

The effect of environmental conditions on diapause in the blister beetle, *Mylabris phalerata* (Coleoptera: Meloidae)

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Abstract. In the field, the blister beetle *Mylabris phalerata* Pallas (Coleoptera: Meloidae) undergoes larval diapause in the ground, which lasts for nearly six months. The effect of the soil environment on this diapause was examined. Final instar larvae kept at temperatures of $\geq 26^{\circ}\text{C}$ do not enter diapause and continued to develop regardless of the soil water content and photoperiod. Below 25°C the final instar larvae entered diapause regardless of soil water content and photoperiod. The early stages, particularly L2, appeared to be more important for diapause induction than the later stages. However, the other instars were also sensitive. Temperature, rather than photoperiod was the main factor influencing pupal duration.

INTRODUCTION

Many animals have evolved to survive seasonally recurring adverse conditions by entering a diapausing stage. To this end, many insects respond to one or many environmental factors as cues for diapause. Photoperiod is the most common environmental factor inducing the onset of diapause in temperate-zone insects (Tauber et al., 1986; Danks, 1987; Saunders, 2002). In many insects temperature is another important factor controlling diapause, especially in insects living in warehouses and underground. Diapause in soil-inhabiting insects can be influenced by soil temperature, moisture and oxygen (Lee & Denlinger, 1990). Diapause in soil-inhabiting insects is an important topic, which is poorly studied. The present study investigates the effects of soil environmental conditions, including temperature, photoperiod and water content, on diapause in *Mylabris phalerata* (Coleoptera: Meloidae).

M. phalerata is found usually on flowers of cowpea (*Vigna unguiculata*) and loofah (*Luffa cylindrica*). Cantharidin from *Mylabris* is used in medicine (Wang, 1989; Hundt et al., 1990; Wang et al., 2000; Xu et al., 2004). In addition, its larvae are predators of eggs of the grasshopper *Chondracris rosea* De Geer (Orthoptera: Acridiidae). As the beetle is now scarce in the field it is important to rear large numbers in the laboratory. Therefore, knowledge of the factors inducing diapause in the final instar larvae is important.

MATERIAL AND METHODS

Insect materials

Mylabris phalerata adults were collected from cowpea flowers in the fields on farms of Huazhong Agricultural University at Wuhan (30.5°N , 114.3°E), Hubei Province, People's Republic of China, in June–July 2003. Adult beetles were

brought to the laboratory and reared at $25 \pm 1^{\circ}\text{C}$, $70 \pm 5\%$ r.h. and 16L : 8D in a wire screen cage (100 cm \times 100 cm at base and 300 cm deep). A plastic container (50 cm \times 25 cm at base and 12 cm deep) was put at the bottom of the cage, which contained moist soil for oviposition and acted as a source of moisture. Adults were fed on cowpea flowers, cowpea pods and flowers of loofah. Daily checks were made and newly laid egg masses of *M. phalerata* were collected and placed in small plastic containers (4 cm wide at base and 10 cm deep). Upon hatching, larvae were placed individually in the same containers filled with fine inorganic soil and a grasshopper egg-pod.

Temperature response experiment

To investigate the effect of temperature on diapause occurrence in *M. phalerata* the larvae were reared at 18, 22, 25, 28, 31 or $34 \pm 1^{\circ}\text{C}$ in soil with a water content of 10% (w : w). 50 individuals were reared at each temperature. Larval moulting and pupation were checked and recorded.

The joint effects of temperature and soil water content

The joint effects of temperature and soil water content on diapause occurrence in *M. phalerata* were determined at 25 and $30 \pm 1^{\circ}\text{C}$ and a water content of 8%, 10% or 12% (w : w). Larval moulting and pupation of 50 individuals were checked and recorded for each treatment.

The joint effects of temperature and photoperiod

The joint effects of temperature and photoperiod on diapause induction in *M. phalerata* were studied by exposing all the immature stages of *M. phalerata* to 22, 24, 25, 26 or $28 \pm 1^{\circ}\text{C}$ at photoperiods of 8L : 16D, 12L : 12D or 16L : 8D. The soil water content was 10% (w : w). The diapause intensity was measured as diapause duration. Larval moulting and pupation of 50 individuals were recorded.

Sensitivity of larvae to photoperiod and temperature

Two experiments investigated the sensitivity of larvae to photoperiod and temperature. The first experiment was on pre-5th instar larvae. Eggs and larvae were kept at 25°C or 30°C and photoperiods of 8L : 16D, 12L : 12D or 16L : 8D, respectively.

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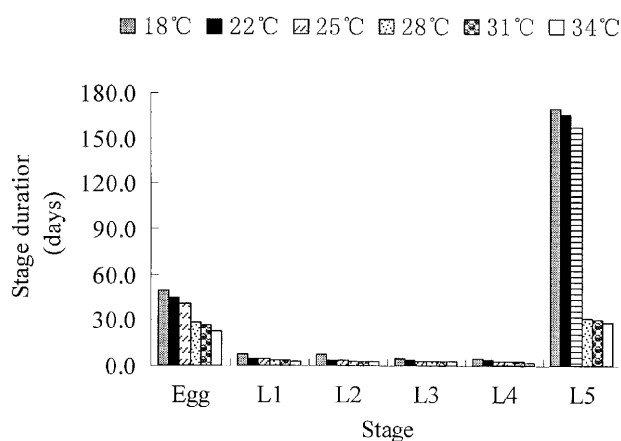


Fig. 1. Duration of development of the immature stages of *Mylabris phalerata* measured at 18, 22, 25, 28, 31 and 34 \pm 1°C. The soil water content was 10% (w : w). The duration of development time is the mean of 50 individuals in each stage.

Larvae kept at 30°C were transferred to 25°C on the first day of the 1st, 2nd, 3rd or 4th instar but kept at the same photoperiod. Similar larvae kept at 25°C were transferred to 30°C as above. In the second experiment, the 5th instar larvae were transferred. Individuals reared at 25°C were transferred to 30°C when they reached 1, 30, 60, 90, 120 or 150 days age. The 5th instar larvae kept at 30°C were transferred to 25°C when 1, 20 or 30 days old. 50 individuals were used in each treatment and the soil water content was 10% (w : w). The control group was not transferred. Diapause occurrence and duration were monitored.

The effect of photoperiod and temperature on pupal duration

Diapausing larvae were kept at 22 or 25°C and a photoperiod of 8L : 16D, 12L : 12D or 16L : 8D and the soil water content was 10% (w : w). The non-diapausing larvae were kept at 28°C. The duration of the pupal stage was recorded in each case.

Diapause identification

The 5th instar larvae wander before entering the soil. This wandering lasts from the time of the moulting of the 5th instar larvae to when they enter the soil. The wandering of non-diapause individuals lasts for \leq 2 days, whereas that of diapause individuals lasts for \geq 4 days.

Data analysis

The difference in the duration of development of each stage in the different treatments was tested for significance by analysis of variance (ANOVA) using SAS (SAS Institute, 1999). The temperature in 2003 and 2004 was monitored by recording daily minimum and maximum temperature. The time required by fifth instar larvae reared under given diapause inducing conditions to reach the pupal stage was used as a measure of diapause intensity.

RESULTS

Effect of temperature on the rate of development

Egg development time decreased with increased in temperature from 18 to 34°C (Fig. 1). Duration of the first to the fourth instar was longer at 18°C than at the other temperatures tested. Larvae kept at temperatures \geq 22°C from the first to the fourth instar took a similar time to complete development. More than 94% of the fifth instar

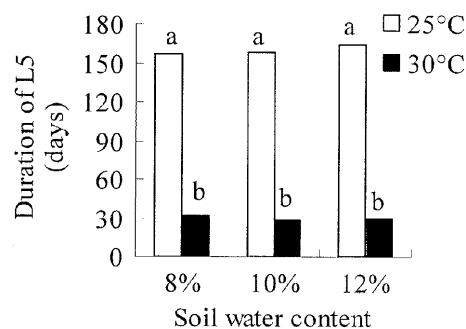


Fig. 2. The average duration of development of 5th instar larvae (L5) of *M. phalerata* reared at different temperatures and soil water contents. Diapause occurred at 25°C. Means followed by the same letter are not significantly different ($P > 0.05$).

larvae kept at low temperatures (\leq 25°C) entered diapause and took five months before they pupated. At 28, 31 and 34°C the L5 larvae did not enter diapause and completed development in 28.4–31.5 days.

The effect of soil temperature and water content

Diapause incidence and duration was not significantly influenced by the water content of the soil. More than 94% of the larvae kept at 25°C entered diapause. At 30°C diapause was averted regardless of the soil water content. The duration of diapause at 25°C was similar whether the water content of the soil was 8%, 10% or 12% (Fig. 2).

The effect of temperature and photoperiod

Most larvae entered diapause at 22, 24 and 25°C, irrespective of the photoperiod. However, 100% of the larvae developed without diapausing at temperatures $>$ 25°C (26°C and 28°C), regardless of the photoperiod (Table 1). The critical temperature for diapause induction was between 25°C and 26°C.

The development of the non-diapausing L5 was five times faster than that of larvae that entered diapause (Table 2). The duration of diapause did not differ significantly at 22, 24 and 25°C; though it was shortest at 25°C. Photoperiod did not significantly influence the duration of diapause, though at 12L : 12D, diapausing larvae required a slightly shorter time to complete development than at 8L : 16D and 16L : 8D.

Sensitivity of larvae to photoperiod and temperature

Rearing eggs and young larvae at different photoperiods did not affect diapause induction. Individuals exposed in the egg stage and L1–L4 to 25°C experienced

TABLE 1. Incidence of diapause in 5th instar larvae (L5) of *Mylabris phalerata* reared at different temperatures and photoperiods in soil with a water content of 10% (w : w).

Temperature (°C)	Photoperiod		
	8L : 16D	12L : 12D	16L : 8D
22	100%	100%	100%
24	98%	96%	98%
25	94%	94%	96%
26	0	0	0
28	0	0	0

TABLE 2. The joint effect of photoperiod and temperature, when the soil water content was 10% (w: w), on the duration of the fifth larval instar of *Mylabris phalerata*.

Temperature °C	Duration of L5 (days)		
	8L : 16D	12L : 12D	16L : 8D
22	165.4 ± 5.1 a	162.5 ± 3.9 a	167.2 ± 4.4 a
24	161.3 ± 3.2 a	158.7 ± 3.7 a	163.1 ± 4.1 a
25	157.5 ± 4.0 a	154.3 ± 3.5 a	158.2 ± 3.3 a
26 (ND)	34.5 ± 3.1 b	33.2 ± 2.9 b	32.5 ± 3.1 b
28 (ND)	30.2 ± 2.6 b	29.1 ± 2.5 b	31.6 ± 6.7 b

Note: Means in a column with the same letter are not significantly different ($P > 0.05$, $n = 50$). ND non-diapause larvae.

diapause in L5, but by such exposure to 30°C diapause was averted (Table 3). Individuals that were reared at 25°C until L1 or L2 and then transferred to 30°C did not enter diapause. Less intense diapause was induced if L3 and L4, or L4 were reared at 30°C. In contrast, diapause of normal length occurred in two treatments in which the larvae were transferred from 30 to 25°C in the first days of L1 or L2. And diapause was averted when they were transferred from 30 to 25°C on day 1 of L3 or L4 (i.e., when egg-L2 or egg-L3 were reared at 30°C). Individuals exposed in egg-L5 to 30°C developed without diapause. Individuals exposed in egg-L5 to 25°C entered diapause and age of L5 did not affect diapause induction. The early

stages, particularly L2 appeared to be more sensitive to diapause induction than the later stages. However, other instars were also sensitive, as shown by the gradual increase in the duration of L5 when transfers from 25 to 30°C occurred at later stage in development (Table 3). It seems that the later exposure to 30°C reverses the previous diapause induction by 25°C; the degree depends on the duration of exposure.

Diapause intensity

Diapause duration of individuals reared from the egg stage at 25°C was 157.5–158.2 days (Table 3). That of individuals exposed to 30°C before the third instar and 25°C throughout their subsequent development lasted 145.3–151.6 days. However, the diapause duration of individuals transferred from 25 to 30°C on the first day of L3 or L4 was shorter than that of individuals reared at 25°C throughout their development. The diapause duration was 67.9–68.5 days when transferred on the first day of L3 and 75.0–76.9 days when transferred on the first day of L4. Individuals transferred on either the first or thirtieth day of L5 from 30 to 25°C took about three months to pupate, those transferred on day 60 took about four months and those on day 90 nearly five months. Development to the pupal stage of L5 kept at 30°C took one month (Table 3).

TABLE 3. Duration of development of L5 of *Mylabris phalerata* reared under different photoperiods and transferred at different stages during their development from 30 to 25°C or from 25 to 30°C ($n = 50$).

Stages exposed to		Duration of stage L5		
25°C	30°C	8L : 16D	12L : 12D	16L : 8D
Egg-L5		157.5	154.3	158.2
L1-L5	Egg	151.6	145.3	144.8
L2-L5	Egg-L1	146.3	145.8	145.7
L3-L5	Egg-L2	36.9	35.7	38.4
L4-L5	Egg-L3	33.8	32.4	32.2
L5	Egg-L4	32.1	31.2	30.8
The other stage of L5	Egg-L5(10)*	32.3	31.3	32.5
The other stage of L5	Egg-L5(20)	30.7	30.1	30.0
	Egg-L5	28.2	29.1	28.4
Egg	L1-L5	32.4	33.3	33.4
Egg-L1	L2-L5	38.6	37.9	37.8
Egg-L2	L3-L5	68.2	67.9	68.5
Egg-L3	L4-L5	76.4	76.9	75.0
Egg-L4	L5	93.1	92.3	94.5
Egg-L5(10)	The other stage of L5	95.5	94.5	97.0
Egg-L5(30)	The other stage of L5	100.1	98.3	99.2
Egg-L5(60)	The other stage of L5	120.9	118.8	120.2
Egg-L5(90)	The other stage of L5	136.2	138.5	139.0
Egg-L5(120)	The other stage of L5	139.9	140.7	141.4
Egg-L5(150)	The other days of L5	155.5	155.8	154.9

*: number in the bracket is the age in days of L5.

TABLE 4. The effect of photoperiod and temperature, when soil water content was 10% (w : w), on the duration of the pupal stage of *Mylabris phalerata*.

Temperature (°C)	Duration of pupal stage (days)		
	8L : 16D	12L : 12D	16L : 8D
22	27.3 ± 2.1 a	27.4 ± 2.3 a	28.8 ± 2.5 a
25	20.9 ± 2.2 b	19.8 ± 3.0 b	21.1 ± 1.9 b
28	17.1 ± 2.2 c	17.0 ± 1.3 c	16.4 ± 1.9 c

Note: Means with the same letter are not significantly different ($P > 0.05$, $n = 50$).

The effect of photoperiod and temperature on the duration of pupal stage

The duration of the pupal stage decreased with increased temperature (Table 4). The larvae that did not diapause at 28°C needed 17 days to complete the pupal stage, which was nearly ten days faster than for the larvae reared at 22°C and that were in diapause in L5. Adult beetles normally emerged 20 days after pupation at 25°C, which was a week faster than at 22°C. Duration of pupal development at each temperature was not significantly different at photoperiod 8L : 16D, 12L : 12D or 16L : 8D (Table 4).

DISCUSSIONS

The present study indicates that diapause in the final instar larvae of *M. phalerata* is induced by temperature rather than the water content of the soil or photoperiod. Diapause induction was averted at temperatures $\geq 26^\circ\text{C}$, but induced by temperatures $\leq 25^\circ\text{C}$. It can be concluded that high temperatures act as a diapause-averting factor in this insect and the critical temperature for diapause induction is between 25 and 26°C as at or below 25°C almost all individuals entered diapause. Temperature-controlled diapause is also reported by Shintani & Ishikawa (1997) in *Psacothia hilaris* and by Ishihara & Shimada (1995) in *Kytorhinus sharpianus*. Xue mentioned that diapause in *Colaphellus bowringi* is induced principally by low temperature and less so by photoperiod (Xue et al., 2002). The same response is seen in *Endopiza viteana* (Tobin et al., 2002). Earlier examples are cited in Beck (1991). The role of temperature in these insects is more important for inducing diapause than regulating development rate.

The duration of diapause in *M. phalerata* reared at the same temperature was not affected by the water content of the soil of 8%, 10% or 12%, or photoperiods of 8L : 16D, 12L : 12D or 16L : 8D. However, the soil temperature greatly influenced diapause duration (Table 2). Diapause duration at 22°C was longer than at 24 and 25°C. Several authors indicate that lower temperatures can prolong development (Ratte, 1985; Sibly & Calow, 1986; Atkinson, 1994; Nylin, 1994; Abrams et al., 1996).

The stages sensitive to induction of diapause of the final larval instars are reported, for instance, by Kurota & Shimada (2001) for *Bruchidius dorsalis* and Milonas & Savopoulou-Soultani (2000) for *Colpoclypeus florus*. *B. dorsalis* enters diapause in the final (late fourth) larval instar under short photoperiods and the stages sensitive to

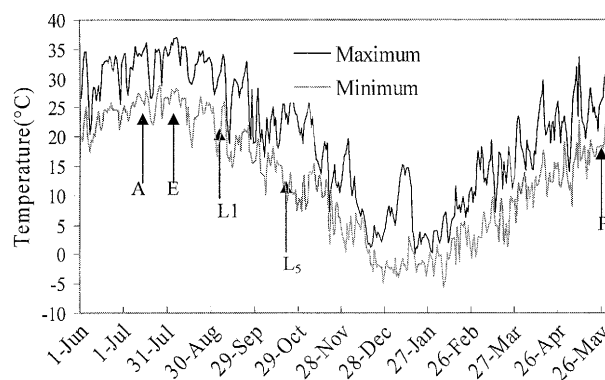


Fig. 3. Life cycle of *Mylabris phalerata* and the daily maximum and minimum temperatures in 2003–2004. Arrows indicate the date of appearance of the life stages of *M. phalerata*. A: adult, E: egg, L1: the first instar larva, L5: the fifth instar larva (overwintering stage), P: pupa

photoperiod are the late egg stage and early first instar larva. The pupa of the maternal generation is the most sensitive stage for the induction of larval diapause in *C. florus*. In this study, exposure of larvae of *M. phalerata* to low temperature ($\leq 25^\circ\text{C}$) from L3 onward results in diapause induction in the final instar (Fig. 3).

In the field the final instar larvae entered diapause at the end of October (Fig. 3). Temperature recordings suggest that the maximum temperature dropped below 25°C in October, which induced the beetles to enter diapause at this time. The minimum temperature in winter is -5°C . As in other insects, diapause enables *M. phalerata* to survive the low temperature conditions prevailing in winter. Temperature remained $\leq 25^\circ\text{C}$ up to the end of May (Fig. 3) and prevented beetles from pupating, which resulted in beetles emerging in early July when cowpea flowers are available in the field.

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