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Larval diapause in the Mediterranean green lacewing *Mallada picteti* (McLachlan) (Neuroptera Chrysopidae): induction by photoperiod, sensitive and responsive stages. (¹)

INTRODUCTION

Fifteen green lacewing species belonging to the genus *Mallada* occur in Europe. The instar in which they overwinter in the field is known for only six of them, namely: *M. clathratus* (Schneider), *M. flavifrons* (Brauer), *M. ibericus* (Navàs), *M. prasinus* (Burmeister), *M. ventralis* (Curtis) and *M. zelleri* (Schneider) (Principi & Castellari, 1970). All do so as free-living larvae, usually in the third-instar before growth is completed, more uncommonly in the second-instar, as sometimes occurs in *M. ventralis sensu lato* (Killington, 1937) or *M. flavifrons* (Principi *et al.*, 1975). Only *M. flavifrons* has been subjected to experimental studies dealing with overwintering and the relevant cues involved (Principi *et al.*, 1975, 1977; Principi & Sgobba, 1987). The larvae exhibited a facultative diapause, induced and possibly broken by photoperiod; but they were always able to end diapause spontaneously, i.e. to resume their development under steady diapausing daylight conditions.

Mallada picteti (McLachlan) is rather common in the west-Mediterranean area, but little is known about its bionomics. Imagos are xerophilous, and occur mainly in shrubby or herbaceous vegetation where they are present from May to October. A preliminary study (unpublished) established that *M. picteti* overwinters, as do its congeners, as free-living larvae. We therefore carried out a study of the effect of photoperiod on the preimaginal development in the laboratory, the results of which are given here.

MATERIAL AND METHODS

The strain of *M. picteti* used in experiments originates from southwestern France, from biotopes previously described by Canard, (1987), which are typical

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of the Quercetum ilicis (evergreen oak) association. Specimens were captured as adults. The recorded data is from the F_1 offspring of 8 pairs.

The larvae were reared singly in 4 ml glass vials stoppered with a cotton-wool plug. They preyed on *Anagasta kuehniella* (Zeller) eggs and on the aphid *Acyrthosiphon pisum* (Harris), both provided in abundance. The temperature was uniformly 21 ± 1°C, the relative humidity ranged from 60 to 80%. A dim illumination was provided by fluorescent bulbs; it was modulated according to an all-or-none type photoperiod, assuming a 24-h cycle. The accuracy of the diel duration was within ± 5 minutes. Three photoperiods were used; the relevant durations of photophases tentitatively simulated the long days of summer solstice L:D 16:8 (condition 1), the daylength of equinox time 12:12 (condition 2), and the shortest days of winter solstice 8:16 (condition 3). Photoperiods were applied continuously during the lifespan (conditions 1 to 3), or with a returnless stepwise transition (transfer) operated at the beginning of one of the three larval stages (conditions 4 to 9).

The development, hatching, ecdysis, spinning of the cocoon and adult emergence, were checked daily at the beginning of the light phase. The larvae were weighed on an electrobalance to the nearest 2 µg, i) just after the two successive ecdysis, ii) at regular times during the third-instar. The cocoons were weighed to the nearest 0.1 mg within two days of spinning. Fat content was quantified after extraction over 24 hours using petroleum ether.

Every experiment was initiated using 24 individuals. When statistically correct, the results were given as means together with standard deviation. Variation in times due to the population polymorphism (inter-individuals) and to the rearing-cabinet accuracy (inter-conditions) never reached 10%; we have therefore rejected from the duration analysis all differences lower than this discriminative value.

RESULTS AND DISCUSSION

Exhibition of diapause

The duration of preimaginal development tested under continuous photoperiods are shown in Table 1 and Figure I. In the long-day regime (condition 1), all stages required the shortest time to achieve the fastest (diapauseless) growth under the above-mentioned thermal and trophic conditions. For this reason, every duration exceeding the maximum of the range in each stage is considered delayed (diapausing), namely when being more than 9-days long in the three larval stages.

The embryogenesis period did not vary with daylength. No significant difference was evident between conditions 1,2 and 3, and the mean was $\bar{x} = 8.9 \pm 0.3$ days ($n = 72$). The first stage manifested a low change in duration, medium and short days inducing a slight lengthening (1.2-fold longer), 58% and 29% of the larvae in medium days and short days, respectively, were affected by this slackening. The second stage was also modified in the same way by daylenght: 1.8-

Table 1 - Durations in days of preimaginal development (mean ± standard deviation) in *Mallada picteti* reared under various constant photoperiods. In brackets: the size of the sample.

Condition	photoperiod (L:D)	duration of						
		embryo	1st stage	2nd stage	3rd stage			prepupa + pupa within the cocoon
					without diapause	diapausing	unachieved	
1	16: 8	9.0 ± 0.2 (24)	8.1 ± 0.4 (23)	7.5 ± 0.7 (23)	8.1 ± 1.1 (23)	–	–	22.1 ± 0.7 (21)
2	12:12	9.0 ± 0.2 (24)	9.6 ± 0.6 (24)	13.2 ± 1.0 (23)	–	21 to 67 (13)	42 to 110 (10)	25.1 ± 2.3 (10)
3	8:16	8.7 ± 0.4 (24)	9.3 ± 0.5 (24)	11.9 ± 1.3 (21)	–	15 to 39 (17)	40 to 62 (4)	25.2 ± 2.1 (16)

fold longer in medium days, and 1.6-fold longer in short days, all individuals being responsive. The third stage was much more significantly affected by daylength. In medium days, 57% of the larvae resumed growth and development, and spun a cocoon within a time range of 3 to 10 weeks; in short days, 81% of the larvae did so within 2 to 6 weeks. All other larvae died (see below).

Mallada picteti thus exhibited a diapause manifested as a retarded development during larval instars, mainly the third, as does *M. flavifrons*. In the former species, all active stages were more or less responsive, the effect in-

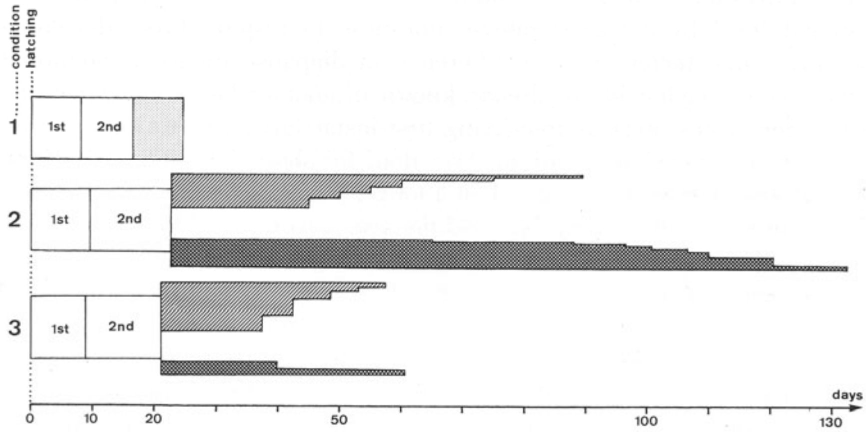

Fig. 1 - Durations of larval development in *Mallada picteti* in relation to the photoperiod. Third-stage without diapause: ░ , in diapause spontaneously broken resulting in cocoon: ▨ , in unlimited diapause resulting in death: ▩ . Diapause data are grouped by 5-day units.

creasing with time: the older the larvae, the stronger the action of the photoperiod. However, *M. picteti* suffered individually from an arrest of development but less intensive than do those of *M. flavifrons* whose third-instars require a longer delay to break diapause spontaneously: 9 to 50 weeks in medium day regime, around an average of 24 to 27 weeks, depending on experiments (Principi *et al.*, 1975, 1977; Principi & Sgobba, 1987). Conversely, the diapause was more severe on the *M. picteti* population, because several individuals never overcame the state of diapause, and died, probably waiting for a cue to restore growth and development. Such a situation was not observed in the Italian *M. flavifrons* strain previously investigated. 43% of the larvae of *M. picteti* survived a long time (6 to 14 weeks) in medium days, and 19% after 6 to 9 weeks in shortdays. Some of them died within a period included in the range of duration required by the homologous larvae to spin a cocoon, especially in short-day conditions (Fig. I). They are expected to fail just before spinning. The others, which survived longer, were active, fed, and did not die from want of food. As in other diapausing larvae, they grew during the third stage and even during the preceding one, but slower than the diapauseless larvae (Fig. II). After living for only 1.5 months from hatching, the mean weight of the irreversibly diapausing larvae reached the maximum weight of the fast-growing larvae, namely 8.6 ± 1.6 mg (n = 17, males and females, undistinguished). Later, the increase gradually diminished, and stopped around an average of 11.5 mg. No weight decrease was recorded before death. The mean rate of fat content was then 28.4 ± 3.4% of the dry body weight (n = 40), 1.5-fold higher than that of the larvae developing fast without diapause. These, checked just before spinning the cocoons, had a fat content of 19.5 ± 4.5% (n = 12).

A peculiarity was manifest in the photoperiodic sensitivity of *M. picteti*, where the medium days (of autumnal type) provided a higher efficiency in inducing diapause, than the short days (of winter type). They revealed in two traits (Fig. II): individually by longer durations of the involved instars, and at the populational level by a higher rate of unfitness to restore direct development without any signal factor. Such a difference in diapause intensity monitored in the same way by daylenght, is already known in another lacewing. *Nineta pallida* (Schneider) overwinters as free-living first-instar larvae; it exhibits a stronger diapause when induced by medium days than by short days (Canard, 1990).

The cocoons of *M. picteti* obtained after longer larval stages were heavier in both sexes than those spun under long day conditions responsible for the shortest developmental time. They weighed 7.4 ± 1.3 mg (n = 35) vs 6.1 ± 0.8 mg (n = 18) in males, and 9.9 ± 1.3 mg (n = 35) vs 8.0 ± 0.9 mg (n = 14) in females. The durations of the lifespan within the cocoon (prepupa plus pupa) were also distributed in the two same classes. Individuals belonging to fastest processes in larval development required 21.5 ± 0.8 days (n = 45), whereas the others completing larval diapause required 24.0 ± 2.2 days (n = 68). The difference (1.1-fold longer) was highly significant. It may be compared to that (1.4-fold longer) recorded in *M. flavifrons* (Principi *et al.*, 1975, 1977), and may be also viewed as a residual effect of the slowing action of diapausing photoperiods.

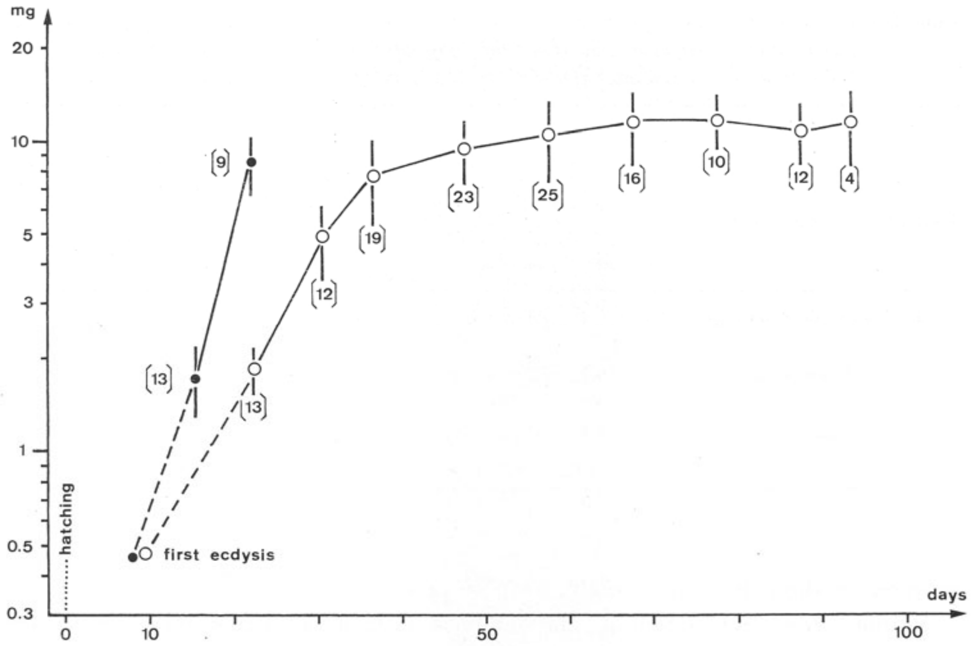

Fig. II - Mean weights of *Mallada picteti* larvae: diapauseless (●), and in unlimited diapause (○), during the second (--) and the third stage (—). In brackets: the size of the sample; vertical bar: standard deviation.

Sensitive stages

In order to determine which stages were sensitive to photoperiod, two types of transfer were operated, subjecting the larvae: either to medium days after long days, or reciprocally, at any step of development. The first category provided data for Table 2 and Figure III. If the single third-instar was subjected to medium days (condition 4), the mean of all durations recorded ($\bar{x} = 8.3 \pm 1.8$ days, $n = 24$) did not differ significantly from that previously observed under the continuous long-day situation (condition 1), mainly because of the high value of the standard deviation. Scrutinizing the distribution of the data however, showed that some individuals (17%) developed in 10 and 11 days, thus more than the predetermined threshold of diapause. They were expected to respond to the sole external factor modified, the experimental photoperiod.

When the transfer was effected at the beginning of the second stage (condition 5), the responses were diversified in three ways. Some larvae (17%) developed without delay, 83% of the larval population entered diapause, but 48% never resumed development and died. When the whole active larval life (from hatching) was completed under medium days (condition 6), the first and second stages were largely slowed by 1.2- to 1.8-fold, respectively. All the third-instars entered diapause, and 68% of the population could not resume development. With respect to the durations recorded, this latter condition was very similar to that induced by station-

Table 2 - Durations in days of preimaginal development (mean ± standard deviation), in *Mallada picteti* after a stepwise transition from long days (L:D 16:8) to medium days (12:12). Italic type: durations after transfer. In brackets: the size of the sample.

Condition	transferred at	duration of 1st stage	 2nd stage	3rd stage without diapause	 diapausing	 unachieved	prepupa + pupa within the cocoon
4	beginning of 3rd stage	8.0 ± 0.4 (24)	7.4 ± 0.8 (24)	*7.9 ± 0.9* (20)	*10.2 ± 0.5* (4)	—	*21.1 ± 1.0* (23)
5	beginning of 2nd stage	8.2 ± 0.7 (24)	*7.0 ± 0.6* (24)	*8.9 ± 0.8* (4)	*33 to 86* (8)	*32 to 107* (11)	*25.1 ± 3.2* (7)
6	hatching	*9.6 ± 0.9* (24)	*12.7 ± 1.3* (22)	—	*30 to 79* (7)	*62 to 104* (15)	*27.9 ± 1.7* 4)

ary medium days. But surprisingly, a higher percentage of individuals in the sample could not achieve development. The decrease of daylength experienced, even very early in larval life, had strengthened the diapause-inducing power of the photoperiod, with respect to the population response only.

The second category of transfer leading the larvae from medium days to long days resulted in the data of Table 3 and Figure IV. When considering the single third-instar (condition 7), the diapausing regime prevalent during the two first stages enabled the third-instars to manifest a generalized diapause, but of low

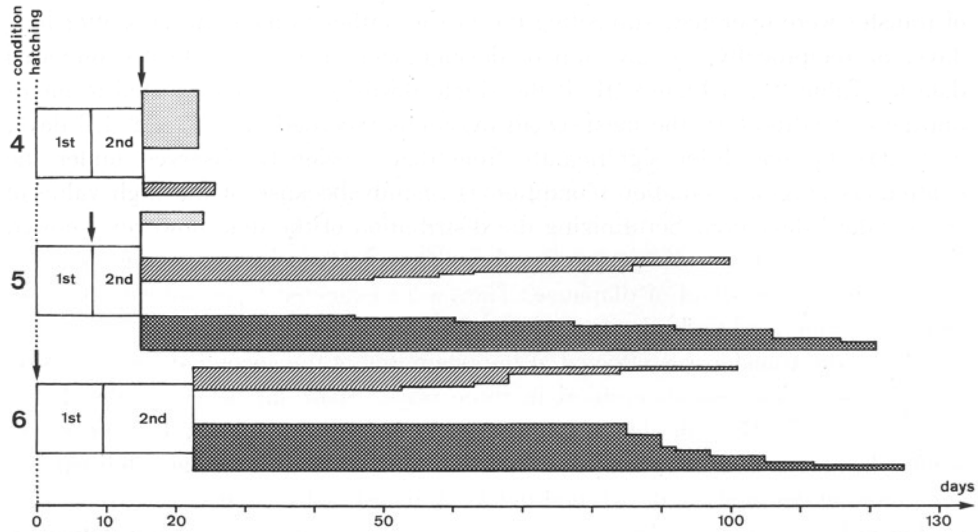

Fig. III - Durations of larval development in *Mallada picteti* when transferred from long days (L:D 16:8) to medium days (12:12). The arrows indicate the time of transfer. Signs as in Fig. I.

Table 3 - As in Table 2, transition from medium days (L:D 12:12) to long days (16:8).

Condition	transferred at	duration of					
				3rd stage			prepupa + pupa within the cocoon
		1st stage	2nd stage	without diapause	diapausing	unachieved	
7	beginning of 3rd stage	9.6 ± 0.7	13.0 ± 1.4	—	*12.6 ± 1.6*	—	*23.2 ± 1.3*
		(24)	(24)		(24)		(22)
8	beginning of 2nd stage	9.3 ± 0.7	*10.8 ± 0.7*	*8.2 ± 0.4*	*11.1 ± 1.6*	—	*22.1 ± 0.9*
		(23)	(23)	(5)	(17)		(17)
9	hatching	*8.6 ± 0.6*	*7.3 ± 0.6*	*7 to 8*	—	—	(not checked)
		(24)	(24)				

intensity; the durations were all between 11 and 17 days. When applied at the beginning of the second stage (condition 8), the transfer towards medium days also allowed the larvae to diapause slightly, but only 74% of the sample did, the other larvae being directed towards fast (diapauseless) development by the new

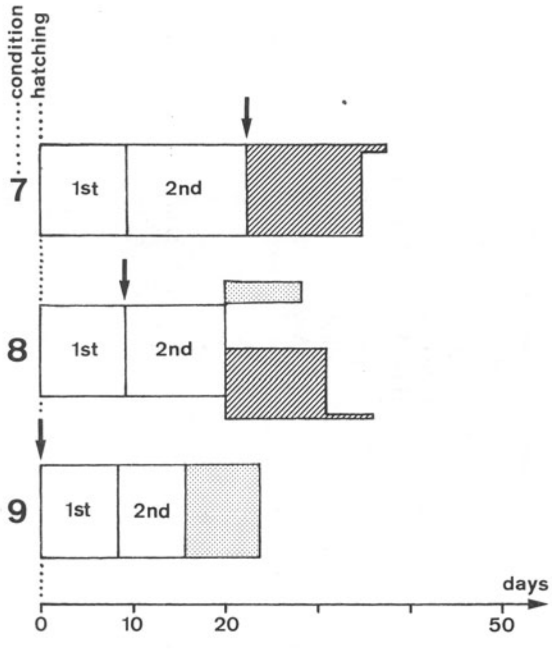

Fig. IV - As in Fig. III, transferred from medium days (L:D 12:12) to long days (16:8).

Table 4 - Compared durations in days of preimaginal development (mean or range) in some *Mallada* spp., from the literature.

Species	temperature (°C)	photoperiod (L:D)	duration of						authority
			embryo	1st stage	2nd stage	3rd stage	prepupa	pupa	
ventralis	unknown	natural (Jun.-Aug.)	7 to 14	5 to 7	18 to 21 or 270 to 300	18 to 21 or 270 to 300	6 to 8	12 to 17	KILLINGTON, 1937
clathratus	24 to 27	natural (Jul.-Aug.)	5 to 7	8 to 16	9 to 14	6 to 11 or 240	6	10 to 11	PRINCIPI, 1956
prasinus	»	»	5 to 6	5 to 5.5	6 to 7	6 to 12 or 140 to 200	5	6 to 10	»
flavifrons	21	16:8	7	19.5	11.8	10.7	7.5	14.6	PRINCIPI *et al.*, 1975
»	»	12:12	7	18.8	19.8	60 to 360	10.4	14.7	»
boninensis	20	9.5:14.5	5.5		← 14 to 18 →		← 13 to 15 →		BRETTEL, 1979
»	24.5	14:10	4.1	4.5	4.8	5.9	← 11.7 →		LEE & SHIH, 1982
ibericus	21	16:8	9.4	8.0	8.8	9.8	8.0	15.9	CANARD, unpublished

long-day regime. This last response involved the whole sampling submitted to long days from hatching (condition 9).

The third-instar of *M. picteti* was in itself insensitive or only slightly sensitive to the diapause-inducing action of the photoperiod, diapause or non-diapause development being pre-determined. The response obtained was not of an «all-or-none» type, and the intensity of diapause largely depended on the conditions the third-instar encountered. The second stage was the key-period for the future of the population, but yet not decisive for a small proportion (about 1/6). Only the cumulative action from the first to the third-instar induced a hard and generalized diapause, as did medium days acting continuously. In these traits, *M. picteti* exhibited the same sensitivity as *M. flavifrons* (Principi *et al.*, 1977, Principi & Sgobba, 1987). It revealed a life cycle regulated by daylength, like other *Mallada* (Table 4) occurring in the temperate region, but different of that of the tropical *M. boninensis* (Okamoto) which can develop fast, independent of short day influence (Brettell, 1979).

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SUMMARY

The lacewing *Mallada picteti* reared in the laboratory at 21°C was subjected to various photoperiods during its preimaginal development. The shortest durations of stages were manifested under the long day regimes (LDs) of summer type L:D 16:8. First stage required 8.1 ± 0.4, second stage 7.5 ± 0.7, and third stage 8.1 ± 1.1 days (n = 23). Medium days (MDs) of autumnal type 12:12 and short days (SDs) of winter type 8:16 induced a lengthening of all stages, except the embryogenesis period which was 8.9 ± 0.5 (n = 72) days long. The first stage increased slightly in time in both acting photoperiods (1.2-fold longer) in only a part of the population. The second stage was also lengthened: 1.8- and 1.6-fold longer in MDs and SDs, respectively. The third stage was more strongly stressed throughout the whole population, whose 43% of larvae remained blocked in arrest of development, never resumed growth and never spun cocoons. They died within 2 to 6 weeks, without decreasing in weight, and showing a fat content rate higher (23.4%) than the fast developing homologous larvae (19.5%).

Instars sensitive to photoperiod were investigated by transferring the larvae from LDs to MDs, and reciprocally, at the beginning of the three stages. Third-instars showed a little or even no sensitivity to daylength, second-instars ran the key time in diapause-inducing action for a large part of the population. First-instars and embryos were slightly sensitive, only in a few individuals.

M. picteti exhibited a larval diapause manifested as a slackening in larval growth and an increase in durations, mainly in the third-stage which could sometimes be definitively arrested. Spontaneous resumption of development was dependent on the inducing conditions, more frequent and shorter in SDs than in MDs.

La diapausa larvale nella Crisopa mediterranea *Mallada picteti* (McLachlan) (Neuroptera Chrysopidae): induzione ad opera del fotoperiodo, stadi sensibili e stadi durante i quali si verifica la reazione

RIASSUNTO

La Crisopa *Mallada picteti* allevata in laboratorio ad una temperatura di 21°C fu sottoposta durante lo sviluppo preimmaginale a vari fotoperiodi. Gli stadi larvali ebbero la durata più breve

con i regimi a giorno lungo (LDs) di tipo estivo (16 h di luce: 8 h di oscurità). Il primo stadio richiese 8,1 ± 0,4, il secondo stadio 7,5 ± 0,7, e il terzo stadio 8,1 ± 1,1 giorni (n = 23). I giorni medi (MDs) di tipo autunnale (12 h di luce: 12 h di oscurità) e i giorni brevi (SDs) di tipo invernale (8 h di luce: 16 h di oscurità) indussero un prolungamento di tutti gli stadi. Non subì alcun prolungamento il periodo dell'embriogenesi, che durò 8,9 ± 0,5 giorni (n = 72). La durata del primo stadio aumentò leggermente sotto l'azione di entrambi i fotoperiodi operanti (1,2 volte più lungo) solo in una parte della popolazione. Anche il secondo stadio fu prolungato con MDs e con SDs e fu rispettivamente di 1,8 e di 1,6 volte maggiore. Il terzo stadio subì un'influenza maggiore in tutta quanta la popolazione; nel 43% delle larve si verificò un arresto dello sviluppo e queste non ripresero più l'accrescimento e non filarono il bozzolo. Esse morirono entro 2-6 settimane senza diminuzione di peso e presentarono un contenuto di grassi più alto (23,4%) di quello delle larve omologhe a sviluppo rapido (19,5%).

La sensibilità dei singoli stadi al fotoperiodo fu indagata trasferendo le larve da LDs a MDs e viceversa, all'inizio di ognuno dei tre stadi. Le larve di terzo stadio non mostrarono che scarsa o nulla sensibilità alla durata del giorno; il fotoperiodo al quale erano sottoposte le larve di secondo stadio era fondamentale per l'induzione della diapausa in una gran parte della popolazione. Una debole sensibilità si rivelò solo in poche larve di primo stadio e in pochi embrioni.

M. picteti ebbe una diapausa larvale che si manifestò con un rallentamento dell'accrescimento larvale e con un prolungamento della sua durata soprattutto nel terzo stadio. In tali larve di terzo stadio l'accrescimento poteva arrestarsi definitivamente. La ripresa spontanea dello sviluppo dipendeva dalle condizioni d'induzione e fu più frequente e più breve a SDs che a MDs.

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