Temperature-Dependent Development of *Lacanobia subjuncta* (Lepidoptera: Noctuidae)

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ABSTRACT Laboratory studies on the temperature-dependent development of *Lacanobia subjuncta* were performed at 10 constant temperatures ranging from 10.0°C to 37.5°C. Lower developmental thresholds for eggs, larvae, and pupae were calculated as 6.6, 6.7, and 4.9°C, respectively. Degree-days required to complete a stage were estimated as 75, 476, and 312, for the egg, larval, and pupal stages, respectively. A comparison of the degree-days required to complete immature development under fluctuating field temperatures indicated the laboratory data could be used to predict results in the field. Larval head capsule measurements indicate distinct size ranges for each larval instar with the exception of a slight overlap for fifth and sixth instars.

KEY WORDS *Lacanobia subjuncta*, apple, phenology model, development, degree-days

Lacanobia subjuncta (GROTE AND ROBINSON) occurs throughout North America and feeds on a wide variety of plants including row crops, shrubs, trees, and several weed species that are found in orchard groundcovers (i.e., dandelion, bindweed, and mallow) (Landolt 1997, McCabe 1980). In recent years, this insect has become a pest of apple in central Washington and parts of northeast Oregon. The increase in pest status is in part the result of a lack of knowledge concerning its phenology and the relatively low susceptibility to pesticides commonly used in orchards (Brunner and Doerr, 2000).

An integrated pest management (IPM) program that incorporates *L. subjuncta* management requires that its phenology be well understood. Landolt (1998) indicated there were two generations on apples in Washington State. Pupae overwintered in the soil, and first-generation adults emerged from May to June. Eggs were laid on the underside of leaves of tree and weed hosts, and larvae were present from early-June through July. The larvae fed primarily on foliage, although in some orchards, significant fruit injury by late instars also occurred. Late instars moved into the soil and pupated. A second adult flight occurred in July and August with larvae feeding on foliage, and later on fruit, in August and September.

The generalized phenology described here is not sufficient for decision-making purposes in IPM. This study was undertaken to provide a more precise understanding of *L. subjuncta* biology, especially related to temperature-dependent development of immature stages.

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Materials and Methods

Rearing Methods. All *L. subjuncta* used in this study were originally collected from field populations located in various apple orchards in Chelan and Douglas counties of Washington in May 2000. Larvae were returned to the laboratory and reared on a combination diet of untreated apple (*Malus domestica* Borkhausen 'Delicious') leaves and an artificial army/cutworm diet (Bio-Serv, F9170, Frenchtown, NJ). Rearing chambers were 18 × 18 × 7 cm clear, plastic containers (1-liter Rubbermaid Servin' Saver; Rubbermaid, Wooster, OH). Food sources were elevated slightly from the bottom of the container by wire mesh material with 5 × 5 mm openings through which mature larvae could drop beneath to pupate. Pupae were collected each week and moved to an oviposition chamber, which was a 37.85-liter glass aquarium (45 × 30 × 35 cm) lined with paper towels on which females laid their eggs. Towels were removed from the chambers daily for egg collection. All temperature threshold experiments were conducted on the F1 generation.

Development Period Studies. Egg masses were carefully removed from the paper towels by hand and placed in a tight-sealing petri dish (Falcon 1006, 50 × 9 mm; Becton-Dickinson Labware, Franklin Lakes, NJ) with a small amount of artificial diet (≈1 cm^3). The petri dishes were placed into growth chambers (Low Temperature Incubator, model 2005, VWR Scientific, Philadelphia) set at 10.0, 12.5, 15.0, 20.0, 25.0, 27.5, 30.0, 32.5, 35.0, or 37.5 ± 0.25°C with a 16 L:8 D light:dark photoperiod. Three to five egg masses containing at least 50 eggs each were placed into each

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growth chamber with the exception of the 30.0°C chamber, which had nine egg masses. Egg masses were checked daily, and hatch was recorded. Because individual eggs in a mass generally all hatched during one 24-h sample period, there was little variation among individuals in a mass. Thus, development time was recorded as the hatching of an egg mass rather than hatching of individual eggs.

Neonate larvae were obtained from eggs collected as described. Following egg hatch, an individual larva was transferred, using a camel's-hair brush, to a 120-ml plastic portion cup (#S400, Prairie Packaging, Bedford Park, IL) with ≈10% of the cup filled with apple leaves. Fresh leaves were placed in the cup at least three times per week and more frequently if needed as the larva matured. Larval arenas were randomly selected and placed in each of the temperature regimes (23–30 arenas per temperature). Each arena was checked daily for evidence of a molt (e.g., cast head capsule or exuviae), and head capsule measurements were made to verify that a molt had occurred. As the larva developed into the sixth stadium, ≈1 cm of sandy soil was added to the arena, providing a suitable substrate for pupation. The time of pupation was recorded, and the arena was checked daily for adult emergence to determine pupal duration.

Estimation of Degree-day Requirements. Development rates for each immature life stage were calculated using the reciprocal of the average number of days (i.e., 1/d) required to complete a particular life stage. The relationship between development rate and temperature was described by a linear model (Arnold 1959) fit through the linear section of the data points (SAS Institute 1995). Temperature data above and below the linear portion of the developmental rate curve were not used to estimate degree-days or the lower threshold. The data above the upper threshold has developmental rates skewed by temperature-induced growth retardation or thermal death (Young and Young 1998), whereas below the linear portions the overall effect on degree-day accumulations is minimal (Pedigo 1999). The lower temperature threshold for development was determined as the *x*-intercept (Arnold 1959).

Fluctuating-temperature Experiment. The rearing procedures for eggs, larvae, and pupae used in the constant-temperature experiments were used to evaluate development under fluctuating-temperature conditions in the field. A white Stephenson weather shelter containing a max–min temperature recorder (Avatel Datascribe Jr., Avatel, Fort Bragg, CA) was placed within the canopy of an apple tree. Rearing arenas were placed inside the Stephenson shelter, and egg development was monitored daily until hatch. Larvae were checked three times per week (Monday, Wednesday, Friday) and larval molts or transformation to pupa or adult recorded. Monitoring continued until all individuals emerged as adults or died. Seventy-three egg masses were collected from the colony and set up from 27 May to 29 June, and 100 larvae from five hatching egg masses were set up in individual arenas for the fluctuating-temperature experiment on 1 June. The experiment was run once from 27 May to 2 August 2000.

Degree-days required for development in the fluctuating-temperature experiment were calculated using a single sine-wave method and daily maximum and minimum temperatures with a horizontal cut-off at the lower and upper thresholds (Baskerville and Emin 1969). The lower and upper thresholds for development of each immature life stage used were derived from the constant-temperature experiment.

Head Capsule Size Measurements. Seventy-five neonate larvae were removed from a laboratory colony and transferred to individual 100-ml plastic portion cup rearing arenas (#S400, Prairie Packaging). These larvae were reared individually on 'Delicious' apple foliage that had never received a pesticide application. The arenas were checked daily for evidence of a larval molt. The head capsule of each larva that successfully molted was measured to the nearest 0.05 mm using a dissecting microscope with an ocular micrometer. Dyar's coefficient was calculated for each of the larval molts (Dyar 1890).

Results

Development Period Studies. The temperatures used in the constant-temperature experiments encompassed the lethal upper temperature for each immature life stage (egg, larva, pupa) and provided estimates of the lower threshold for development (Table 1; Fig. 1). The fastest egg development occurred at 27.5 and 30.0°C. The development rate of eggs decreased slightly at 32.5°C, which was near the lethal limit. Little or no egg mortality was noted at temperatures from 10.0 to 32.5°C, but 100% mortality was observed at 35.0 and 37.5°C (Table 1).

Larvae successfully completed development of all instars in constant-temperature regimes of 10.0 to 30.0°C with fastest development at 30.0°C (Table 1). Mortality was high (92.3%) at 10.0°C and low to moderate (12.5–47.1%) at temperatures from 12.5 to 30.0°C, with the lowest mortality observed at 25°C. Larvae were able to complete development only through instars L4 and L3 at 32.5 and 35.0°C, respectively. No larval development occurred at 37.5°C.

The fastest pupal development occurred at 27.5°C and then decreased slightly at 30°C (Table 1). Pupal development at 10.0°C was faster than at 12.5 or 15.0°C, but these data are based on only two individuals from larvae that completed development at that temperature. High pupal mortality occurred at 12.5 or 15.0°C again providing few individuals on which to base development time. No pupal development data were available at 32.5, 35, or 37.5°C because no larvae completed development at those temperatures.

Degree-day Estimates and Fluctuating-temperature Comparison. The estimated lower thresholds for development varied between life stages but were similar for eggs and total larval development (6.6 and 6.7°C, respectively) (Table 2; Fig. 1). The estimated lower threshold for pupae was 4.9°C, but was strongly

Table 1. Mean duration (d ± SE) of *Lacanobia subjuncta* life stages when reared under constant temperature regimes

Life stage	Parameter	Rearing temperature, °C									
		10.0	12.5	15.0	20.0	25.0	27.5	30.0	32.5	35.0	37.5
Egg		13.3 (0.4)	11.7 (0.1)	9.7 (0.3)	5.2 (0.1)	4.0 (0.0)	3.7 (0.1)	3.7 (0.3)	4.5 (0.3)	—	—
	No.	3	3	3	5	4	3	9	4	4	4
	% Mortality	0	0	0	0	0	0	20.0	0	100.0	100.0
Larva											
L1		26.9 (1.0)	16.4 (0.2)	13.3 (0.6)	4.4 (0.1)	3.9 (0.2)	2.8 (0.2)	3.1 (0.1)	3.7 (0.5)	3.4 (0.2)	—
L2		18.2 (1.1)	15.2 (0.5)	5.8 (0.7)	4.6 (0.4)	2.7 (0.2)	3.8 (0.2)	2.1 (0.1)	2.2 (0.3)	3.1 (0.3)	
L3		22.5 (1.4)	11.8 (0.4)	7.3 (0.3)	3.9 (0.5)	3.8 (0.5)	4.8 (0.7)	3.8 (0.3)	3.3 (0.3)	3.8 (0.9)	—
L4		20.7 (0.7)	13.4 (0.9)	6.6 (0.7)	3.3 (0.3)	4.9 (0.4)	3.2 (0.5)	3.6 (0.4)	4.3 (1.3)	—	—
L5		12.5 (7.5)	15.6 (0.8)	4.5 (0.4)	3.8 (0.4)	2.9 (0.3)	2.4 (0.4)	3.3 (0.3)	—	—	—
L6		15.0 (2.0)	28.2 (1.7)	21.9 (0.8)	9.4 (0.3)	7.7 (0.2)	7.6 (0.4)	7.7 (0.3)	—	—	—
Total L1–6		123.5 (1.5)	100.8 (1.90)	59.4 (0.8)	29.5 (0.3)	25.8 (0.5)	24.5 (0.6)	22.6 (0.4)	—	—	—
	No.	23	23	24	24	24	23	53	30	30	30
	% Mortality	91.3	26.1	33.3	29.2	12.5	39.1	47.1	100.0	100.0	100.0
Pupa		73.5 (1.5)	104.8 (2.7)	129.7 (16.6)	20.5 (0.7)	15.5 (0.3)	13.7 (0.2)	14.9 (0.5)	—	—	—
	No.	2	15	16	17	21	13	27	0	0	0
	% Mortality	0	66.7	57.1	5.9	0	0	33.3	—	—	—

—, 100% mortality occurred before this stage was completed. In case of pupae, this stage was never reached.

influenced by a lack of sufficient development data at lower temperatures (Table 1).

Degree-day estimates for eggs, total larval, and pupal development in the constant-temperature experiments were 74.6, 476.2, and 312.5, respectively (Tables 2 and 3). Degree-day estimates in the fluctuating-temperature experiments for eggs, total larval, and pupal development periods were 72.7, 518.6, and 298.3, respectively (Table 3). The average number of degree-days accumulated per day while individuals of each life stage were present in the fluctuating-temperature experiment (Table 3) provides some measure by which to understand the different estimates of degree-days required for each life stage in the two experiments.

Head Capsule Measurements. Larval head capsule width increased by a factor of ≈1.5 for successive stadia (Table 4). This is in agreement with Dyar's rule, which states that the increase in an insect's head capsule follows a geometric progression. There was no overlap in head capsule size ranges for instars L1 to L5; however, there was a slight overlap in the range of head capsule sizes between instars L5 and L6. These data were used to verify larval molts for all development data collected in this study.

Discussion

Before 1995, *L. subjuncta* was not recognized as a pest in Washington apple orchards. However, since that time, it has occurred in high and sometimes damaging densities in several apple production regions of Washington (Landolt 1997). Because of the sudden rise in pest status of *L. subjuncta* in central Washington and northeastern Oregon apple orchards, growers and researchers have attempted to manage this new pest with little detailed information on its basic biology or phenology. An important component of a better management system for *L. subjuncta* is the ability to predict development of field populations based on temperature data.

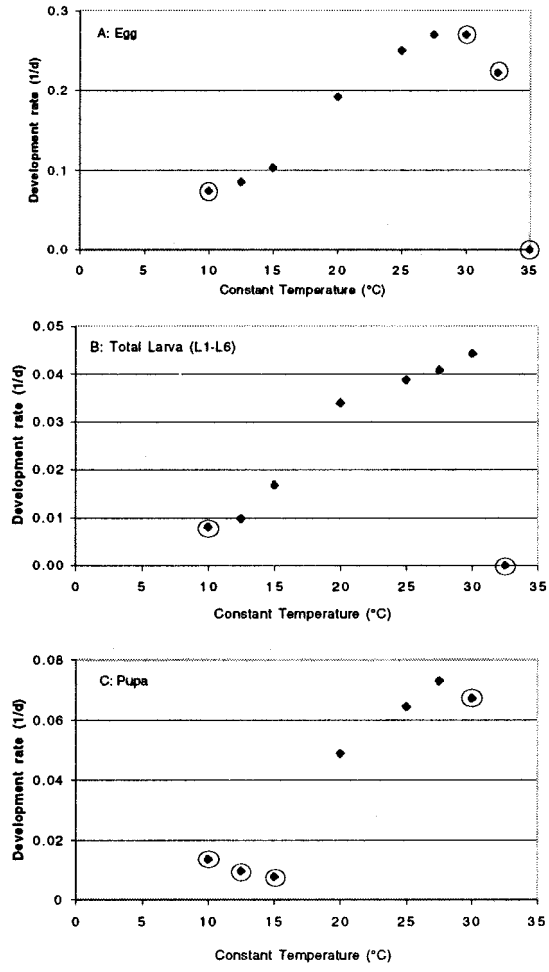

Fig. 1. Development rates for egg (A), larva (B), and pupa (C) of *Lacanobia subjuncta* reared under constant temperatures in the laboratory. Circled data points not included in linear regression.

Table 2. Linear regressions of development rate on rearing temperature used to estimate the lower developmental threshold and degree-days (DD) for development of *Lacanobia subjuncta*

Life stage	No.	Model	*F*	*P*	r^2	Lower development threshold, °C[a]	Estimated stage duration in DD[b]
Egg	5	$y = 0.0134x–0.0885$	92.2	0.011	0.98	6.6	74.6
L1	5	$y = 0.0192x–0.1895$	55.9	0.005	0.95	9.9	52.1
L2	6	$y = 0.0188x–0.1466$	18.1	0.013	0.82	7.8	53.2
L3	5	$y = 0.0159x–0.1047$	37.4	0.009	0.926	6.6	62.9
L4	6	$y = 0.0138x–0.0703$	11.8	0.026	0.75	5.1	72.5
L5	5	$y = 0.0200x–0.1387$	31.4	0.011	0.91	6.9	50.0
L6	5	$y = 0.0070x–0.0508$	47.9	0.006	0.94	7.3	142.9
Total larva	6	$y = 0.0021x–0.014$	43.6	0.007	0.94	6.7	476.2
Pupa	3	$y = 0.0032x–0.0156$	2850.3	0.012	0.999	4.9	312.5

No., Number of temperature data points along linear portion of development curve used in equation.
[a] Lower threshold for development calculated as *x*-intercept of regression model.
[b] DD, Degree-days calculated as 1/slope (m) of regression model.

There was reasonable concurrence between degree-day estimates from constant-temperature and fluctuating-temperature experiments for each life stage. The observed difference in degree-day estimates in the two experiments was probably more an artifact of differences in the intervals used to evaluate development than in any real disagreement between the methods used to calculate degree-days. In the constant-temperature experiment, and in the egg development under fluctuating-temperature experiment, evaluations were made daily to assess changes from one life stage to the next. For larvae and pupae in the fluctuating-temperature experiments, development was assessed every two or three days. The different sampling protocols combined with varying degree-day accumulations between sample dates in the fluctuating-temperature experiment, based on daily maximum and minimum temperatures, made a direct statistical comparison between the two experiments impossible.

If one takes into account the degree-days accumulated during the development of each life stage in the fluctuating-temperature experiment, the differences amount to only 1 or 2 d from predictions based on the constant-temperature experiment. In the case of total larval development, the difference of 42.4 degree-days (476.2 versus 518.6 degree-days) between the two methods amounts to only 3 d over a 40-d larval development period. It is possible that the upper threshold (estimated at 31°C) used to calculate degree-days from the fluctuating-temperature data could have influenced the accumulation of degree-days. However, daily maximum temperatures exceeded the estimated upper threshold for development on only 5 of the 70 d during the experiment. The lack of data exceeding the upper threshold for development also limited our ability to validate this proposed upper threshold for development and to adequately determine whether a horizontal or vertical cutoff method would best model development of immature *L. subjuncta.*

To complete the description of *L. subjuncta* phenology, the effects of temperature on development of diapausing pupae and on the reproductive behavior of subsequent adults, specifically the preoviposition and oviposition periods, must be characterized. After the temperature-dependent development of each life stage is characterized, a predictive degree-day model can be developed and validated by comparing model predictions with observations of *L. subjuncta* development under field conditions at several locations. The information on head capsule size of different larval stadia of *L. subjuncta* will assist in classifying the

Table 3. Degree-days required for *Lacanobia subjuncta* to complete development in laboratory at constant temperature and in field at fluctuating temperatures

Life stage	Constant temperatures	Fluctuating temperatures			Absolute value of difference [DDc–DDf][a]
	Degree-days required	Degree-days required (±SE)	No.	Degree-days per day	
Egg	74.6	72.7 (2.3)	73	11.6	1.9
L1	52.1	60.6 (1.3)	100	7.1	8.5
L2	53.2	66.7 (1.8)	100	10.3	13.5
L3	62.9	43.8 (1.0)	100	17.2	19.1
L4	72.5	71.4 (2.8)	100	16.8	1.1
L5	50.0	81.2 (2.3)	99	14.1	31.2
L6	142.9	165.8 (2.9)	86	14.1	22.9
Total larva	476.2	518.6 (3.9)	86	12.8	42.4
Pupa	312.5	298.3 (4.7)	43	18.4	14.2

No., Number of individual *L. subjuncta* per life stage.
[a] Absolute value of the difference between degree-day requirements calculated in the laboratory constant-temperature experiment (DDc). and the field-fluctuating temperature experiment (DDf).

Table 4. Mean (± SE) *Lacanobia subjuncta* larval head capsule widths and size range

Instar	No.	Mean width, mm	Range, mm	Dyar's coefficient
1	65	0.30 (0.002)	0.28–0.35	
2	63	0.51 (0.004)	0.48–0.65	1.69
3	61	0.82 (0.01)	0.65–0.90	1.59
4	49	1.25 (0.02)	1.05–1.50	1.54
5	37	1.92 (0.04)	1.50–2.50	1.54
6	32	2.78 (0.48)	2.25–3.45	1.44

age distribution of populations encountered in the field.

The information presented in this article forms the basis for developing a more complete understanding of *L. subjuncta* development. These data will be useful in creating a temperature-based degree-day model for predicting the occurrence of key life stages in the field. An accurate predictor of a pests' phenology can be very important in developing sampling protocols, timing insecticide applications, or implementing a biological control strategy targeting susceptible life stages (Brunner et al. 1982, Beers et al. 1993, Pedigo 1999).

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