Effect of Temperature and Photoperiod on the Larval Development and Diapause Induction in the Onion Fly, *Hylemya antiqua*

MEIGEN (Diptera: Anthomyiidae)¹

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Larvae of the onion fly, *Hylemya antiqua* were reared under several combinations of temperatures (10–22°C) and photoperiods (16L–8D and 12L–12D) to determine the effect of these factors on the larval development and diapause induction. Photoperiod little affected the developmental zero temperature for larval development (4.3°C under 16L–8D, and 4.6°C under 12L–12D), whereas the total effective temperature necessary for such development was a little greater in the short day (257 day-degrees under 16L–8D, and 278 day-degrees under 12L–12D). When pupae obtained from different conditions were kept at 25°C, 16L–8D, most flies emerged 9–18 days after pupation, but some were observed to emerge 40–60 days after pupation. This latter was particularly common in cultures at the lower temperatures and short photoperiod. We regarded the individuals emerging within 20 days after pupation as “non-diapausing” and the rest as “diapausing”. The temperature inducing diapause in 50% of individuals was found to be significantly affected by photoperiod: 14.0°C under 16L–8D, and 18.5°C under 12L–12D.

INTRODUCTION

Calculation of total effective temperature (TET) has been proved useful for predicting the emergence of onion flies, *Hylemya antiqua* MEIGEN. This method is used for pest management in an early warning system for onion growers in New York, U.S.A. (ECKENRODE et al., 1975), and also for timing insecticide sprays in Ontario, Canada (LIU et al., 1982). In Japan, prediction by the TET method was tested but not proved accurate enough to be entirely relied on (TOMIOKA, 1977).

For more accurate forecasting of the fly emergence in early spring, much should be studied on the diapause of this insect in general: conditions for the induction and maintenance of diapause as well as those for the initiation of post-diapause development. One of the problems encountered in estimating the developmental zero for larval development is that at low temperature, the yield of pupae on onion becomes so low that it is difficult to obtain enough data for analysis (ISHIKAWA, unpublished observa-

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tion). We found that the yield was improved at low temperature when larvae were reared on an artificial diet (ISHIKAWA et al., 1983). In this study, we have determined the conditions inducing diapause in the onion fly by rearing the larvae on the artificial diet under several combinations of temperatures and photoperiods.

MATERIALS AND METHODS

Adult culture. Onion flies were originally collected at Sapporo, Hokkaido, and their offspring have been reared on an artificial diet (ISHIKAWA et al., 1983) for about 15 generations in our laboratory. Flies were reared in a cage (30×30×30 cm) with a supply of water, cube sugar and Bacto® yeast extract. Cages were kept in an air-conditioned room (1.8×2.7×1.8 m, 23±1°C, 16L-8D and R.H. 60-80%). Eggs were obtained as described in a previous paper (ISHIKAWA et al., 1983). About 1,000 eggs were put on a moistened filter paper in a petri dish (9 cm in diam.) and kept at 25°C, 16L-8D. Eggs hatched within 2 days under these conditions.

Rearing of larvae. Fifty larvae less than 24 hr old were inoculated on an artificial diet (ISHIKAWA et al., 1983) in a petri dish (9 cm in diam.). The rearing dishes were divided into ten groups and put in growth chambers (TG-100-A, Nihon Ikakikai) under different conditions: combinations of 5 constant temperatures; 10, 13, 16, 19 and 22°C, and 2 different photoperiods; 16L-8D (long day) and 12L-12D (short day). From 10 days after inoculation, pupation was checked daily, and the new pupae were transferred into small vials (2.5 cm diam.×5.0 cm) and kept in a chamber of 25°C, 16L-8D irrespective of the larval rearing conditions. The number of newly emerged flies was recorded daily. Seventy-five days after pupation, all the remaining pupae were dissected to see if they were alive or dead.

Criterion of diapause. As described later, the individuals emerging within 20 days after pupation were regarded as “non-diapausing”, and those emerging hereafter and remaining in pupal stage as “diapausing”.

RESULTS

Effect of temperature and photoperiod on the larval growth and pupation

Figure 1 shows the relationship between the rearing temperature and the develop-

Fig. 1. The rate of larval development in relation to temperature under 16L-8D and 12L-12D,

Table 1. Larval growth of *Hylemya antiqua* under different temperatures and photoperiods

Photoperiod	Temperature (°C)	No. larvae inoculated	No. pupae obtained	Pupation rate (%)	Mean larval period (days)
16L-8D	22	300	229	76.3	14.4
	19	150	101	67.3	17.3
	16	200	106	53.0	21.3
	13	350	107	29.8	32.5
	10	250	68	21.5	41.5
12L-12D	22	300	183	61.0	14.3
	19	200	74	37.0	22.5
	16	200	101	50.5	24.5
	13	200	73	36.5	36.3
	10	200	120	60.0	41.5

Fig. 2. Cumulative percentage of emergence in *H. antiqua*, larvae of which were reared at 5 different temperatures under 16L-8D (2A) or 12L-12D (2B). Pupae were kept under 25°C, 16L-8D irrespective of the larval rearing conditions.

mental rate (100/larval period). The regression was highly significant in the 16L-8D culture, showing a relation $Y = -1.70 + 0.39X$, $r^2 = 0.99$, where X and Y indicate the temperature and rate of development, respectively. The developmental zero was estimated from this equation as 4.3°C, and a sum of 257 day-degrees was necessary to complete larval development under 16L-8D. Under 12L-12D, the larval growth at 13-19°C seemed somewhat retarded. The relationship was represented by $Y = -1.65 + 0.36X$, $r^2 = 0.90$, the developmental zero (4.6°C) being almost the same as that under 16L-8D. The total effective temperature (278 day-degrees), however, was a little greater than that under 16L-8D. Therefore, it seemed that photoperiod did not affect the developmental zero, but at certain temperatures it affected the larval growth probably by inducing diapause. The developmental zeros obtained in this experiment are very close to 4.4°C for the onion fly pupal development (ECKENRODE et al., 1975), and are also close to 3.9°C for the larval development of the seed-corn fly, *Hylemya platura* (CHIBA and SUZUKI, 1980).

The pupation rate varied from 21.5% to 76.3% (Table 1). Under 16L-8D it increased as the temperature rose, but there was no such correlation under 12L-12D. The cause of these fluctuations was not clear, but it seemed that low temperature was

Table 2. Number of flies emerging within 20 days after pupation and percentages of pupae in diapause^a

Photoperiod	Temperature (°C)	No. healthy pupae	No. adults	Pupae in diapause (%)
16L-8D	22	167	155	7.2
	19	65	50	23.1
	16	96	73	24.0
	13	88	30	65.9
	10	55	5	90.9
12L-12D	22	131	119	9.2
	19	66	38	42.4
	16	82	18	78.0
	13	46	7	84.8
	10	105	3	97.1

^a See text for definition of diapause in this paper.

Fig. 3. The relationship between the rearing temperature and incidence of pupal diapause. Dotted line indicates the critical temperature.

unfavorable for the growth of larvae. In this experiment, since a minimum of 68 pupae were obtained per culture, the results obtained could be analysed reliably.

Emergence of flies

Emergence patterns of adults reared under different conditions in their larval stage are shown in Fig. 2. The emergence rate here is based on the number of pupae excluding dead ones. Almost all the flies emerged 9–18 days after pupation in the 22°C, 16L-8D culture (Fig. 2 A). In contrast, few emerged in this period and a considerable number were observed to emerge 40–60 days after pupation in the 10°C, 12L-12D culture (Fig. 2 B). The patterns of adult emergence under the other conditions were intermediate between these two (Figs. 2 A and 2 B). The emergence rate did not exceed 90% except in the 22°C, 16L-8D; 22°C, 12L-12D; and 19°C, 16L-8D cultures. The emergence of adults under other conditions seemed to be suppressed, with the suppression more pronounced in the lower temperatures and short day-length.

In many species the pupae (or eggs) in diapause have to experience a certain period of low temperature, e.g. more than 22 weeks of 0–10°C in the cabbage root fly, *Hylemya brassicae*, for their diapause development, and hence to terminate diapause (COLLIER and FINCH, 1983 a, b; TAUBER and TAUBER, 1976). Since the pupae in this experiment were transferred to 25°C, 16L–8D on the day of pupation, there was no time for the pupae in diapause to complete the diapause development. Meanwhile, non-diapausing pupae were able to develop into adults. It is considered, therefore, that the individuals emerging within 20 days after pupation were “non-diapausing” and those remaining in pupal stage were “diapausing”.

Effect of temperature and photoperiod on diapause induction

Table 2 shows the relationship between the rearing conditions of larvae and the rate of diapause. As dissection showed that some pupae were dead, the rate of diapause was calculated by excluding these dead ones. A possible cause of the pupal mortality is considered to be that the diapausing pupae could not adapt well to the high temperature, or that the great change of temperature (up to 15°C within one day) which would seldom be encountered in nature in the soil may have induced metabolic disorder.

Pupae obtained from the cultures of lower temperatures and short photoperiod showed higher diapause rates. The relationship between the rate of diapause and rearing temperature is shown in Fig. 3. The effect of photoperiod on diapause induction was apparent at the temperatures between 10°C and 19°C, and was greatest at 16°C: diapausing pupae were less than 25% under 16L–8D, whereas more than 90% under 12L–12D. On the contrary, the effect of photoperiod seemed almost nil at 22°C: less than 10% diapause was observed in both 16L–8D and 12L–12D. At 10°C the diapausing rates in 16L–8D and 12L–12D were almost the same, but the effect of photoperiod was clearly shown in the increase of late-emerging flies in 12L–12D (Fig. 2).

The critical temperature (50% diapause induction) was estimated to be 14.0°C under 16L–8D, and 18.5°C under 12L–12D. The rate of diapause induction changed sharply around the critical temperatures under both 16L–8D and 12L–12D (Fig. 3).

DISCUSSION

The developmental zero temperature for larval development was estimated as 4.3°C under 16L–8D, and 4.6°C under 12L–12D (Fig. 1). These values are very low compared with those of other insects studied to date: the developmental zeros range from –1.1 to 19.5°C, and their frequency distribution shows a normal curve, the mean being ca. 11°C (UTIDA, 1957). This may indicate that the onion fly has its origin in a northern region as does the seed-corn fly, whose developmental zero is 4.4°C (CHIBA and SUZUKI, 1980).

Among related species of the onion fly, the conditions for the induction and termination of diapause were investigated in detail in the cabbage root fly. In this species, several factors are known to affect diapause induction: temperature, photoperiod during the larval and adult stages, light intensity, and direction of changes *per se* in temperature and photoperiod (READ, 1969). Opinions of the relative importance of these factors are conflicting among investigators (HUGHES, 1960; READ, 1969; SONI, 1976), but on the whole, photoperiod less than 12 hr and temperature below 15°C is considered to induce diapause in nature. The individuals which pupate in late summer

and autumn maintain diapause until the temperature falls below 10°C. Then diapause development is initiated and completed during the winter. When the temperature rises above 6°C in early spring, the pupae begin post-diapause development to become adults (COLLIER and FINCH, 1983 a, b).

In this study, a joint effect of temperature and photoperiod on diapause induction was observed (Fig. 3). As day-length and temperature change in close association with each other in nature, the joint effect would be related to adaptation to the environment. The effect of temperature on the photoperiodic induction of diapause is demonstrated in many species, e.g., *Pieris brassicae*, *Leptinotarsa decemlineata* and *Acronycta rumicis* (DANILEVSKI et al., 1970; TAUBER et al., 1986). The effect of temperature is greatest when the photoperiod is in the threshold range of these species (DANILEVSKI et al., 1970). In the onion fly, the temperature seemed to play a primary role in diapause induction, and the photoperiod to have additional regulatory effect (Fig. 3). The effect of photoperiod was greatest around the critical temperature.

In Sapporo, Hokkaido, Japan, onion flies have 3 generations a year, and some individuals of the 2nd generation (laid as eggs in July–August) and all of the 3rd generation (laid as eggs in late August–September) are considered to enter diapause (TOMIOKA, 1977). As the mean air temperature decreases below 17°C and the day length shortens to less than 13 hr in September in Sapporo, the environmental conditions are very close to the diapause inducing ones as interpolated from the result of this experiment (Fig. 3). This seems to be in good accordance with the bionomics of onion flies in Hokkaido.

A considerable number of flies emerged 40–60 days after pupation from the cultures under short photoperiod and lower temperatures (Fig. 2 B). The diapause in some pupae might not be fully established, and the incomplete diapause might be broken by exposure to a high temperature (25°C). As a considerable percentage of diapausing pupae did not become adults even 70 days after pupation, the normal diapause may be quite firm. Another possibility is that the pupal diapause was disrupted because of the great change in temperature within a day causing disorder in metabolic and endocrine systems. At any rate, further study is needed to fully explain this phenomenon.

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