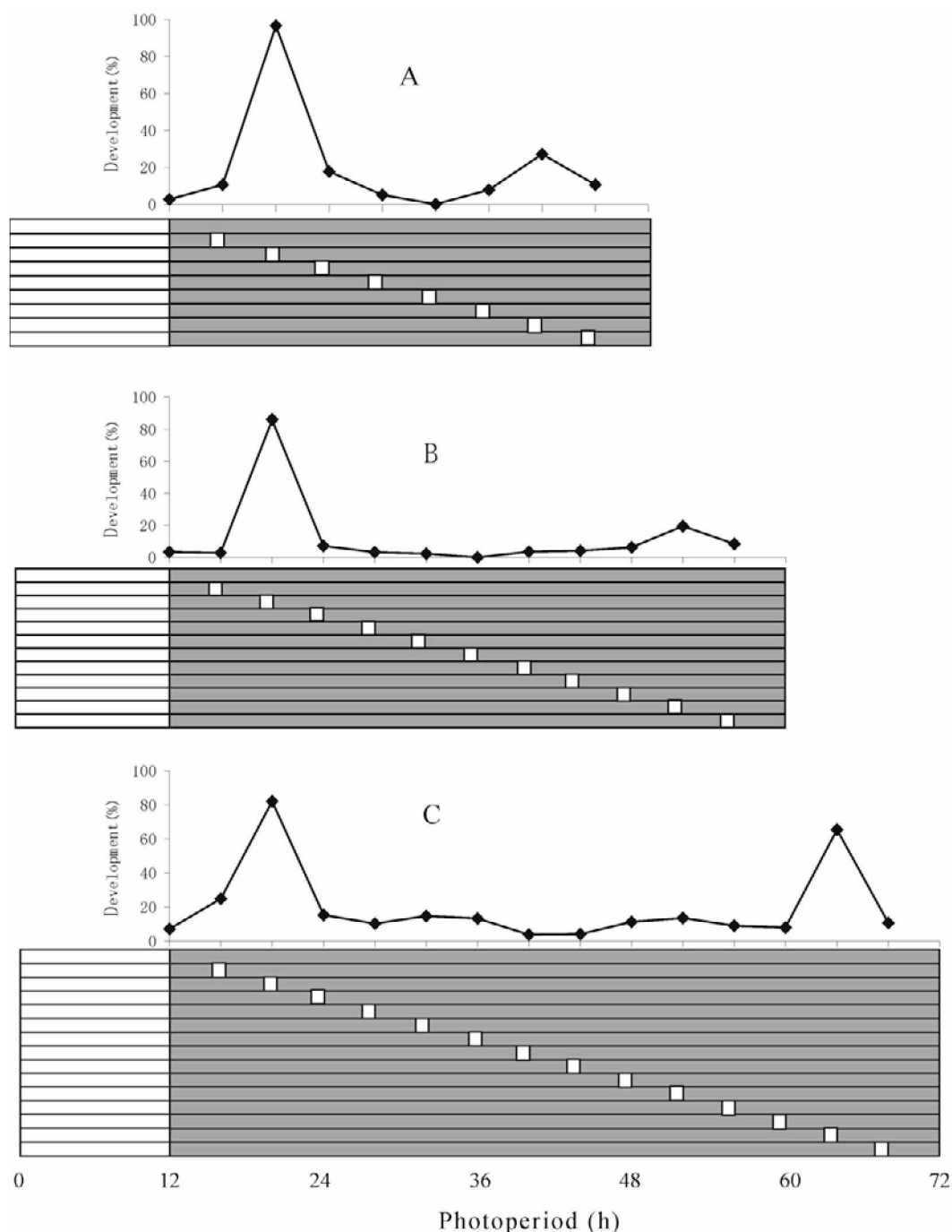
Fig. 7. Diapause termination of *P. fasciata* in Bünsow experiments at a daily mean temperature of 27.5°C with a constant photophase of 12h and an extended scotophase of 36 h (A), 48 h (B) or 60h (C) which was systematically scanned by a 1-h light pulse.

most crucial event for the photoperiodic time measurement in this moth is whether the duration of the scotophase exceeds the CNL or not.

Length requirement for night interruption in diapause induction has been investigated in several insects (Beck, 1962; Barker, 1963; Takeda, 1985), but not studied in diapause termination. In *P. fasciata* a 10-min light pulse falling 8 h in the darkness prevented diapause in over 50% of the individuals (Wei et al., 2001) whereas the same length of light pulse terminated diapause only in

18.8% (Fig. 3). This may suggest that the length requirement of light pulse in diapause termination is longer than that in diapause induction. In Fig. 3 a 15-min light pulse induced development in 93.7% of the individuals, indicating that diapause termination in this species is also very photosensitive.

Resonance experiments for diapause induction have now been conducted with a number of insects and three mites (see Vaz Nunes & Saunders, 1999), whereas resonance experiments for diapause termination have been

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conducted only in five species, *Plodia interpunctella* (Takeda & Masaki, 1976), *P. brassicae* (Claret, 1985), *O. nubilalis* (Skopik & Takeda, 1986), *Pimpla instigator* (Claret & Arpagaus, 1994) and the mite *T. urticae* (Veerman & Koveos, 1989). The first four species showed a negative resonance effect (i.e. no periodic response occurred). *T. urticae* showed a positive resonance effect (i.e. response curve displayed periodic peaks). In addition, in two species (*P. brassicae* [Claret, 1985] and *O. nubilalis* [Skopik & Takeda, 1987]), diapause termination appeared to be controlled by an hourglass, whereas induction had a circadian involvement. In the Russian strain of *T. urticae*, a circadian influence was found to be present on both induction and termination of diapause (Veerman & Koveos, 1989). In *P. fasciata*, the resonance experiments for diapause induction showed a weak circadian periodicity at temperatures of 24.5 and 26°C, but not at 30.5 and 23.3°C (Wei et al., 2001). In *P. fasciata*, however, the resonance experiments for diapause termination with a constant photophase of 12 h or 16 h (Fig. 4) showed that the response curves depended completely on the measurement of CNL; all scotophases longer than the CNL effectively kept larvae in diapause. In the reverse experiment with constant scotophase (Fig. 5, 6), all photoperiods terminated diapause. No periodicity was found. It is very clear that the photoperiodic time measurement for diapause termination in this moth is apparently accomplished according to an hourglass principle. More recently, Veerman (2001) declares that the expression of a circadian rhythm found in Nanda-Hamner experiment does not mean that this rhythm reflects the operation of the photoperiodic clock. The rhythm may be the expression of a circadian disturbance, possibly by internal desynchronization in the unnatural regimes used in Nanda-Hamner experiments. He concludes that photoperiodic time measurement in insects and mites is performed by a non-circadian "hourglass" clock.

So far, Bünsow experiment for diapause termination was done only in the Indian meal moth, *Plodia interpunctella* (Takeda & Masaki, 1976). It showed a negative circadian effect. In *P. fasciata*, the Bünsow experiments showed that the photoperiodic response curves for diapause termination had two apparent peaks: First major peak occurred 8 h after lights-off, second minor peak occurred 8 h before lights-on. However, there was no rhythmicity thereafter and the photoperiodic time measurement may function on the hourglass principle. Such two peaks locked to 8 h after dusk and 8 h before dawn were also found in diapause induction for the codling moth, *Carpocapsa pomonella* (Hamner, 1969) and the aphid, *Megoura viciae* (Lees, 1973).

All experimental results from both diapause induction and termination lead to the conclusion that the same photoperiodic clock mechanism (a long-night measuring hourglass) is involved in both diapause induction and termination in *P. fasciata*.

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