

Photoperiodic response of diapause induction in the pine caterpillar, *Dendrolimus punctatus*

Lili Huang^{1,2}, Fangsen Xue², Guohong Wang³, Ruidong Han¹ & Feng Ge^{1,*}

¹State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Science, Beijing 100080, China; ²Institute of Entomology, Jiangxi Agricultural University, Nanchang 330045, China;

³College of Bioengineering, Fujian Normal University, Fuzhou 350007, China

Accepted: 23 June 2005

Key words: photoperiod, temperature, diapause induction, Lepidoptera, Lasiocampidae, circadian oscillator, non-oscillatory hourglass timer

Abstract

The pine caterpillar, *Dendrolimus punctatus* (Walker) (Lepidoptera: Lasiocampidae), is a multivoltine pest of pine trees in China, overwintering as larvae. Winter diapause was induced by short day length. The critical night length was about 10 h 40 min at 25, 28, and 31 °C in the field, showing a temperature-compensated diapause induction. Transfer experiments from a short night (L16:D8) to a long night (L12:D12) or vice versa at different times after hatching showed that sensitivity to day length was restricted to the first 14 days; the required day number for a 50% response at 25 °C was about 3.5 days for short nights but 7.5 days for long nights, indicating that short nights are photoperiodically more effective. When four successive short nights (L16:D8) were used to interrupt the long-night regime (L12:D12) at different development stages and vice versa, the results showed that the highest sensitivity to photoperiod occurred on the 4th–8th day, corresponding to the second larval instar. Experiments of alternating short-night (L16:D8) and long-night (L12:D12) cycles during the larval period showed that the information of short nights as well as long nights could be accumulated. By rearing the larvae under conditions other than 24-h light–dark cycles, we clearly showed that the dark period (scotophase) played a major role in the determination of diapause. The Nanda–Hamner and Bünsow experiments failed to reveal rhythmic fluctuations with a period of about 24 h in the occurrence of diapause. Therefore, the photoperiodic clock in *D. punctatus* is an hourglass timer or a damped circadian oscillator.

Introduction

Photoperiodic regulation is widespread in terrestrial organisms, including flowering plants, fungi, birds, mammals, mollusks, and arthropods (Hastings & Follett, 2001). Among insects, such seasonality has now been recorded in over 500 species from 17 orders (Nishizuka et al., 1998). Photoperiodic clocks allow organisms to predict the coming season. In insects, the seasonal adaptive response mainly takes the form of diapause. The photoperiodic clock in insects has been extensively studied primarily by a ‘black-box’ approach, resulting in numerous cybernetic models (see Vaz Nunes & Saunders, 1999). Over several decades, formal experiments measuring diapause responses to variable light

inputs have indicated that photoperiodic time measurement in insects is accomplished either by oscillatory components of the circadian system or by a non-oscillatory hourglass mechanism. The two most indicative tests for a rhythmic basis of photoperiodic time measurement are the Nanda–Hamner protocol (Nanda & Hamner, 1958) and the Bünsow protocol (Bünsow, 1960). In the Nanda–Hamner protocol, the duration of the light phase is fixed and various dark intervals are used to produce a set of varying cycle lengths (ranging between 16 and 84 h). In the Bünsow protocol, a fixed light interval is used with a long dark period (e.g., 36, 48, or 60 h), and the dark phase is systematically interrupted by short (1–2 h) pulses of light. If in such protocols the incidence of diapause rises and falls within a period of about 24 h, it is generally interpreted as being the involvement of a circadian clock. If the photoperiodic response with either protocol does not show rhythmic fluctuations within a period of about 24 h, it

*Correspondence: State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Science, Beijing. E-mail: gef@panda.ioz.ac.cn

suggests an hourglass clock or a rapidly damping oscillator (Saunders & Lewis, 1987). However, the question of whether the photoperiodic clock in insects is a circadian oscillator or an hourglass has not been settled. Some studies have supported the former (Bünning, 1936; Pittendrigh & Minis, 1964; Saunders, 2002; Saunders et al., 2004), while others have supported the latter (Lees, 1973; Veerman, 2001).

The pine caterpillar, *Dendrolimus punctatus* (Walker) (Lepidoptera: Lasiocampidae), is one of the most destructive insect pests of the pine tree, *Pinus massoniana*, in agroforestry systems in China (Cheng, 1990). *Dendrolimus punctatus* shows typical facultative diapause. There are 2–3 generations per year near the 30°N region in China (Li et al., 1993; He, 1995). However, there have as yet been few studies on photoperiodic induction of diapause in *D. punctatus* (Li & Gia, 1991; Li et al., 1994). The purpose of the present study was twofold: (1) to determine the role of photoperiod and temperature in the induction of diapause by rearing the pine caterpillar at various photoperiods and temperatures, as this information is useful for forecasting the onset and termination of diapause in the field; and (2) to examine the nature of photoperiodic time measurement of diapause induction by using Nanda-Hammer and Bünsow experiments, in order to better understand the mechanism of photoperiod.

Materials and methods

Insect cultures

The insects used in the experiments were collected from the pine forestry project in Xinjian County (28°46'N, 115°50'E), Jiangxi Province, China, in 2003. The larvae were allowed to form cocoons and to emerge under natural conditions. Eggs used in all experiments were obtained from insectary-reared females. The larvae were fed with fresh pine leaves of *P. massoniana*. At least 50 larvae were used for each treatment unless otherwise noted.

Experimental conditions

The photoperiodic experiments were conducted in illuminated incubators (LRH-250-GS, Guangzhou) equipped with eight fluorescent 30 W tubes controlled by an electric timer. Light intensity at the level of the caterpillars was 700–1000 lux and the variation in temperature was ± 1 °C. The scotophase was controlled manually by enclosing the rearing boxes in opaque hoods.

Diapause identification

The diapausing larvae were easily recognizable by their small size and yellow body color. They settled in the pine needle axil and ceased feeding (Li & Gia, 1989, 1991).

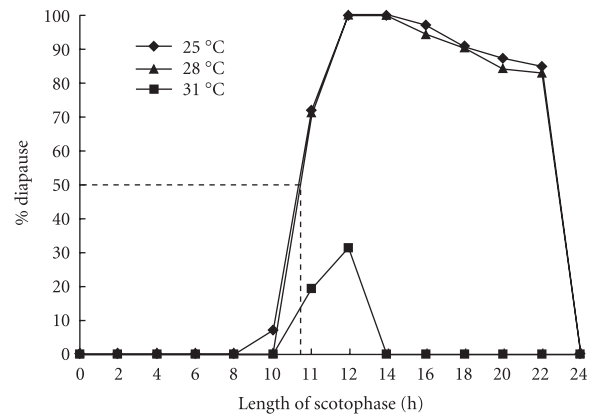


Figure 1 Photoperiodic response curves for the induction of diapause in *Dendrolimus punctatus* under 24-h light–dark cycles at constant temperatures of 25, 28, and 31 °C.

Results

Photoperiodic induction of diapause

Photoperiodic response curves were investigated for the induction of diapause at various temperatures. Figure 1 shows that the photoperiodic response curves were similar, with a critical night length of about 10 h 40 min at 25 and 28 °C. Photoperiods with scotophases from 12 to 22 h induced diapause effectively; scotophases shorter than 12 h prevented diapause; no diapause was induced at continuous darkness. At 31 °C, only the scotophases of 11 and 12 h induced a low incidence of diapause (19.2% and 31.2% diapause, respectively); the others resulted in 100% development without diapause. However, the critical night length at 31 °C was also 10 h 40 min. According to field observations, the night length on August 9 (10 h 42 min) caused about half of the larvae to diapause (Table 1). The results showed that the critical day length obtained with various photoperiods was nearly identical to the critical day length in nature.

Table 1 Incidence of overwintering diapause larvae of *Dendrolimus punctatus* in the field (n = sample size)

Date hatched	n	Diapause		Day length ^a	
		Number	%	h	min
25 July	51	5	9.80	13	36
3 August	52	10	19.23	13	28
9 August	34	18	52.94	13	18
15 August	40	36	90.00	13	13
25 August	42	42	100.00	13	01

^aNot including twilight.

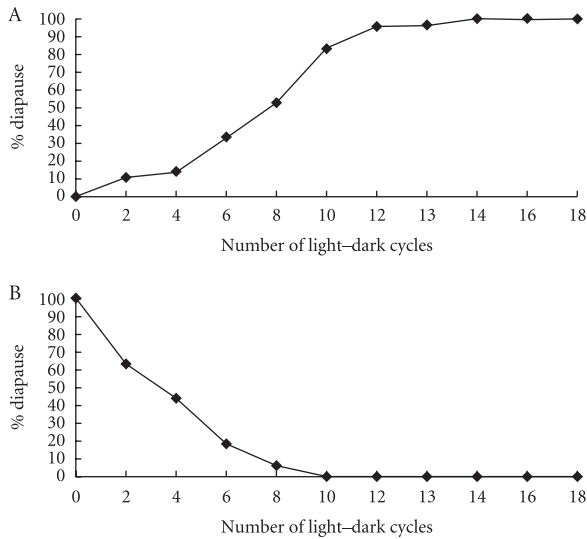


Figure 2 Incidence of diapause in *Dendrolimus punctatus* at 25 °C: (A) when larvae were exposed to different numbers of long-night cycles (L12:D12) then moved to short-night cycles (L16:D8); (B) when larvae were exposed to different numbers of short-night cycles(L16:D8) then moved to long-night cycles (L12:D12).

Sensitive stage for diapause induction

By transferring larvae from a short night (L16:D8) to a long night (L12:D12) or vice versa at different times after hatching, the stage sensitive to photoperiod occurred during the first 14 days of the larval period; the required day number (RDN) was about 7.5 days for long nights and about 3.5 days for short nights (Figure 2). To find the highest sensitive stage, four sequential short nights (L16:D8) were used to interrupt the long-night regime (L12:D12) or vice versa, at various development stages (Figure 3).

When four sequential short nights (L16:D8) were used to interrupt the long-night regime (L12:D12), the highest diapause-averting effect occurred on the 4th–8th day (only 28.1% diapause in Figure 3A), corresponding to the second larval instar. When four sequential long nights (L12:D12) were used to interrupt the short-night regime (L16:D8), the highest diapause-inducing effect also occurred on the 4th–8th day (35.1% diapause in Figure 3B).

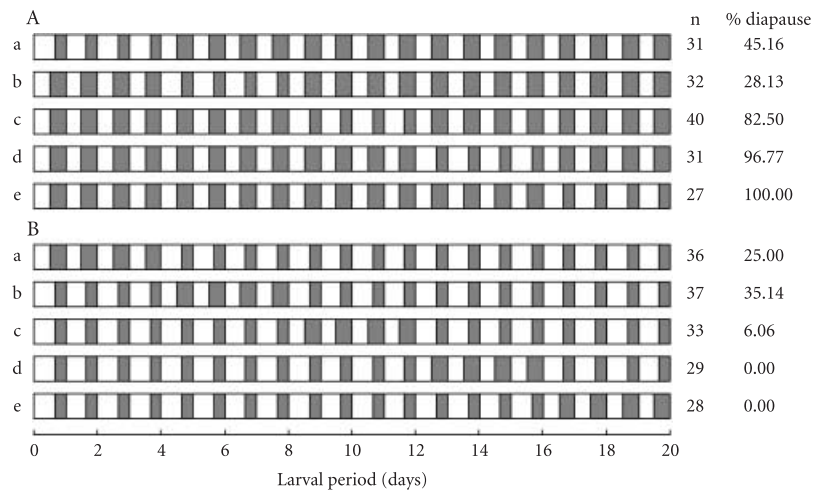
Accumulation of photoperiodic information

When one short-night cycle (L16:D8) was alternated with one long-night cycle (L12:D12) or vice versa during the larval period, the diapause-averting effect of short nights was clearly expressed (only 16.4% diapause in Figure 4C and 10.5% in Figure 4D). However, when four successive short-night cycles were alternated with four successive long-night cycles or vice versa, the diapause-inducing effect of long nights was only partly expressed (43.3% diapause in Figure 4E and 60.8% in Figure 4F). These results illustrate that the information of short nights as well as long nights could be accumulated in different ways.

Photoperiodic responses under non-24-h light-dark cycles

We further carried out experiments with four different photophases ranging from 10 to 16 h, each of which was combined with different scotophases extending from 4 to 24 h (Figure 5). In these experiments, it was obvious that the dark period is of central importance to the timing mechanism. When scotophases were 4 and 8 h, all individuals developed without diapause, regardless of the length of the photophase. With scotophases from 12 to 24 h, however, the individuals, except for those under photoperiods of L10:D24 (Figure 5A) and L12:D24 (Figure 5B), entered diapause independently of the length of the photophase.

Figure 3 Incidence of diapause in *Dendrolimus punctatus* at 25 °C: (A) when four sequential short nights (L16:D8) were used to interrupt the long-night regime (L12:D12); (B) when four sequential long nights (L12:D12) were used to interrupt the long-night regime (L16:D8).



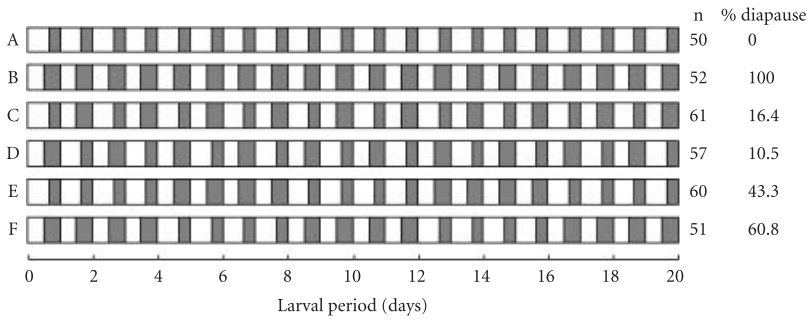


Figure 4 Incidence of diapause in *Dendrolimus punctatus* in response to alternating one or four sequential short-night cycles (L16:D8) and long-night (L12:D12) at 25 °C.

Nanda-Hamner (or resonance) experiment

In the Nanda-Hamner experiments, constant photophases of 12 and 16 h were combined with serially extended scotophases at intervals of 4 h at 25 °C. (Figure 6). The

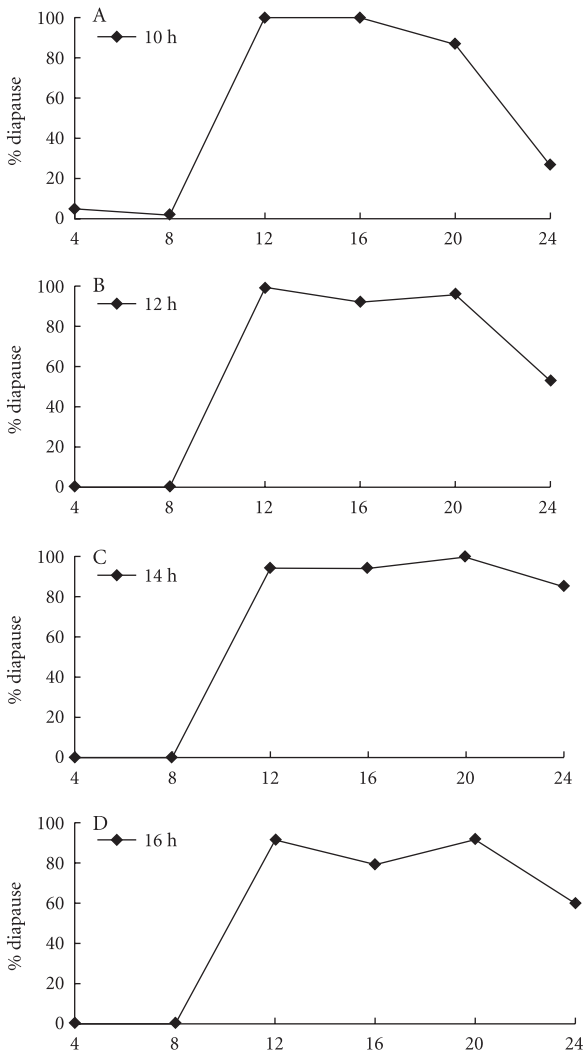


Figure 5 Diapause incidence in *Dendrolimus punctatus* under four different non-24 h photoperiodic cycles at a constant temperature of 25 °C.

diapause rate was high when scotophases were 12–72 h, except for photoperiods of L12:D24 and L16:D24. No periodic rhythmicity was found in the two photoperiodic responses.

Bünsow experiments

Bünsow experiments with a constant photophase of 12 h were combined with scotophases of 36, 48, and 60 h, and the scotophases were scanned by 1-h light pulses at 4-h intervals (Figure 7). The results showed that the diapause incidence dropped slightly when a light pulse was given 12 h after lights-off. The other light pulses resulted in higher diapause incidence.

Discussion

Photoperiodic response curves in *D. punctatus* showed a critical night length of 10 h 40 min at 25, 28, and 31 °C, although only few larvae entered diapause at 31 °C (Figure 1). This pine caterpillar is a good example of temperature compensation in diapause induction. Temperature-compensated

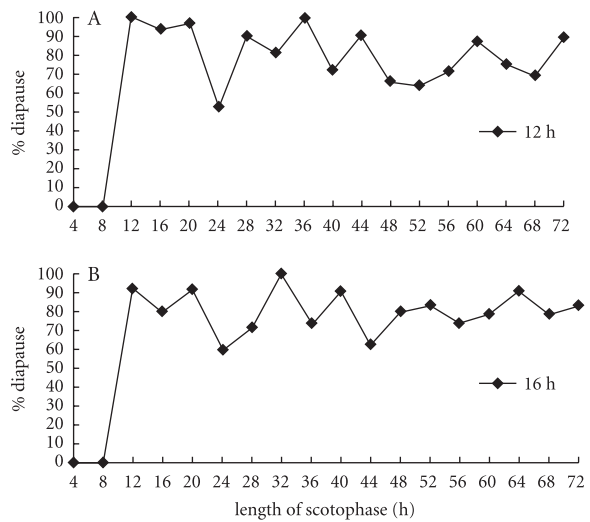


Figure 6 Incidence of diapause in *Dendrolimus punctatus* in Nanda-Hamner resonance experiments using a constant photophase of 12 h and 16 h at a constant temperature of 28 °C.

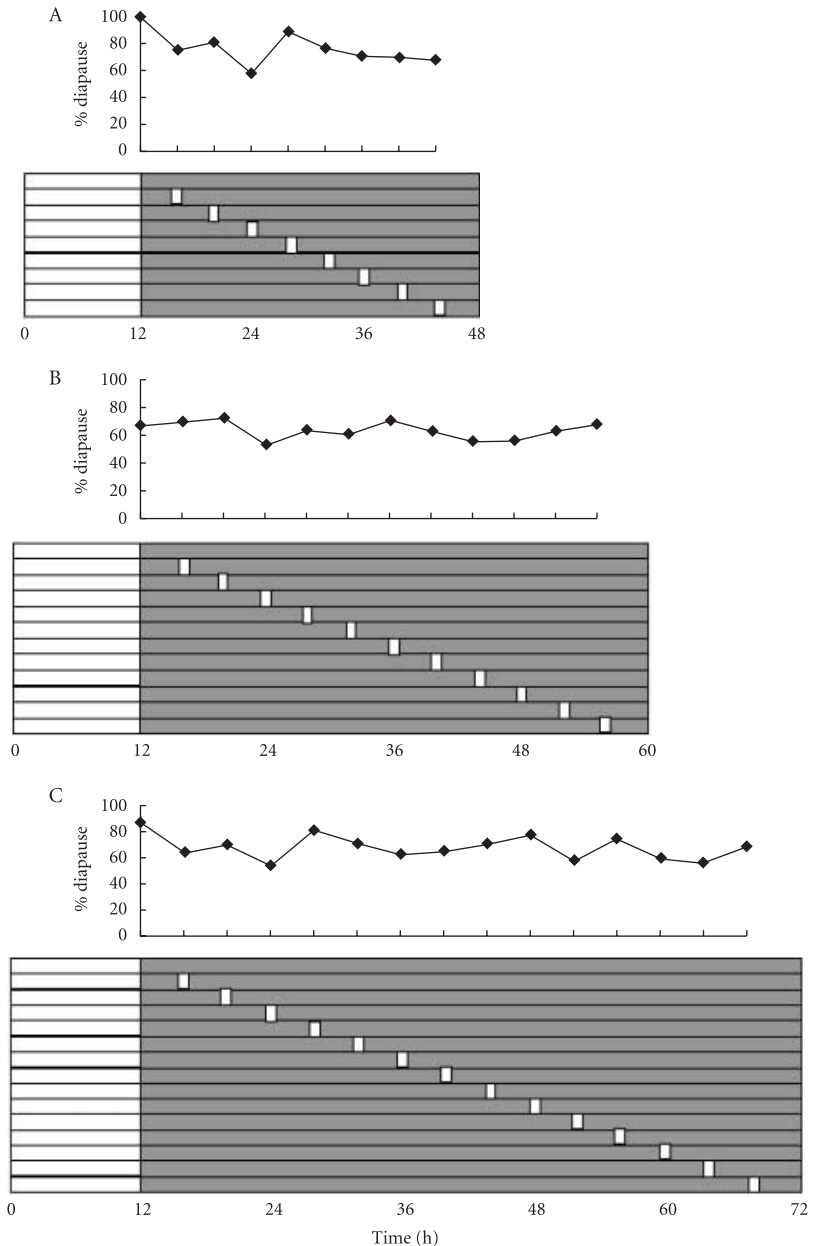


Figure 7 Incidence of diapause in *Dendrolimus punctatus* in Bünsow experiments at 28 °C with a constant photophase of 12 h and a scotophase of 36 h (A), 48 h (B), and 60 h (C), which was systematically scanned by a 1-h light pulse at 4-h intervals.

diapause induction plays an important role in the life history of this insect. It ensures that larvae that hatch between late July and early August under high temperature conditions will enter diapause, thus avoiding producing a next generation that might fail in the region because of the subsequent low temperatures. In fact, most larvae in the field entered diapause in response to high temperatures and declining day lengths rather than to low temperatures. Temperature-compensated diapause induction was also found in the large white cabbage butterfly, *Pieris brassicae*, and the flesh fly, *Sarcophaga argyrostoma* (Saunders, 1971).

The transfer experiments of insects from short-night to long-night cycles during development and vice versa have been used to map out the sensitive period and to determine the number of cycles required for diapause induction. It has been shown that the induction effect of a given number of long nights may be different from the diapause-averting effect of the same number of short nights. Most often, a greater number of days' exposure is required for induction of diapause than for its elimination. This has been indicated in species as diverse as *Aedes atropalpus*, *Megoura viciae*, *Pieris rapae*, *Lobesia botrana*, and *Papilio xuthus* (see Danks,

1987). In *D. punctatus*, such a comparison of the reciprocal transfers indicates that short nights (L16:D8), against a background of long nights (L12:D12), are more potent in their induction of diapause at 25 °C (RDN = 3.5 days) than are long nights on a background of short nights, in the induction of diapause (RDN = 7.5 days).

It has been shown that the accumulation of photoperiodic information can be achieved in different ways in different insects. In the aphid *Megoura viciae*, long-night cycles are accumulated in a straightforward fashion, independent of the accompanying photophase (Hardie & Vaz Nunes, 1994), whereas the effect of short nights is dependent on the photophase length (Hardie, 1990). In the flesh fly, *Sarcophaga argyrostoma*, and the black bean aphid, *Aphis fabae*, long-night accumulation is temperature compensated, but short-night accumulation is not (Saunders, 1992; Vaz Nunes & Hardie, 1999). In the spider mite, *Tetranychus urticae*, the large white butterfly, *P. brassicae*, and the cabbage beetle, *Colaphellus bowringi*, long-night and short-night cycles seem to be accumulated in different ways (Veerman & Vaz Nunes, 1987; Dumortier, 1994; Wang et al., 2004). In the cabbage moth, *Mamestra brassicae*, however, only long nights are accumulated, not short nights (Goryshin & Tyshchenko, 1973). In the pine caterpillar, *D. punctatus*, when one short-night cycle (L16:D8) is alternated with one long-night cycle (L12:D12) during the larval period or vice versa, the diapause-averting effect of short nights is strongly expressed (Figure 4C,D), showing that the information of a short-night cycle can be accumulated effectively one by one; when four short-night cycles alternate with an equal number of long-night cycles, the diapause-inducing effect of short nights is partly expressed (Figure 4E,F), showing that a minimum of consecutive exposures to long-night cycles is required for diapause induction. This indicates that information about short nights as well as long nights can be accumulated during the larval period in this species, but in different ways.

The Nanda-Hamner and Bünsow experiments in *D. punctatus* did not show any rhythmic fluctuations with a period of about 24 h in their photoperiodic response curves. The species usually showed higher incidence of diapause when the scotophase exceeded the critical night length. The results may suggest that the photoperiodic time measurement of diapause induction in this moth is based on a non-circadian hourglass timer (according to the interpretation of Veerman, 2001) or a damped circadian oscillator (according to the interpretation of Saunders, 2004).

Acknowledgements

The research was supported in part by Key Program of National Natural Science Foundation of China (30330490).

References

- Bünning E (1936) Die endogene Tagesrhythmik als Grundlage der photoperiodischen Reaktion. *Berichte der Deutschen Botanischen Gesellschaft* 54: 590–607.
- Bünsow RC (1960) The circadian rhythm of photoperiodic responsiveness in *Kalanchoe*. Cold Spring Harbor Symposium on Quantitative Biology 25: 257–260.
- Cheng CJ (1990) Integrated Management of Pine Caterpillars in China. China Forestry Publishing, Beijing.
- Danks HV (1987) *Insect Dormancy: An Ecological Perspective*, Vol. 1. Biological Survey of Canada, Ottawa.
- Dumortier B (1994) The 'circadian paradigm': a test of involvement of the circadian system in the photoperiodic clock. *Journal of Theoretical Biology* 166: 101–112.
- Goryshin NI & Tyshchenko GF (1973) Accumulation of photoperiodic information during diapause induction in the cabbage moth, *Barathra brassicae* L. (Lepidoptera, Noctuidae). *Entomological Review* 52: 173–176.
- Hardie J (1990) The photoperiodic counter, quantitative day-length effects and scotophase timing in the vetch aphid *Megoura viciae*. *Journal of Insect Physiology* 36: 939–949.
- Hardie J & Vaz Nunes M (1994) The aphid photoperiodic counter. *Individuals, Populations and Patterns in Ecology* (ed. by SR Leather, AD Watt, NJ Mills & KFA Walters), pp. 13–23. Intercept, UK.
- Hastings MH & Follett BK (2001) Toward a molecular biological calendar. *Journal of Biological Rhythms* 16: 424–430.
- He QQ (1995) Inquiry to law of differentiation in second and third generations of *Dendrolimus punctatus* Walker. *Journal of Zhejiang Forestry Science and Technology* 15: 38–41.
- Lees AD (1973) Photoperiodic time measurement in the aphid *Megoura viciae*. *Journal of Insect Physiology* 19: 2279–2316.
- Li ZL & Gia FY (1989) Photoperiodic reaction of the pine caterpillar *Dendrolimus tabulaeformis* Tsai et Liu. *Acta Entomologica Sinica* 32: 410–417.
- Li ZL & Gia FY (1991) The photoperiodic reaction (PhPR) of the pine caterpillar: studies on the mechanism of the second generation differentiation. *Forest Research* 4: 409–413 (in Chinese).
- Li ZL, Gia FY, He Z & Hou WW (1993) Effect of photoperiods on larval growth and development of *Dendrolimus punctatus*. *Forest Research* 6: 276–281 (in Chinese).
- Li ZL, Gia FY, Hou WW, He Z, Wen ZZ & Wen XS (1994) Photoperiodic reaction of the pine caterpillar *Dendrolimus punctatus* (Walker). *Acta Entomologica Sinica* 37: 31–37.
- Nanda KK & Hamner KC (1958) Studies on the nature of the endogenous rhythm affecting photoperiodic response of Biloxi soybean. *Botanical Gazette* 120: 14–25.
- Nishizuk M, Azuma A & Masaki S (1998) Diapause response to photoperiod and temperature in *Lepisma saccharina* Linnaeus (Thysanura: Lepismatidae). *Entomological Science* 1: 7–14.
- Pittendrigh CS & Minis DH (1964) The entrainment of circadian oscillation by light and their role as photoperiodic clocks. *American Naturalist* 98: 261–294.
- Saunders DS (1971) The temperature-compensated photoperiodic clock 'programming' development and pupal diapause

- in the flesh fly, *Sarcophaga argyrostoma*. *Journal of Insect Physiology* 17: 801–812.
- Saunders DS (1992) The photoperiodic clock and 'counter' in *Sarcophaga argyrostoma*: experimental evidence consistent with 'external coincidence' in insect photoperiodism. *Journal of Comparative Physiology* 170: 121–127.
- Saunders DS (2002) *Insect Clocks*, 3rd edn. Elsevier Science, Amsterdam.
- Saunders DS, Lewis RD & Warman GR (2004) Photoperiodic induction of diapause: opening the black box. *Physiological Entomology* 29: 1–15.
- Saunders DS & Lewis RD (1987) A damped circadian oscillator model of an insect photoperiodic clock. Circadian and 'hour-glass' response. *Journal of Theoretical Biology* 128: 73–85.
- Vaz Nunes M & Hardie J (1999) The effect of temperature on the photoperiodic 'counters' for female morph and sex determination in two clones of the black bean aphid, *Aphis fabae*. *Physiological Entomology* 24: 339–345.
- Vaz Nunes M & Saunders DS (1999) Photoperiodic time measurement in insects: a review of clock models. *Journal of Biological Rhythms* 14: 84–104.
- Veerman A (2001) Photoperiodic time measurement in insects and mites: a critical evaluation of the oscillator-clock hypothesis. *Journal of Insect Physiology* 47: 1097–1109.
- Veerman A & Vaz Nunes M (1987) Analysis of the operation of the photoperiodic counter provides evidence for hourglass time measurement in the spider mite *Tetranychus urticae*. *Journal of Comparative Physiology* 160A: 421–430.
- Wang XP, GE F, Xue F & You L (2004) Diapause induction and clock mechanism in the cabbage beetle, *Colaphellus bowringi* (Coleoptera: Chrysomelidae). *Journal of Insect Physiology* 50: 373–381.