

DEVELOPMENTAL BIOLOGY OF THE IMMATURE STAGES OF *ELDANA SACCHARINA* WALKER (LEPIDOPTERA: PYRALIDAE)

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Abstract

The relationship between temperature and rate of development for *Eldana saccharina* Walker (Lepidoptera: Pyralidae) under controlled laboratory conditions is described. Thermal constants and development thresholds were calculated from these data. Thermal constant estimates for the egg, larval and pupal stages were 119, 618,6 and 160,3°D, above average thresholds of 5,3, 10,2 and 10,7°C, respectively. Thermal constants for the seven larval instars were 80, 70, 69, 74, 86, 129 and 116°D, respectively. Measurements are given for identifying larval instars on the basis of head capsule width. The influence of diet on the duration of the larval stage was investigated. When nitrogen was reduced in the larval diet the development period of the larvae increased.

Introduction

For successful study of *Eldana saccharina* Walker (Lepidoptera: Pyralidae) it is desirable to be able to predict the age of individuals, and to determine the time taken for the insect to develop from one life stage to another. To do this, the developmental periods of the life stages need to be quantified on the basis of a physiological time scale. Physiological time is a measure of the amount of heat required over time for an insect to complete a stage of development. It is defined as the cumulative product of total development time and temperature above a development threshold temperature, and is considered to be a thermal constant (Dent, 1991). Before physiological time can be calculated for *E. saccharina*, an estimate of the rate of development at various temperatures is needed.

There appears to be great variability in the development time of the larval stage of *E. saccharina* (Atkinson, 1980). Development time appears to depend on both the quality of the food supply and the ambient temperature (Dent, 1991). Atkinson and Nuss (1989) showed that nitrogen levels in the larval diet influenced development, and this aspect was investigated further in the present study.

Thermal constant data have many applications at the South African Sugar Association Experiment Station. For example, laboratory cultures of *E. saccharina* are manipulated by altering the temperature in the rearing rooms to ensure that the insects will be at the desired life stage when they are required, based on the accumulation of degree-days above the threshold (Conlong, personal communication). In addition, in the varietal resistance programme, sampling is based on the accumulation of 500°D after inoculation (Leslie and Keeping, 1995).

There were several aims in this study; to confirm the present values of the thermal constants for the life stages of *E. saccharina*. Since Atkinson (1980) published thermal constant data, *E. saccharina* has been reared for many generations in the laboratory and, furthermore, the artificial medium on which larvae are reared has been modified. These

factors may influence developmental rates. Another aim was to obtain estimates for the thermal constants for each of the larval instars of *E. saccharina*. These data may have immediate applications for the control of *E. saccharina*. It is proposed to use degree-days to help in the correct timing of insecticide treatments aimed at first instar larvae, should this approach prove useful. Further aims of this study were to clarify aspects of the biology of *E. saccharina*, such as the number of larval instars, and the size of the head capsules of successive instars. Although several researchers have already reported on these aspects for *E. saccharina* (Waiyaki, 1974; Atkinson, 1980) the results remain unclear.

Eggs, larvae and pupae were reared under environmentally controlled conditions in the laboratory and development rates were determined. These data were used to calculate thermal constants and provide an estimate of the development threshold temperatures of these life stages.

Methods

E. saccharina eggs were taken from a colony bred in the laboratory for three years. The adults in this colony were periodically mixed with adults collected from the field to reduce the effects of inbreeding. Experiments were conducted in constant temperature cabinets set at seven constant temperatures, namely 13, 15, 20, 23, 25, 30 and 35°C ($\pm 1^\circ\text{C}$). Batches of approximately 400 eggs on paper towelling, that are naturally laid in clusters of about 20 eggs each were incubated at each temperature and their development monitored daily until hatching; the resulting larvae were reared individually on an artificial diet (Graham and Conlong, 1988) in 5 ml vials; larval development was monitored until pupation and pupae were then observed daily for eclosion.

To determine the influence of nitrogen on the duration of larval development, larvae were reared on two diets; one with the standard level of nitrogen, and the other with a reduced nitrogen level. The lower level of N was obtained by halving the proportion of ingredients which provide most of the dietary nitrogen, namely crushed cane, casein and chick pea.

Thermal constants were calculated by iteration in the formula: $K = D(T - T_t)$ (Wilson and Barnett, 1983) where K = thermal constant, or heat units measured in degree-days ($^\circ\text{D}$), D = duration of the insect life stage measured in days, T = the temperature at which the insect was reared ($^\circ\text{C}$) and T_t = the assumed threshold temperature for development ($^\circ\text{C}$). Temperature thresholds were arbitrarily inserted into the formula, and the thermal constant value with the lowest standard deviation taken as the most likely value. This procedure provided an estimate of the threshold temperature for development, which was confirmed using a lin-

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ear regression x-axis method. Accordingly, the developmental rate was obtained by plotting the reciprocal of duration against temperature. This relationship approaches a straight line, and the threshold for development was the point at which the regression line crossed the x-axis. The thresholds obtained from these two methods were then averaged.

Results and Discussion

Establishment of degree-days and thresholds

The incubation period for each egg batch varied from 15-18 days (mean = $16,8 \pm 0,9$ days) at 13°C, to between three and eight days (mean = $4,6 \pm 1,0$ days) at 35°C (Table 1). Eggs hatched at all experimental temperatures, although it was noted that a smaller proportion of the total eggs laid at 35°C hatched, suggesting that the upper threshold temperature was being approached. To hatch, eggs required a mean of $119 \pm 7,2^\circ\text{D}$ (111 to 133°D) above an average threshold temperature of 5,3°C. Thresholds of 6°C and 4,5°C were calculated using the formula and the regression analysis methods, respectively. This threshold is half the threshold determined by Atkinson (1980) and Shanower *et al.* (1993). In this study egg development rate (using data from all temperatures) was highly correlated with temperature ($r = 0,97$).

Table 1

Average incubation periods and thermal constants for batches of *E. saccharina* eggs reared at seven constant temperatures

Temperature (°C)	Number of egg batches	Incubation period (days)	Standard deviation	Incubation period range (days)	Thermal constant (°D)
13	17	16,8 a	±0,9	15-18	118
15	30	13,2 b	±1,5	11-16	119
20	30	8,6 c	±0,6	8-10	120
23	13	6,5 d	±0,3	6-7	111
25	30	6,1 d	±0,9	4-9	116
30	10	4,8 e	±0,6	4-6	115
35	30	4,6 e	±1,0	3-8	133
Average STD					119,0 7,2

Values followed by the same letter were not significantly different at the 95% confidence level.

Total larval development time varied from 86-172 days (mean = $122 \pm 25,6$ days) at 15°C, to between 27 and 37 days (mean = $30 \pm 2,6$ days) at 30°C. At 35°C development time increased to between 30-40 days (mean = $34 \pm 3,6$ days) (Table 2). It thus took longer at 35°C for larvae to develop than at 30°C. At all temperatures investigated, development periods for female and male larvae were not significantly different. To pupate, larvae required an average of $618,6 \pm 20,0^\circ\text{D}$ (598 to 645°D) above an average threshold of 10,2°C. Thresholds of 10°C and 10,3°C were calculated using the formula and the regression analysis methods respectively. This threshold is lower than the 15°C threshold estimated by Atkinson (1980). However, Shanower *et al.* (1993) showed, by regression analysis and using the data of Atkinson (1980), that an estimate of 9,2°C was obtained,

Table 2

Average total development time of *E. saccharina* larvae reared at six constant temperatures, with the calculated thermal constants at each temperature

Temperature (°C)	Number larvae	Development period (days)	Standard deviation	Larval period Range	Thermal constant (°D)
15	67	122 a	25,6	86-172	610
20	53	64 b	8,4	53-73	640
23	12	46 c	3,6	41-52	598
25	47	43 c	6,0	35-52	645
30	12	30 d	2,6	27-37	600
35	36	34 d	3,6	30-40	-
Average STD					618,6 20,0

Values followed by the same letter were not significantly different at the 95% confidence level.

and was similar to the estimation of 10,6°C made by Shanower *et al.* (1993). Larval development rate, within the range 15-30°C, was highly correlated with temperature ($r = 0,98$). Larval development time at 35°C was excluded from the regression because there appeared to be a nonlinear relationship between temperature and development between 30°C and 35°C. This suggested that the upper threshold temperature for larval development may have been approached.

Development times varied for the larval instars I-VII. The longest development time (36 days) was recorded for the second larval moult at 15°C, and the shortest (4,1 days) for the first moult at 30°C. For instars V to VII, development times remained the same or decreased with increased temperature at all the experimental temperatures. This trend was the same for instars I-IV except at 35°C, when development times increased (Table 3). The thermal constants for instars I-V were similar, but increased for instars VI-VII.

Pupal development time varied from 28-46 days (mean = $38,1 \pm 4,9$ days) at 15°C, to 5-13 days (mean = $6,1 \pm 1,8$ days) at 35°C (Table 4). Pupae required a mean of $160,3 \pm 14,5^\circ\text{D}$ (144,2 to 183,6°D) above an average threshold temperature of 10,7°C, to develop into adults. Thresholds were 11°C and 10,4°C using the formula and regression analysis methods respectively. This threshold approximates the estimations reported by Atkinson (1980) (10°C) and Shanower (1993) (8,8°C). Pupal development rate was highly correlated with temperature ($r = 0,97$) in this study.

Clearly, temperature influenced the development rate of *E. saccharina*. The development time for *E. saccharina* obtained in this study is similar to that of other studies in southern Africa (Dick, 1945; Atkinson, 1980), in West Africa (Betbeder-Matibet, 1977; Kaufmann, 1983; Sampson and Kumar, 1985; Shanower *et al.*, 1993) and in East Africa (Waikayi, 1974; Girling, 1978). These thresholds may be over-estimations of the actual values because the development rate curve is sigmoidal (Price, 1984). Despite these short-comings, these data represent fairly accurately the development rate of *E. saccharina* on a temperature dependent time-scale.

Based on the above data, *E. saccharina* is theoretically capable of developing throughout the year in all regions of

Table 3

Average development time of the larval instars of *E. saccharina* at five constant temperatures; the calculated thermal constants for each instar are included

Temperature (°C)	Development time in days						
	Instar						
	I	II	III	IV	V	VI	VII
15	23,1 ± 8,7	36,0 ± 13,7	26,0 ± 12,1	30,4 ± 15,8	31,5 ± 12,7	27,0	-
20	17,2 ± 5,8	9,56 ± 5,9	9,56 ± 5,4	9,8 ± 5,3	11,2 ± 4,8	13,1 ± 4,3	16,4 ± 3,4
25	7,1 ± 2,2	6,0 ± 1,1	6,3 ± 1,8	6,4 ± 2,5	7,5 ± 2,5	8,4 ± 2,4	8,0 ± 3,1
30	4,1 ± 1,0	4,4 ± 1,0	4,2 ± 1,0	4,2 ± 1,0	4,7 ± 1,0	6,7 ± 2,0	7,3 ± 1,0
35	5,06 ± 1,1	4,88 ± 1,1	4,94 ± 1,1	5,00 ± 1,2	4,70 ± 1,1	5,20 ± 2,0	5,00 ± 1,4
Thermal constant (°D)	80	70	69	74	86	129	116
Range	45-83	60-80	52-84	63-80	72-104	104-140	108-135

Table 4

Average development time of *E. saccharina* pupae reared at six constant temperatures, with the calculated thermal constants at each temperature

Temperature (°C)	n	Development time in days	Standard deviation	Range in days	Thermal constant (°D)
15	23	38,1 a	± 4,9	28-46	160,4
20	17	19,6 b	± 1,0	17-32	183,6
23	11	12,7 c	± 0,7	11-15	148,8
25	27	9,8 d	± 2,0	6-16	144,2
30	9	8,0 e	± 0,6	7-9	152,0
35	14	6,1 e	± 1,8	5-13	172,8
Average STD					160,3 14,5

Values followed by the same letter were not significantly different at the 95% confidence level.

the South African sugarcane industry, including the Natal Midlands (except very occasionally in winter) because the estimated development thresholds are below average winter (July) temperatures. Nevertheless, the rate of development will vary from region to region depending on ambient temperatures, particularly during the larval stage. Under the experimental conditions in this study, viz. constant temperatures and an artificial larval diet, *E. saccharina* required 880°D to develop from egg to adult stage. The larval stage required the highest number of degree-days (68% of the total), whereas eggs and pupae required only 14% and 18% of the total degree-days, respectively. Degree-day data may help explain the spread of *E. saccharina* in the Natal Midlands. The current theory proposed to explain the spread of *E.*

saccharina in this area is that the insect can mate in laboratory experiments at 15°C and that winter temperatures have increased (Way, 1994). It is suggested that the development duration of *E. saccharina* has been affected also by the increased winter temperatures, to the extent that the insect's life cycle has become shorter, contributing to the increase of this pest in the Midlands.

Effect of nitrogen in the larval diet

Larval development time between different dietary regimes was significantly different at the 95% confidence level, except at 35°C (Table 5). In this study, larvae reared on the standard and reduced nitrogen diets required an estimated 641,2 ± 110,7°D and 772,7 ± 81,6°D, respectively, to complete development (Table 5). These findings concur with work done by Atkinson and Nuss (1989) and Shanower *et al.* (1993). From this it can be concluded that the diet used in these laboratory experiments should reflect, as closely as possible, the diet of the insect in the field if these data are applied in the field (Dent, 1991). This result also concurs with the hypothesis by Atkinson and Nuss (1989) that *Eldana saccharina* develops more rapidly in stressed cane than in healthy cane because the former utilises less nitrogen which becomes available for insect growth.

Table 5

Average development time of the larval stage of eldana reared on two diets at four different constant temperatures, the thermal constants are included

Temperature (°C)	Development time in days		Thermal constant (°D)	Development time in days		Thermal constant (°D)
	Standard diet	Standard deviation		Reduced nitrogen diet	Standard deviation	
15	114,4 a	+11,3	537,7	152,7 a	± 17,9	717,7
20	68,5 b	± 6,8	664,5	77,5 b	± 11,9	751,8
25	37,4 c	± 1,6	549,8	48,3 c	± 7,8	710,0
35	32,9 c	± 3,5	812,6	36,9 c	± 4,7	911,4
Average STD			641,2 110,7	Average STD		772,7 81,6

Values followed by the same letter were not significantly different at the 95% confidence level.

Determining larval instars based on head capsule widths

Larvae usually completed five to six instars, although several individuals moulted seven times. Both females and males were produced by fifth as well as sixth stage larvae, with females tending to moult more often than males. These results are in agreement with data from Atkinson (1980) who recorded six or seven larval moults for the female larva, and five or six for the male. Atkinson (1980) stated that it was difficult to find the head capsule of the first instar larva because it was often lost in the diet and damaged. During this study the first instar head capsules were recovered because the amount of diet given to first instar larvae was restricted, while ensuring that sufficient diet was present to allow development. Larger larvae tended to damage the discarded head capsules, and it is suggested that the differences in instar numbers recorded during this study and by Atkinson (1980) may be attributed to the difficulty in recovering head capsules.

Table 6

Average head capsule width of the larval instars of female (n=6) and male (n=14) eldana, at 25°C, including the proportional increase in head capsule width in successive instars

Instars	Head-capsule width (mm)						
	I	II	III	IV	V	VI	VII
Combined ♀ & ♂	0,32550	0,44475	0,64917	0,98921	1,54600	2,68676	3,08667
Standard deviation	±0,02288	±0,02398	±0,06545	±0,11352	±0,10733	±0,30781	±0,37754
Proportional increase		1,4	1,5	1,5	1,6	1,7	1,2

Increments in the widths of head capsules were similar in successive moults at all the temperatures used; therefore only the measurements from 25°C are given in Table 6. The results from those trials where it appeared as if instars had been missed were not included. In this study there was less individual variation in head capsule sizes of first instars than in later instars. Singh and Rembold (1992) reported similar results for *Heliothis armigera* larvae reared under controlled conditions. To calculate the rate of growth of larvae, Dyer's Law was used: the mean head capsule width for each larval instar was divided by that of the preceding instar (Richards, 1949). Most of the larval growth rates were close to 1,4 which agrees with Dyer's Law. The higher values for later instars may be explained by the fact that individuals were exposed to the artificial diet for longer in the later instars, and therefore had more time to show different growth rates compared to the earlier instars. It was deduced from the data that no larval instars had been omitted in this study.

Acknowledgements

Dr TC de K van der Linde (University of the Orange Free State) and anonymous referees provided helpful comments on the manuscript. N Govender and S Zuma provided technical assistance.

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