

**Recurrent photoperiodic response in *Graphosoma lineatum*  
(Heteroptera: Pentatomidae)**

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**Heteroptera, *Graphosoma lineatum*, adult diapause, recurrent photoperiodic response**

**Abstract.** Photoperiodic response was studied in a pentatomid bug, *Graphosoma lineatum* (L.). When insects were reared from eggs under constant temperature of 26°C and long-day (18L:6D) conditions, they developed into reproductive adults and started oviposition about 30 days after ecdysis. Short-day (12L:12D) conditions and 26°C induced adult diapause. Both diapausing and reproductive adults displayed the photoperiodic response. Reproductive adults stopped oviposition about 10 days after the transfer to short-day conditions, and diapause adults started oviposition about 40 days after the transfer to long-day conditions.

Adults from the field were exposed either to short-day or long-day conditions. In late summer, adults that had already entered diapause responded to long-day photoperiod and started oviposition about 65 days after transfer. Thus, diapause induced under natural conditions appeared more intensive than the short-day “laboratory” diapause. Overwintered adults collected in spring started oviposition in both regimens about 7 days after the transfer. The absence of photoperiodic response indicated that diapause was over. However, under short-day photoperiod, females ceased oviposition about 11 days after its onset and re-entered diapause.

Czech population of *G. lineatum* has a facultative adult diapause induced by short-day photoperiod. The adults are sensitive to photoperiod before and in early hibernation and the completion of diapause is accompanied by a reversible loss of photoperiodic response. After a short period of oviposition the photoperiodic response is resumed. Thus, *G. lineatum* shows the recurrent photoperiodic response.

INTRODUCTION

*Graphosoma lineatum* (L.) (Heteroptera: Pentatomidae), which feeds on seeds of Umbelliferae, is a common species in Europe. However, diapause has been studied in Russia only (Popov, 1971) where the induction of diapause by short-day photoperiod was reported. In this study, the role of photoperiod in a Czech population of *G. lineatum* also in diapause termination and re-induction was examined.

Usually, after termination of adult diapause, insects do not respond to the environmental factors which induced diapause, but some insects resume photoperiodic response after a short period of oviposition (Hodek & Hodková, 1992). Several heteropteran species restore photoperiodic sensitivity in spring (Hodek, 1971a, 1977; Numata, 1987; Muraji et al., 1989; Ikeda-Kikue & Numata, 1992). Therefore the photoperiodic sensitivity of *G. lineatum* adults in different physiological conditions was examined, not only before and during, but also after diapause.

## MATERIAL AND METHODS

Adults of *G. lineatum* were collected from the fields along the river Vltava in České Budějovice (49°N, 15°E), Czech Republic, from late May to August in both 1994 and 1995. Insects were reared as male-female pairs in petridishes (8 cm in diameter, 1.5 cm in depth). Long-day (18L:6D) or short-day (12L:12D) photoperiod at 26°C were used throughout the experiments. Eggs were placed in long-day or short-day conditions within 24 h after oviposition. Early instar nymphs at an initial density of about 14 were reared in petri dishes, and older nymphs were transferred to larger glass containers (460 ml). When the last instar nymphs ecdysed to adults, the experimental animals were kept in petri dishes as male-female pairs. Adults were transferred from long-day to short-day conditions or vice versa within 24 h or 45 days after ecdysis. All insects received dried seeds of various Umbelliferae and water ad libitum. The mortality and oviposition of adults were recorded daily. The time of field sampling is given in the results.

Analysis of difference in mean values of the two samples was performed using t-test.

## RESULTS

### Photoperiodic response of laboratory reared adults

Thirty six of 42 adults reared from eggs under long-day conditions started oviposition  $28.9 \pm 12.1$  (mean  $\pm$  S.D.) days after adult ecdysis (Fig. 1A) and continued egg-laying until death. Most adults reared from eggs under short-day conditions did not start oviposition and subsequently died although 12 of 66 started oviposition  $89.1 \pm 8.1$  days after adult ecdysis (Fig. 1B). Two long-day and 7 short-day adults were alive at the age of 210 days when the experiments were discontinued.

Thus, the adult diapause of *G. lineatum* is facultative and is induced by short-day photoperiod and prevented by long-day photoperiod. In 18.2 % of females the "laboratory" diapause was terminated after about 3 months without any change of conditions.

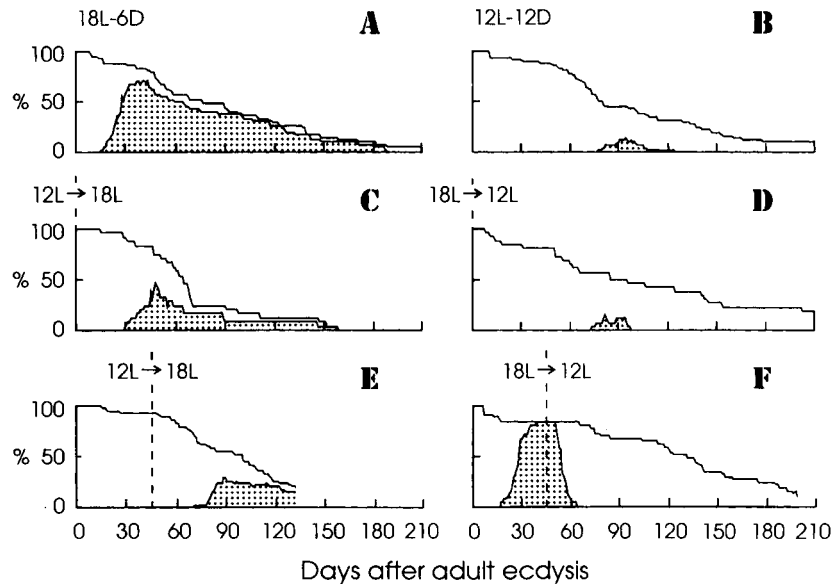


Fig. 1. Effect of photoperiod on the oviposition of *Graphosoma lineatum* adults in the laboratory at 26°C. Vertical axis: survival (open), incidence of ovipositing females (shaded). The vertical lines (----) show the day of change in experimental photoperiod; n = 24–66.

When insects were transferred from short-day to long-day conditions within 24 h after adult ecdysis, 16 of 24 adults started oviposition and the pre-oviposition period was  $42.3 \pm 8.8$  days (Fig. 1C). The oviposition delay was significantly longer and shorter than that of adults reared continuously from eggs under long-day and short-day, respectively ( $p < 0.001$ ). When 26 pairs were transferred from long-day to short-day conditions within 24 h after adult ecdysis, most of them died without ovipositing but 6 females started oviposition  $80.7 \pm 5.3$  days after ecdysis (Fig. 1D). Six females were alive at the age of 210 days when the experiment was discontinued. The bugs keep the photoperiodic sensitivity after adult ecdysis and, thus, the sensitive period for the induction of diapause covers both the nymphal and adult periods.

Twenty one diapausing adults were transferred from short- to long-day conditions 45 days after ecdysis from nymphs. Thirteen of them started oviposition  $43.7 \pm 13.4$  days after the transfer to long-day conditions (Fig. 1E). This oviposition delay was not significantly different from the pre-oviposition period of adults transferred from short-day to long-day conditions within 24 h after adult ecdysis ( $p > 0.05$ ). Eight females were alive 135 days after adult ecdysis (90 days after transfer) when the experiment was discontinued. Thirty seven reproductive adults reared for 45 days under long-day condition were transferred to short-day. They stopped oviposition  $10.7 \pm 3.4$  days after their transfer (Fig. 1F). Six females were alive 195 days after adult ecdysis (150 days after transfer) when the experiment was discontinued. Thus, it was shown that both diapausing and reproductive adults were sensitive to the photoperiod.

#### Photoperiodic response of field-collected adults

Adults collected in the field on 26 August were transferred to long-day conditions. Thirty two of 36 females started oviposition  $64.7 \pm 37.9$  days after the transfer (Fig. 2A). Evidently they entered diapause already before their collection. The oviposition delay after

transfer was longer than in two experiments where the laboratory reared adults were transferred from short-day to long-day conditions within 24 h (pre-oviposition period lasted 42.3 days) ( $p < 0.01$ ) (Fig. 1C) and 45 days after adult ecdysis (pre-oviposition period was 43.7 days) ( $p < 0.05$ ) (Fig. 1E). Thus, diapause induced in the field appeared more intense than that in the laboratory. One female died as late as 359 days after collection (not shown in the figure).

In spring, adults were collected from 26 to 29 May. The outdoor photoperiod was around 16.5L : 7.5D (see Beck, 1980). All females started oviposition several days after transfers to both long-day ( $n = 23$ ) and short-day ( $n = 30$ ) conditions (average pre-oviposition period was  $6.9 \pm 5.8$  and  $7.5 \pm 4.8$  days, respectively) and there was no

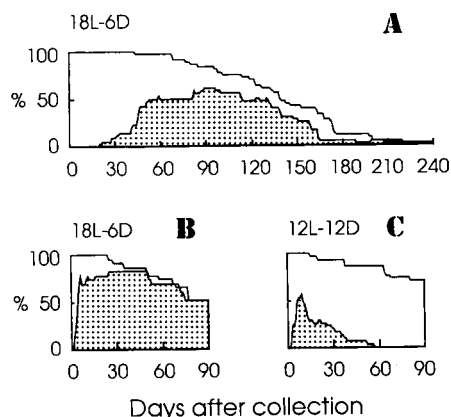


Fig. 2. Effect of photoperiod at 26°C on the oviposition of *Graphosoma lineatum* adults collected from the field. Adults were collected in late August (A) or late May (B, C). Vertical axis: survival (open), incidence of ovipositing females (shaded);  $n = 23-36$ .

significant difference between the two conditions ( $p > 0.05$ ) (Fig. 2B, C). It was evident that at least some of the females had begun their oviposition outdoors. Under long day, they continued oviposition, but under short day they stopped oviposition  $18.8 \pm 13.6$  days after transfer. The oviposition period lasted significantly longer than when the laboratory females were transferred from long-day to short-day conditions at the adult age of 45 days ( $p < 0.01$ ). Twelve adults in long-day and 21 adults in short-day were alive 90 days after collection when the experiments were discontinued.

To check whether the photoperiodic response was lost before spring, next year the adults were kept outdoors until early February and then transferred to short- ( $n = 20$ ) and long-day ( $n = 20$ ) conditions. Their reproduction was recorded for 70 days. As the adults were not followed till death, only the number of egg batches produced during the experiment were compared. Under long-day conditions 3.24 (1–12) egg batches were laid, and under short-day conditions 3.46 (1–13) egg batches. Thus, the photoperiodic response of hibernating *G. lineatum* is lost already in early February.

#### DISCUSSION

Adult diapause of *G. lineatum* was induced and averted in the laboratory by short-day and long-day photoperiod, respectively (Fig. 1A, B). Diapausing adults, collected in the field before hibernation also displayed photoperiodic sensitivity and diapause was terminated under long-day conditions, but the diapause in nature was more intense than that in the laboratory (Fig. 2A).

In spring, it is probable that the oviposition had started already outdoors; the oviposition delay after the transfer to laboratory was about a week in both photoperiods (Fig. 2B, C). Generally, the disappearance of photoperiodic response is considered to be a criterion of diapause termination (Lees, 1955; Hodek, 1971b; Tauber et al., 1986; Danks, 1987). Thus, the adults sampled in May had already completed diapause. However, after a period of reproductive activity these post-diapause females stopped oviposition under short-day conditions and entered diapause again (Fig. 2C). This indicates that they could respond to diapause-promoting photoperiod rather early after diapause termination.

In many insects, once adult diapause is terminated, photoperiod that induced diapause has no influence on the post-diapause oviposition (Tauber et al., 1986). However, some species can respond to the diapause-promoting photoperiod again after starting oviposition in spring (see Hodek & Hodková, 1992). Hodek (1971a) reported this phenomenon in the pentatomid *Aelia acuminata* and termed it “recurrent photoperiodic response” (Hodek, 1979). In addition, among heteropterans, also *Dolycoris baccarum* (Hodek, 1977), *Riptortus clavatus* (Numata, 1987), *Microvelia douglasi* (Muraji et al., 1989) and *Eurydema rugosa* (Ikeda-Kikue & Numata, 1992) show recurrent photoperiodic responses. The present results indicate that *G. lineatum* also exhibits this type of photoperiodic response.

Recurrent photoperiodic response enables an insect to enter diapause more than once within its life (Hodek & Hodková, 1992). In central Europe, a single season – particularly with a cooler summer – does not permit full realisation of reproductive potential of the long-lived *A. acuminata*. The species lives long periods in the laboratory at constant high temperature (24–26°C) and has a high fecundity (Hodek, 1977). Thus, recurrent photoperiodic response has an important role for its life cycle in that oviposition may be discontinued by shortening photophase in late summer and resumed next spring after the second

hibernation. Adults of *G. lineatum* were also long-lived even under the long-day photoperiod (Fig. 1A) and, therefore, they are likely to enter the second diapause in nature. To enter diapause more than once appears to be essential for the life cycle strategy of those long lived insects which are distributed in the regions where the period suitable for reproduction is not long enough.

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