

PHOTOPERIODIC CONTROL OF DIAPAUSE INDUCTION IN THE LARVA OF *LUCILIA CAESAR* L. (DIPTERA: CALLIPHORIDAE)

By R. A. RING

Entomology Research Institute, Central Experimental Farm, Ottawa, Ontario

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INTRODUCTION

Diapause in insects is herein defined as a state of arrested growth and development which, unlike quiescence, persists until the animal undergoes a certain, well-defined sequence of physiological events that enables development to be resumed. In the green blowfly, *Lucilia caesar* L., a facultative diapause occurs in the 3rd-instar larva after cessation of feeding but before puparium formation. The 1st-instar larva hatches within 24 hr. of oviposition and at 22° C. this stadium lasts for about 24 hr. The 2nd instar also lasts about 24 hr. at this temperature, while the 3rd instar lasts 7-10 days. The first 3 days of the final instar is a feeding period, the remaining 4-7 days being spent as a non-feeding prepupa. As Fraser (1957) points out this is not, strictly speaking, a true prepupa since the prepupal stage is an evanescent 4th instar within the puparium. The non-feeding stage of the 3rd instar, however, has by convention become known as the prepupa. After cessation of feeding, the 3rd-instar larva becomes negatively phototropic, positively geotropic, and under natural conditions burrows into the soil up to a depth of about 6 in. It may then either complete development or enter a prolonged state of diapause, depending upon the season of the year, among other factors.

A number of authors (e.g. Roubaud, 1922; Cousin, 1932; Mellanby, 1938; Cragg & Cole, 1952; Fraser, 1957; Fraser & Smith, 1963) have shown that environmental factors such as low temperature, desiccation, lack of aeration and competition for available food act upon the larvae of *Lucilia* spp. to induce diapause. There is, however, little information available on the effect of photoperiod. Dickson (1949) indicated that this factor did not influence the tendency of *L. sericata* Mg. larvae to enter diapause, but his results with this species were incidental to the main theme of his research, the induction of diapause in *Grapholitha molesta* Busck, and were based on only a few observations. Cragg & Cole (1952) found that the egg-batches from wild *L. sericata* females caught in late summer produced a high proportion of diapausing larvae even when reared under laboratory conditions favouring normal development. Although they accept that unfavourable environmental conditions acting on the larvae may induce diapause, they concluded that diapause in this species may also be of maternal origin. Fraser & Smith (1963) reached a similar conclusion while maintaining cultures of *L. caesar*.

This paper offers preliminary information concerning the direct effect of photoperiod on diapause induction in larval *L. caesar*, such information being a prerequisite for the understanding of a maternal influence on larval diapause (the subject of a subsequent communication).

* Present address: Department of Biology, University of Victoria, Victoria, B.C.

METHODS

The larvae used in this experiment were offspring of adults captured at the Glasgow University Field Station, Rosdhu Estate, Dunbartonshire, during the breeding seasons of 1962 and 1963. The adult flies were housed in one or other of two constant temperature rooms maintained at 22° C. and 60–70% relative humidity. Illumination was provided by 'warm white' fluorescent tubes which, at cage level, produced a light intensity of approximately 100 foot-candles as measured by an E.E.L. photometer. In one room a time switch supplied a long photoperiod regime of 20 hr. of light alternating with 4 hr. darkness (LPR); in the other a time switch gave a short photoperiod regime of 12 hr. of light alternating with 12 hr. of darkness (SPR).

The eggs obtained from stock cultures hatched on slices of liver, and the larvae from several egg batches, each laid by a different female, were allowed to intermingle before being placed in groups of approximately 50 into 500 ml. rearing flasks. Each flask contained 2 in. of moist peat moss to discourage the newly introduced larvae from wandering, and raw meat was added at the rate of about 1 gm. for every two larvae. Two or three days after introduction of the larvae each flask was filled with moist peat moss and then closed with muslin. The larvae were exposed to the same photoperiod conditions as their parents until the desired stage of development had been reached, when they were transferred to the alternative photoperiod regime.

The conditions described constituted the so-called optimal environment with respect to temperature, adequate food supply, high moisture content, good aeration and freedom from crowding (Fraser & Smith, 1963). Any excess food was left in the flask after the larvae had stopped feeding, because to remove it at this time would have disturbed the larvae when they were 'assessing' the attributes of the environment (Fraser, 1957) and when they may have been hypersensitive to stimuli. Since non-diapausing larvae pupate within 4–7 days of cessation of feeding, those individuals still in the larval stage of development after 14 days were arbitrarily regarded as being in diapause (Cragg & Cole, 1952; Fraser, 1957).

RESULTS

A comparison was made of the diapause incidence of larvae reared continuously under long or short photoperiod conditions with that of larvae initially reared under one regime but later transferred to the other where they completed development. The effects of transferring larvae from LPR to SPR are represented in Table 1, and of transference from SPR to LPR in Table 2. In obtaining these data two, or three where possible, culture flasks were prepared from each sample of larvae derived from a single stock cage. One group (group A) was retained as a control in the original photoperiod regime under which the parents had been reared, while the other (group B) was transferred to the alternative regime. Where three culture flasks were available, then the third (group C) was retained along with control group A. In order to determine the larval stage responsive to photoperiod, groups were transferred at various stages in development between eclosion and the late 3rd instar feeding period. Transfers were made during the 1st instar, within 24 hr. of eclosion; or during the 2nd instar, 24–48 hr. after eclosion; or in the 3rd instar, 48–96 hr. after eclosion, that is,

Table 1. *Diapause rate of groups of larvae transferred from long to short photoperiod conditions*

Transferred to SPR in 1st instar				Transferred to SPR in 2nd instar				Transferred to SPR in 3rd instar				
Control Group A		Group B		% Diff. between diap. and non-diap. insects		Control Group A		Group B		% Diff. between diap. and non-diap. insects		
No. larvae entering diap.	No. larvae entering diap.	No. larvae entering diap.	No. larvae entering diap.	No. larvae entering diap.	No. larvae entering diap.	No. larvae entering diap.	No. larvae entering diap.	No. larvae entering diap.	No. larvae entering diap.	No. larvae entering diap.	No. larvae entering diap.	
35	3	31	28	+81	50	26	48	48	38	0	44	0
42	16	40	40	+68	55	11	50	32	50	47	51	50
56	11	50	43	+66	105*	14	54	37	54	9	59	10
Mean difference = +72 %					48	1	46	28	96*	0	50	2
					92*	46	47	42	57	0	61	1
					59	6	37	23	+52	45	48	48
					44	1	49	19	+37	32	49	38
					Mean difference = +48 %							
							49	40	49	48	50	39
							51	48	51	48	39	39
							49	6	49	6	48	4
							100*	84	100*	84	51	45
							96*	6	96*	6	51	6
							100*	0	100*	0	54	6
							Mean difference = +5 %					

* Group C retained with group A.

Table 2. *Diapause rate of groups of larvae transferred from short to long photoperiod conditions*

Transferred to LPR in 1st instar				Transferred to LPR in 2nd instar				Transferred to LPR in 3rd instar			
Control Group A		Group B		Control Group A		Group B		Control Group A		Group B	
No. entering diap.	No. entering larvae	No. entering diap.	% Diff. between diap. and non-diap. insects	No. entering diap.	No. entering larvae	No. entering diap.	% Diff. between diap. and non-diap. insects	No. entering diap.	No. entering larvae	No. entering diap.	% Diff. between diap. and non-diap. insects
100*	50	37	-26	71*	32	29	-9	50	46	34	-26
92*	49	32	-35	46	53	33	-36	103*	58	49	-16
49	51	29	-41	39	38	20	-37	88*	45	39	-13
40	57	32	-44	46	32	12	-29	50	42	14	-54
103*	44	16	-64	98*	38	12	-68	47	41	16	-50
46	37	6	-51	51	55	53	-2	51	11	0	-22
55	43	18	-44	96*	50	48	-4	103*	48	36	-25
Mean difference = -44%				49	50	36	-18	50	50	42	-14
				89*	47	44	-7	105*	39	0	-37
				50	40	36	-10	56	49	39	-20
				74*	41	19	-22	Mean difference = -28%			
				37	29	3	-90				
Mean difference = -28%											

- Group C retained with group A

during the first and second days of the feeding period of this instar. Since later instars would have had less time to respond to a change in the length of the photoperiod the possibility of obtaining less critical results with such groups was partially offset by employing a greater number of replicates in the 2nd and 3rd instars. The difference in the diapause incidence of the two groups from the same parentage was calculated as percentage difference, and the mean percentage difference for groups transferred in each instar is shown. Transference of larvae from LPR to SPR during the 1st instar resulted in a mean increase of 72 % in diapause rate in experimental groups over control groups. This value became progressively smaller with increasing age of the larvae at the time of transfer, being 48 % in the 2nd instar and only 5 % in the 3rd instar. With the reverse procedure there was a relatively large overall reduction in the diapause rate, but the age at transference did not seem to be so critical. Removal from SPR to LPR in the 1st instar resulted in a 44 % decrease in the diapause rate, in the 2nd instar a 28 % decrease, and in the 3rd instar a similar 28 % decrease.

DISCUSSION

Differences between control and experimental groups of larvae from the same females indicate that when larvae were transferred from long to short photoperiod conditions there was a general increase in the diapause incidence. Conversely, transfer from short to long photoperiod produced a general decrease in diapause incidence. The response to photoperiod seems to be a cumulative one which depends upon the number of illuminatory cycles experienced by the larvae, since the earlier they were transferred from one photoperiod regime to the other, the greater the contrast between experimental groups and controls. Fraser (1957) concluded that the fate of an individual with respect to diapause tendency was determined by the 24th hour after cessation of feeding. Thus, when a larva is transferred during the 1st instar, it will experience a maximum of six illuminatory cycles during which it can respond to the new photoperiod regime. On the other hand, when a larva is transferred on the second day of the 3rd instar then it will experience only two such cycles. However, even at this stage the larva is still sensitive to a change in the absolute length of the photoperiod, and particularly so when the change is from a short to a long photoperiod regime. This rapid response to a change in the absolute length of the photoperiod is in contrast to the delayed response to photoperiod of the mother in inducing larval diapause via a maternal influence. Ten to sixteen days are required in the case of adult *L. sericata* (Cragg & Cole, 1952) and 8–10 days in *L. caesar* (Ring, 1967). The present results show, therefore, that there is no critically sensitive stage to photoperiod limited to a single instar, or phase of an instar, but instead there is a response spread over all larval instars.

The results of this experiment do not conform with those of Dickson (1949). On the contrary, it is evident that larvae of *L. caesar* are sensitive to photoperiod throughout their feeding life, and possibly also in the immediate post-feeding phase. Cragg & Cole (1952) noted Dickson's results with *L. sericata* and stated that 'considering the normal habitat of blowfly larvae this result is not unexpected'. It is interesting that larvae, normally in a darkened environment, nevertheless do respond.

There are several other examples of insects which, like blowfly larvae, are shielded

from direct light but which are nevertheless capable of reacting to photoperiod, For instance, the larvae of the Oriental Fruit Moth, *Grapholitha molesta*, will respond to a low light intensity (1-3 foot-candles) reaching the surface of immature apples in which they are tunnelling (Dickson, 1949). In some parasitic insects, it has been shown that the parasite has its own independent photoperiodic response and diapause can be induced without interfering with the development of the host (deWilde, 1962). Also, Williams & Adkisson (1964) have shown that the pupa of *Antheraea pernyi* can respond to photoperiod even though it is enveloped in a stout-walled cocoon.

Further studies of photoperiodic reactions in *L. caesar* will have to take into account this hitherto unrecorded larval sensitivity.

SUMMARY

1. It has been shown that photoperiod has a direct effect on the larva of *Lucilia caesar* L. in the induction of diapause.

2. Transference of larvae from long to short photoperiod conditions during the 1st, 2nd, or 3rd instar increases their tendency to enter diapause. Conversely, transfer from short to long photoperiod conditions decreases their tendency to enter diapause.

3. Larvae are sensitive to changes in the absolute length of the photoperiod during all instars. The reaction is not restricted to any one stage but tends to be cumulative; thus the earlier the larvae are transferred from one photoperiod regime to another then the greater the contrast in diapause incidence between experimental groups and controls.

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