



長日と低温によるタバコシバンムシ幼虫休眠の終結 と休眠誘導の阻害

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Inhibition and Termination of Larval Diapause and Low-Temperature Tolerance in the Cigarette Beetle, *Lasioderma serricorne* (Coleoptera : Anobiidae)

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Abstract

Diapause larvae of the cigarette beetle, *Lasioderma serricorne* (F.) reared under a short-day (LD 12 : 12) at 25°C, were exposed to a long-day (LD 16 : 8) at the same temperature, 51 and 71 days after hatching, for the rest of their life. Since under LD 16 : 8 at this temperature they started pupating around 50 days after hatching and none started pupating when they were reared continuously under LD 12 : 12 at this temperature, it was considered that these larvae in diapause responded to the photoperiodic shift and started emerging 8 days following the shift. When diapause larvae were exposed to more than 6 cycles of LD 16 : 8, 20 days after the entry to the third larval instar, the shifts accelerated pupation significantly. Six days were sufficient to change the program for developmental determination. After the program was altered, 2 more days were required to pupate and 8 more days for adult development. Under LD 16 : 8, a small fraction of larvae developed at a slower rate than the majority but at a faster rate than diapause larvae reared under LD 12 : 12. The development of this fraction was also accelerated by the photoperiodic shift. This species thus responds not only to a constant photoperiod but also to a shift of photoperiod from LD 12 : 12 to LD 16 : 8. If the photoperiodic shift was made later, the effect was much smaller, indicating that the intensity of diapause increased, or otherwise, the sensitivity to photoperiodic shift decreased while diapausing. Photoperiodic shifts from LD 16 : 8 to LD 12 : 12, made at different times before and after the third larval ecdysis, demonstrated that photoperiodic sensitivity remained till the entry of the fourth instar, since more than 50% of larvae entered diapause when the shift was placed before the fourth instar. Upon the entry to the fourth instar, they lose the sensitivity to photoperiod or the ability to enter diapause. The tolerance of this species to 5°C was weak except at the diapause stage, since the exposure of 10 days to this temperature killed all eggs, 30 days exposure killed all second instar larvae and 97.4% of the third instar. In contrast, the fourth instar larvae survived this treatment. While no pupae survived this temperature, 65% adults survived this low temperature. Although 10°C was much milder than 5°C, the treatment of this temperature for 75 days killed about a third of the total number of non-diapause 4th instar larvae exposed. Low temperature was harmful but effective in synchronizing post-diapause emergence, since the emergence under LD 12 : 12 at 25°C after chilling of two months at 5°C was as fast as after the transfer to LD 16 : 8, 25°C. High humidity and the presence of cocoon helped survival at low temperature.

Key words : *Lasioderma serricorne*, Cigarette beetle, Diapause termination, Stored product pest, Photoperiodism

Introduction

Urban environment such as granaries, warehouse and household is a new habitat for some insects. Such environment can be characterized to be resource-rich, warmer and prone to disturbances to compare with natural environment (Howe, 1957 ; Tsuji, 1963 ;

Naeemullah and Takeda, 1998 ; Naeemullah *et al.*, 1999, 2003). The insects colonize this kind of habitat by changing their life cycle properties to adapt and cope with such characteristics. We have demonstrated that *Lasioderma serricorne* (F.), a representative of such pests, enters diapause at full-grown larval stage under short-day conditions at intermediate temperatures

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(Higashi *et al.*, 2004). Once larvae entered diapause, they gradually resume their development if they are kept in the same conditions as they were reared till diapause stage. The time of diapause termination determines the time of reproduction in spring and therefore it has a serious consequence in the subsequent population growth. This investigation focuses on the mechanism that terminates and inhibits diapause as a fine-tuning mechanism of the life cycle. Effect of photoperiodic shift made at various stages of larval development was investigated to know the number of long-day cycles required to inverse diapause determination, and the persistence of sensitivity to photoperiod. The cold tolerance, the effects of humidity and presence of cocoon on survival, and the effect of low temperature on diapause termination were also examined, since these factors determine the survival rate of diapausing insects in the field was controlled environmental condition. The present investigation in the constant laboratory condition that could probe the insect life cycle in a controller environmental condition.

Materials and Methods

1) Effect of shifts in photoperiod on larval diapause

The experimental animals were provided from our laboratory stock designated as the S stock, as described before (Higashi *et al.*, 2003). General characteristics in temperature and photoperiodic responses of this stock have been described in Higashi *et al.* (2003). Forty individuals were used for each experiment at 25°C. In the following experiments, they were reared individually in the glass vial. The first experiment employed a shift of photoperiod from LD 12 : 12 to LD 16 : 8, 51, 72 and 103 days after oviposition and adult emergence patterns were observed thereafter to investigate the effect of long-day to reverse diapause program, as shown in Higashi *et al.* (2003) that this stock enters diapause by the 50-th day of larval development at 25°C. Normally more than 80% of individuals pupate within 50 days under LD 16 : 8 at 25°C. The minimal days to induce pupation was investigated by observing days of pupation after the photoperiodic shift from LD 12 : 12 to LD 16 : 8.

Then the sensitive period to photoperiodic shift was investigated in the second experiment by transferring larvae from LD 16 : 9 to LD 12 : 12. Photoperiod was shifted before and after the third molt ; namely 1st, 3rd, 4th, 5th, 6th, and 7th days of third instar and 0, 1st, 2nd,

3rd, 4th and 5th days of 4th instar.

In the third experiment, the effects of long-day exposures of 2, 4 and 6 days were investigated on the termination of diapause. The transfer was made 20 days after the third larval molt. Diapause rate was determined as percentage of larvae remaining as 4th instar larvae when all long-day reared individuals completed larval development.

In the fourth experiment, different developmental stages of *L. serricorne* were exposed to 5°C for cold tolerance. Eggs were cooled (5°C) for 1, 10 or 30 days. One day old larvae were transferred to sample tube individually and reared at 25°C under LD 16 : 8 until the third day of the second instar. They were exposed for 30 day to 5°C. Likewise, 3 days old larvae of the third instar were exposed to 5°C for 30 days. Larvae that were reared in mass at 25°C under LD 16 : 8 were individually transferred to a new vial and they were transferred to 20°C under LD 16 : 8. When they span cocoon, they were transferred to 18°C and then 5°C and kept there for 30 days. Fourth instar larvae prepared in the same way were transferred to 25°C when they pupated and then to 5°C where they were kept for 30 days. Fourth instar larvae prepared in the same way were transferred to 20°C upon eclosion from the cocoon and then to 5°C and kept there for 30 days. Temperature was gradually lowered by 5°C for two days at each step. Between 20 and 15°C, an acclimation step of 18°C was employed for one day. The temperature step up took the reverse course to the step down.

In the fifth experiment, several conditions were applied, either cocoon was removed or kept intact and minimum temperature or the duration was varied. The presence or absence of humidification is the other variable. For high humidity condition, saturated NaCl solution was used. Low humidity condition was not humidified heated laboratory condition during winter months. The exposure of diapause larvae to 5 or 10°C for designated periods began 91 days after the egg-laying until larvae were reared under LD 12 : 12 at 25°C.

Results

Figures. 1 and 2 show the pattern of pupation after the exposure to LD 16 : 8 in larvae reared under LD 12 : 12. Pupation started to occur 8 days after the exposure made 51 days after egg-laying. The long-day exposure at later stage, 72 days after egg-laying, was

equally effective to induce pupation but ca. 20% of the individuals stayed in diapause. The rise of pupation occurred more slowly than after the shift made on day 51 (**Fig 1**, closed circles). Under LD 16 : 8, a small fraction of larvae developed at a slower rate than the majority but at a faster rate than diapause larvae kept under LD 12 : 12. The development of this fraction was also accelerated by the photoperiodic shift. The result indicates that species responds not only to a constant

photoperiod but also to a shift of photoperiod. When the shift was made later (**Fig 1**, triangles), the effect was much less, indicating that diapause was intensified or that the photoperiodic sensitivity was reduced. **Figure 2** shows that the transfer to LD 16 : 8 induced pupation a week or two after the transfer, that is followed by pupal eclosion 5-6 days later, while they stayed in diapause under LD 12 : 12.

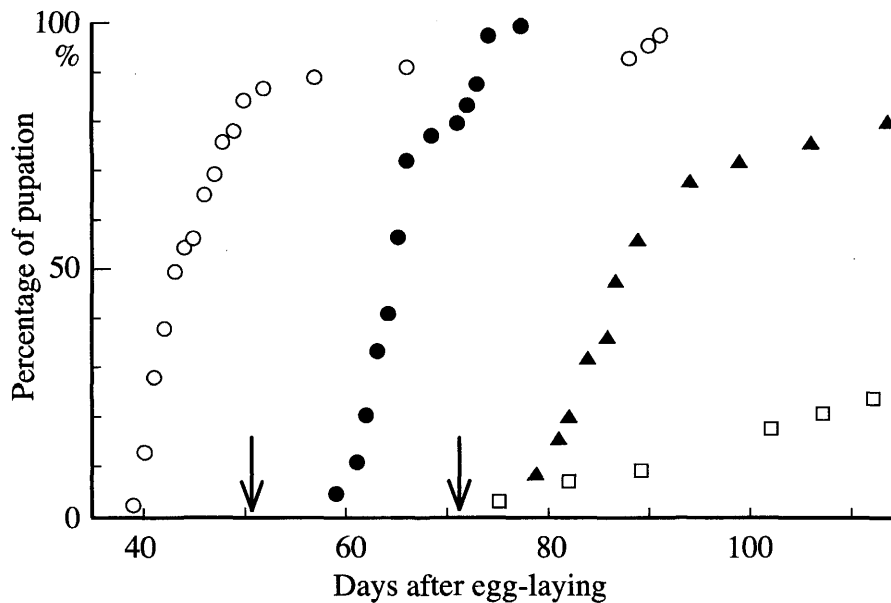


Fig. 1 Cumulative percentage of the number of pupation after transfer from LD12 : 12 to LD16 : 8. Open circles show pupation under LD16 : 8 constant; open squares under LD12 : 12 constant; closed circles transferred on the 51st day (arrow); closed triangles transferred on 72nd day (arrow).

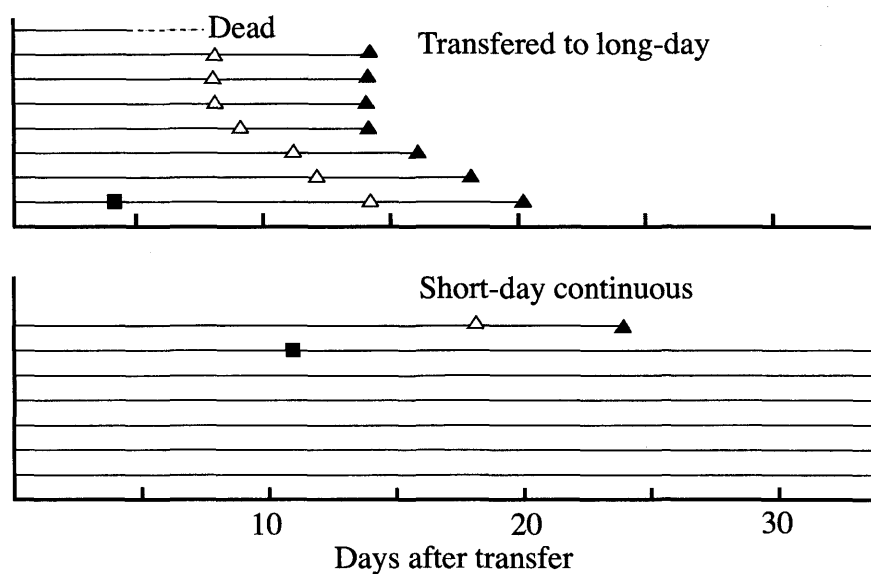


Fig. 2 Timing of metamorphic events occurring in individuals transferred to LD 12 : 12 or 16 : 8 103rd day after egg-laying. Closed squares show larval molting, open triangles pupation and closed triangles pupal eclosion.

Figure 3 shows a surtch in developmental fate by the transfer from LD 16 : 8 to LD12 : 12 at different times after the third larval molt. The result showed that the transfer to LD 12 : 12 was effective to induce diapause until 1-3 days before the molt but the transfer thereafter could not keep them from pupating. Diapause program was more solidly committed and

enforced if the transfer was made in later time.

Figure 4 shows the result of experiment that examined the effect of long day treatment on diapause-destined larvae 20 days after the third larval molt for diapause termination. Pupation seemed to be accelerated by the treatment of 6 days but the treatment of 2-4 days failed to reverse the

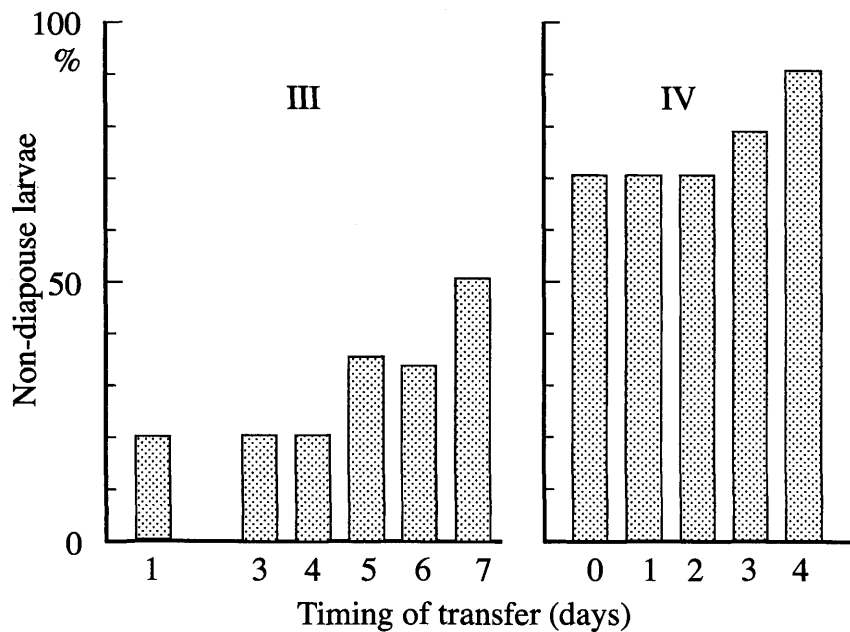


Fig. 3 Percentage pupation without diapause after transferred from LD 16 : 8 to LD12 : 12. Numbers indicate age in days after molting to 3rd (III) or 4th (IV) instar.

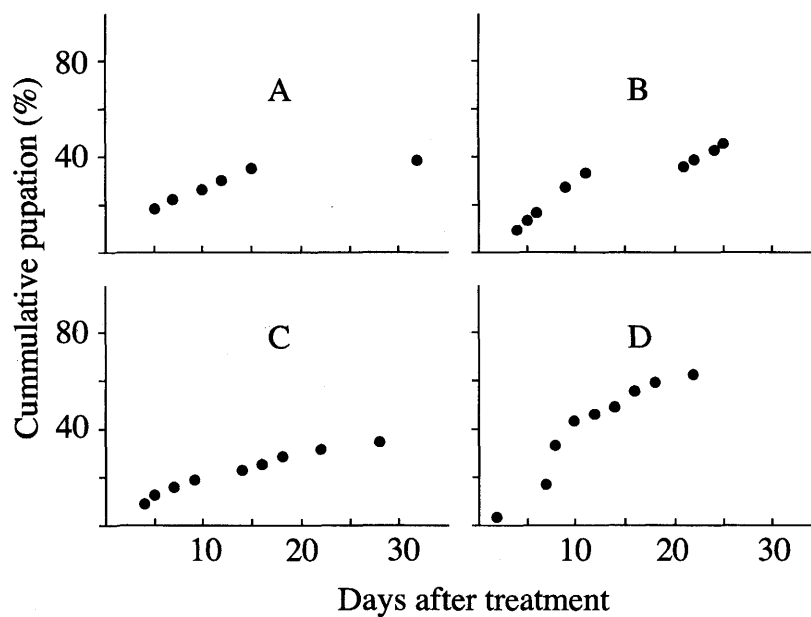


Fig. 4 Effects of periods of long-day exposure on diapause termination in *L. serricorne*. Dots show cumulative percentage of pupation. The insects were exposed to LD 16 : 8 on the 20th day after the third larval molting. A, LD12:12 continued without long-day exposure; B, two days exposure to LD 16:8; C, 4 days exposure; D, 6 days exposure.

determination made by the preceding photoperiodic condition.

The effect of low temperature exposure in different conditions and at different developmental times is given in **Tables 1** and **2**. The exposure of eggs to 5°C for one day killed about 10% of individuals but the exposure for 10 days or longer killed all. Second and third instar larvae reared under LD 16:8 were also not much cold-hardy. However, only 3.6% were killed by 30

days exposure of 4th instar larvae. Pupae were not cold-hardy but adults endured of cold more than other stages but 4th instar larvae. The presence of cocoon and humidification improved survival.

When diapause larvae were exposed to 5°C for two months, 91 days after the egg-laying, the pupation was very synchronous when they were returned to 25°C and 50% of individuals pupated within 10 days after the transfer to 25°C even under LD 12 : 12 (**Fig.5**).

Table 1. Mortality at each developmental stage after exposed to 5°C.

Developmental stage	Period of exposure (days)	Mortality (%)	N
Egg	1	10.5	76
	10	100	78
	30	100	90
2 nd larval instar	30	100	40
3 rd instar	30	97.4	39
4 th instar	30	3.6	28
pupa	30	100	40
Adult	30	35.0	40

All but 4th instar larvae were kept in humid condition. N, the number of samples.

Table 2. Mortality of full grown larvae reared under LD16:8 or 12:12 (*) at 25°C after the exposure to low temperatures, 5 or 10°C, for a variable period

Temperature (°C)	Period	Humidity	Cocoon	Mortality (%)	N
5	60	hi	present	69	32
5*	60	hi	present	53	32
5	30	hi	present	21	34
5	30	hi	removed	32	38
5	30	lo	removed	81	37
10	75	lo	present	31	13
10	61	lo	present	0	22

The low temperature exposures began 91st days after egg-laying. N, the number of samples. High humidity condition is made by saturated salt solutions, while low humidity means an unhumidified condition.

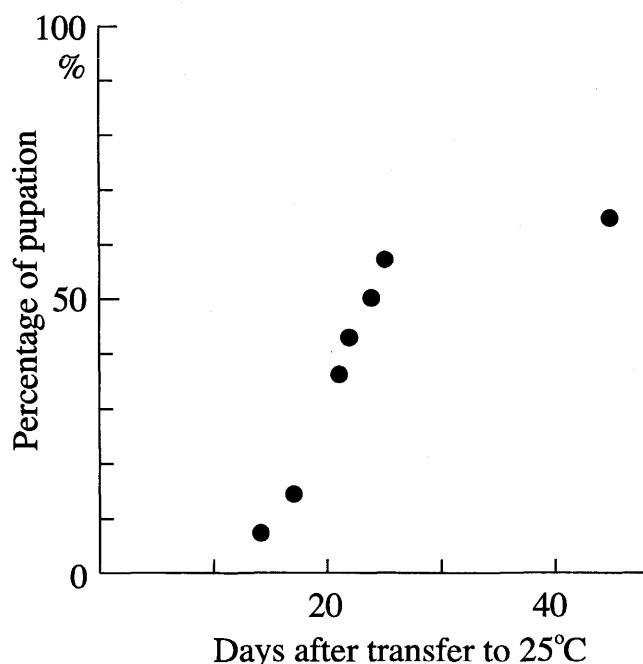


Fig. 5 Cumulative % pupation after the exposure to 5°C for two months. Diapause larvae were transferred to 25°C under LD 12 : 12 after the cold exposure.

Discussion

Developmental responses of *L. serricorne* to various environmental conditions are complicated and unique (Higashi *et al.*, 2005). Diapause of *L. serricorne* that occurs at 4th larval instar was terminated by long-days and low temperature though the former seems more effective than the latter. This stage is by nature tolerant to low temperature since about 50% non-diapause larvae at this stage survived this temperature of two months. Diapause at this stage is thus the adaptation to overwinter. The adult stage is also somewhat more tolerant than the other stages but 4th instar larva. However, it is not certain whether adults can overwinter indoors, since Howe (1957) has reported that adults cannot survive 4°C for more than 6 days, though our strain survived 5°C for 30 days. There may be a strain difference. The sensitive stage to photoperiod may include all larval stage before diapause stage but the number of short day cycles required for diapause induction should be more than the period of fourth larval instar. On the other hand, the long-day exposure of 6 days or longer terminated or blocked diapause effectively. A variety of control channels for development as well as a long period of

diapause that is sporadically terminated makes the life cycle patterns more variable. Photoperiodic response has most frequently been analyzed at constant photoperiods but a most elaborate set of responses to photoperiod has been demonstrated in the regulation of prepupal diapause in the tailed zygaenid moth, *Elcysma westwoodii* (Gomi and Takeda, 1991) that includes the response to changing photoperiods. *L. serricorne* also has such an adaptation, though less dramatical.

The drugstore beetle *Stegobium paniceum* that belongs to the same Anobiidae as *L. serricorne* also shows an extension of the fourth larval instar at 20°C (Momoi and Sadamori, 1982). *S. paniceum* is more tolerant to low temperature than *L. serricorne*. The former has been found in bird nests of temperate areas (Kiritani, 1959; Yoshida, 1978), whereas *L. serricorne* has seldom been observed outdoors. Momoi, S. has failed to detect photoperiodic response in *S. paniceum* (unpublished data). Both *L. serricorne* and *S. paniceum* have colonized Japan in warehouse habitats in the same way from tropical forest environment relatively recently. Comparison of life cycle traits of these beetles may provide interesting clues to the evolutionary process of colonization of the new environment in these species. The life cycles of these beetles may depend on various physiological, developmental and ecological constraints such as history of colonization, the stability of the new habitat, the presence of the competitors, the periodicity of stress conditions, physiological limit of low temperature tolerance and degree of migration. Clarifications of these factors in relation to life cycle traits are desperately needed for the implementation of effective urban pest management.

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長日と低温によるタバコシバンムシ幼虫休眠の終結と休眠誘導の阻害

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25℃短日 (LD12:12) で個体飼育したタバコシバンムシの幼虫を色々な時期 (産卵後 51 日目, 71 日目および 103 日目) に長日 (LD16:8) に移し, 休眠期間の調節と休眠覚醒機構を検討した。いずれの時期でも, 長日処理で休眠間発育が促進され, 処理後約 10 日で羽化した。51 日目で長日に移した場合と比べ, 71 日目に移した場合では蛹化がゆっくり起こった。このことは, 休眠時間とともに, 休眠が深化するか, 短日に対する感受性が低くなるためと考えられる。長日で一部発育遅延がみられたが, 短日 51 日目から長日に移し変えた場合には, これが見られなかった。このことは, 一定の光周期だけではなく, 光周期の変化にも本種は反応している可能性を示す。長日 6 日目でかなりの個体が羽化したことから, 休眠覚醒には 6 日間の処理で十分であることが判った。次に 4 齢脱皮を挟んでその前後を 25℃長日で飼育したものを, 短日へ移しかえたところ, 短日に対する感受性は幼虫の 4 齢に達するまで持続することが判った。日本の冬で遭遇する低温 5℃に 10 日間暴露すると, 全ての卵が死亡し, 蛹, 2 齢及び 3 齢幼虫では 30 日間の暴露で, それぞれ 100 %, 100 % および 97.4 % の死亡率となったから, この発育ステージでは本種の低温に対する耐性は低いと結論された。しかし, 4 齢幼虫は低温耐性が高く, 成虫では 65 % がこの処理 (5℃, 30 日間) に耐えた。4 齢幼虫では, 繭の存在と高湿度で低温条件での生存率が高くなった。5℃に比べ 10℃は厳しい条件ではないが, 低温 75 日間処理すると 3 分の 1 は死亡した。2 ヶ月 5℃で処理すると, 休眠が早期終結する効果を示した。