

Development of the First-Instar Spruce Budworm (Lepidoptera: Tortricidae)

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ABSTRACT Development of the 1st-instar (L1) of the spruce budworm, *Choristoneura fumiferana* (Clemens), was studied at different temperatures under laboratory conditions. The development process was divided into different phases based on 4 distinct events observed during L1 development: hatching of L1, construction of hibernacula, excretion of green pellets, and molt. The green substance in newly hatched L1 was found to be proteinaceous. The proportion of L1 that successfully completed development at various temperatures indicated that the optimal temperature range for L1 development was between 18 and 27°C. Larval mortality, particularly at low temperatures, occurred mainly before construction of hibernacula and excretion of green pellets. Low temperatures had a weak effect on development time from larval hatching to construction of hibernacula, but the duration of subsequent development periods were substantially increased under these temperatures. Temperature threshold ($TT \approx 6^\circ\text{C}$), degree-day requirement ($DD \approx 87$), and other developmental parameters were estimated.

KEY WORDS spruce budworm, first instar, temperature, developmental rate

THE 1ST-INSTAR (L1) of the spruce budworm, *Choristoneura fumiferana* (Clemens), not only marks the beginning of larval development in this insect, it is also the last developmental stage to be completed in a calendar year before the insect undergoes overwintering diapause as a 2nd instar. Unlike the other larval stages of spruce budworm, L1 exhibit distinct behavioral and morphological features, notably the construction of a hibernaculum and a change in larval color. Although Harvey (1957) provided a detailed description of the behavior of L1, these special features of development have since attracted little attention. Temperature-dependent developmental rate of spruce budworm has been investigated extensively for almost all developmental stages (Rose and Blais 1954, Bean 1961, Cameron et al. 1968, Miller et al. 1971, Régnière 1987). L1 developmental rate, however, has been overlooked. This apparent data gap may reflect a lack of interest in this relatively short developmental phase, which has seemed to be of little ecological significance as far as the life cycle of the insect is concerned. The potential importance of L1 development has been raised by recent studies on the timing of spruce budworm diapause initiation (Han and Bauce 1998), and by suggestions that the L1 development period might provide a suitable time window for insecticide applications (Retnakaran et al. 1999). Here, we divide L1 development into subphases based on morphological changes and behavioral events observed during the development of the stadium. We examine the effects of temperature on development time and survival rate for each development phase.

We also estimate the temperature threshold and degree-day requirement for L1 development.

Materials and Methods

Insect Material and Rearing Condition. Egg masses were provided by the Insect Production Unit, the Canadian Forest Service, Sault Ste Marie, Ontario. They were collected within 72 h of oviposition at 20°C and were received within 10 h in our laboratory. The egg masses were placed at 18°C for hatching. Newly hatched L1 were transferred individually within 24 h into plastic containers (4 by 2.5 by 1.5 cm) whose lids were lined with cheese cloth. These larvae were placed at 7 different temperatures (8, 10, 13, 18, 23, 27, and 30°C), 55–65% RH, and a photoperiod of 16:8 (L:D) h.

Developmental Phases. The development process of L1 was observed daily using a dissecting microscope. Based on changes in morphological and behavioral features, 4 sequential events were identified and the development of L1 was divided into sub-phases accordingly.

Extraction of Green Substance from L1. To determine the nature of the green substance in L1, ≈ 200 newly hatched larvae were homogenized in 1.5-ml microfuge tubes containing 550 μl of either 60 mM Tris-HCl buffer (pH 6.8) or a lipid extraction solution (chloroform/methanol/water, 2:2:1.5). The homogenate was centrifuged at $13,000 \times g$ for 3 min. Trichloroacetic acid (20 μl , 100% wt:vol), a protein precipitant, was added to 200 μl of the Tris-HCl buffer

supernatant, followed by centrifugation at $13,000 \times g$ for 3 min.

L1 Development Time and Survival. Daily observations were made throughout L1 development to determine the time required to reach each developmental event at different temperatures. The proportion of larvae that failed to complete development to a given event was estimated in terms of mortality. Non-parametric analysis of variance (ANOVA) (PROC RANK followed by PROC GLM and PROC MEAN, SAS Institute 1990) was used to assess the statistical significance of differences in mean development time between different temperature treatments. Pair-wise comparisons were made when necessary, using the modified Fisher exact test (Zar 1984) to compare differences in L1 mortality among insects submitted to different temperatures.

Estimation of Developmental Rate. Estimation of developmental parameters was carried out according to Lamb (1992). Developmental rate (1/d) is presented as the percentage of development per day. The relationship between developmental rate and temperature was described by a nonlinear regression model:

$$R(T) = R_m \exp[-0.5[(T - T_m)/T_o]^2],$$

where $R(T)$ is the developmental rate with respect to rearing temperature T ; T_m is the temperature at which the developmental rate is highest; R_m is the maximum developmental rate at T_m ; and T_o ($^{\circ}\text{C}$) is a shape parameter giving the spread of the curve. The model was fitted by nonlinear regression analysis (PROC NLIN, SAS Institute 1990). The coefficient of determination (r^2) was estimated as:

$$r^2 = 1 - (RSS/CTSS),$$

where RSS is the residual sum of squares and $CTSS$ denotes the corrected total sum of squares.

The temperature threshold (TH) and the mean degree-day requirement (DD) for L1 development were estimated as

$$TH = T_m - 2.23 T_o.$$

$$DD = 100 T_o/0.483 R_m.$$

Results and Discussion

Developmental process. The 1st instar is distinguished from other spruce budworm larval stadia in that distinct changes, at both behavioral and morphological levels, take place during its development. The following four sequential events were observed during this stadium (Fig. 1). (1) Hatching of green larvae: L1 hatch from egg masses; their green color results from the presence of a green substance evenly distributed in their bodies. (2) Construction of hibernaculum: L1 spin silk to build hibernacula while concentrating the green substance in the gut region. The occurrence of this event was defined by the 1st appearance of a complete hibernaculum form, although the insect may continue thickening the walls of the hibernaculum. (3) Excretion of green pellets: L1 usually expel 2 green pellets inside the hibernaculum and, as a result, their

body color changes from green to yellow. (4) Completion of L1 development: L1 shed head capsule and exuvium within the hibernaculum; the presence of the shed head capsule was taken as the sign that L1 development is completed. Although these 4 developmental events occur one after another, they are not completely separate development processes. For instance, the preparation for excretion of green pellets (e.g., concentration of the green substance into gut region) started at the same time or even before, depending on rearing temperatures, the larvae constructed their hibernacula.

Green Substance. Extraction of the green substance from newly hatched L1 by ternary mixtures of chloroform-methanol-water showed that yellowish-green substances only appeared in the upper methanol-water phase and in the tissue-residue interphase, whereas no visible color was observed in the lower chloroform phase. This indicates that the green substance in L1 larvae was not lipid in nature. The green substance, however, was recovered from supernatant of the buffer extraction and was completely precipitated by trichloroacetic acid, suggesting that the green substance is proteinaceous or is closely associated with proteins. A bile pigment chromoprotein was identified in the hemolymph of mature spruce budworm larvae and adults (Stehr 1959, Schmidt and Young 1971), and the green substance found in L1 larvae could be derived from the parental adults. Color change in L1 was described as a "yolk conversion" process (Harvey 1957). Han and Bauce (1993) reported that the excretion of green substance resulted in a lower supercooling point as the insect prepared for overwintering. It is also possible that the green substance serves primarily as a camouflage for egg protection as spruce budworm eggs are laid on green needles and may be vulnerable to predation. The function, as well as the nature of chromoproteins, merits further investigation.

Temperature and L1 Survival. L1 development proceeds better at moderate to high temperatures as indicated by the L1 mortality data (Table 1). The optimal temperature range was between 18 and 27 $^{\circ}\text{C}$, which corresponds well to the field situation where larval hatching would normally occur in the summer. L1 mortality, particularly at low temperatures, was mainly caused by developmental failure before the construction of hibernacula and excretion of green pellets events, which constituted 68% or more of L1 mortality. Mortality occurring at molting was relatively low and similar at different temperatures, except for the case of 10 $^{\circ}\text{C}$. Thus, those larvae that survived the first 2 events after hatching would usually survive the last event.

The high mortality at construction of hibernacula at 13 $^{\circ}\text{C}$ was not consistent with the overall mortality pattern. Although, larval mortality was largely attributed to rearing temperatures in this study, other factors, such as egg and substrate quality, might interact with temperature and cause variation. For example, Thomas (1977) reported a much higher mortality estimate near the optimal temperature (e.g., 55% at

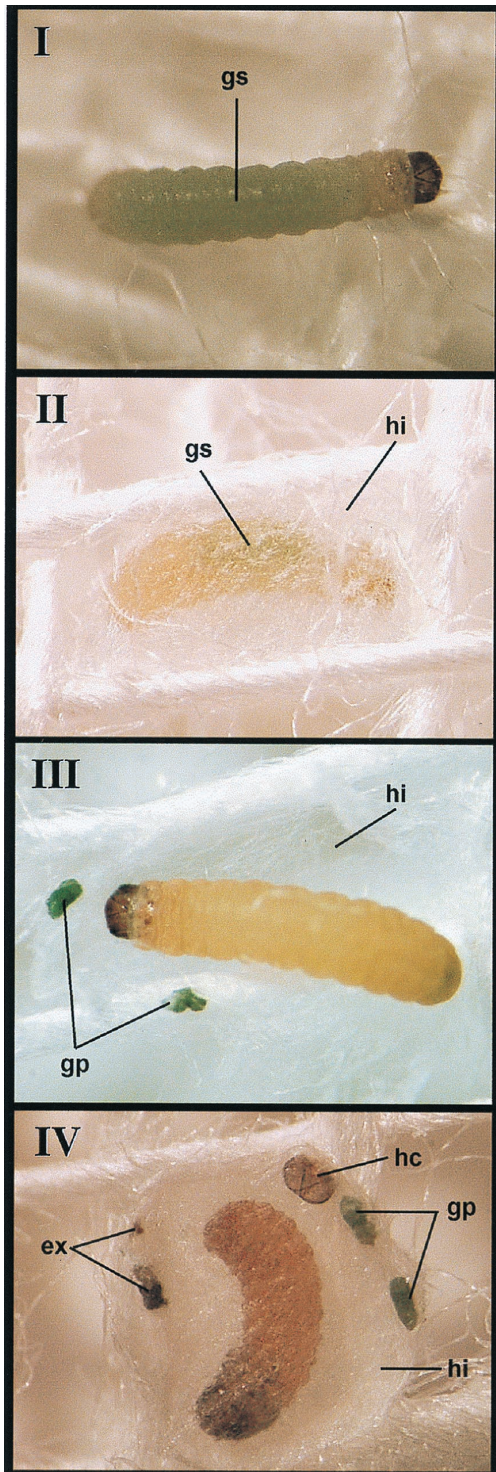


Fig. 1. Sequential events during L1 development of *C. fumiferana*. (I) Hatching of L1 with green substance (gs) inside. (II) Construction of hibernaculum (hi). (III) Excretion of green pellets (gp) (the top of the hibernaculum was opened to show the green pellets). (IV) Molt into 2nd instar. hc, Head capsule; ex, exuvium. Normal size of L1 is ≈ 2 mm long. See text for more details.

Table 1. Proportion (percent) of 1st-instar (L1) *C. fumiferana* that died before a given event during L1 development under various temperatures

Temp, °C	Construction of Hibernacula	Excretion of green pellets	Molt into 2nd instar	Total mortality	n
8	29.73	21.62	2.70	54.05	74
10	14.67	9.33	10.67	34.67	75
13	36.00	6.67	2.67	45.33	75
18	4.17	11.11	1.39	16.67	72
23	3.95	5.26	2.63	11.84	76
27	12.16	1.35	2.70	16.22	74
30	13.70	15.07	4.11	32.88	73

23°C), which may have occurred because the egg masses were "heavily parasitized with microsporidia." Régnière and Duval (1998) showed that failure of hibernacula construction by L1 could result from unsuitable spinning substrates.

Temperature and L1 Development. L1 development time was significantly increased as rearing temperatures decreased (Table 2). However, the relative increase in development time differed among developmental events. For example, the time required for L1 to reach the events of excretion of green pellets or molt into L2 at 13°C was almost 3 times as long as at 23°C, whereas it was only twice as long in the case of the construction of hibernacula. At moderate and high temperatures, hibernacula were constructed within 2 d or less of hatching. In fact, L1 reached the hibernaculum construction event so fast that the daily observation interval was insufficient to detect time differences between the 27 and 30°C treatments. This is in accordance with the observation that most L1 spun hibernacula within 24 h at 20°C on suitable shoot substrates (Régnière and Duval 1998). The rapid development at this stage indicates that L1 have a propensity to search and establish overwintering sites soon after they hatch. If this finding can be applied to the field situation, the effective window would be

Table 2. Development time in days (mean \pm SD) from larval hatching to each of the 3 subsequent events during development of the 1st-instar *C. fumiferana* at different temperatures

Temp, °C	Construction of Hibernacula	Excretion of green pellets	Molt into 2nd instar
8	6.1 \pm 3.2a (n = 34)	17.1 \pm 1.8a (n = 30)	32.9 \pm 4.2a (n = 34)
10	5.7 \pm 2.0a (n = 49)	13.8 \pm 2.5b (n = 49)	21.0 \pm 4.5b (n = 49)
13	3.8 \pm 1.2b (n = 41)	8.7 \pm 2.3c (n = 41)	14.7 \pm 3.0c (n = 41)
18	2.1 \pm 0.7c (n = 60)	5.0 \pm 0.6d (n = 60)	7.8 \pm 0.8d (n = 60)
23	1.8 \pm 0.7d (n = 66)	3.1 \pm 0.6e (n = 67)	5.0 \pm 0.8e (n = 67)
27	1.1 \pm 0.4e (n = 61)	2.4 \pm 0.5f (n = 59)	4.0 \pm 0.2f (n = 62)
30	1.0 \pm 0.1e (n = 48)	2.1 \pm 0.3g (n = 42)	3.7 \pm 0.5g (n = 49)

Mean values for development time followed by the same letter are not significantly different (nonparametric analysis followed by Tukey test on ranked data $P > 0.05$).

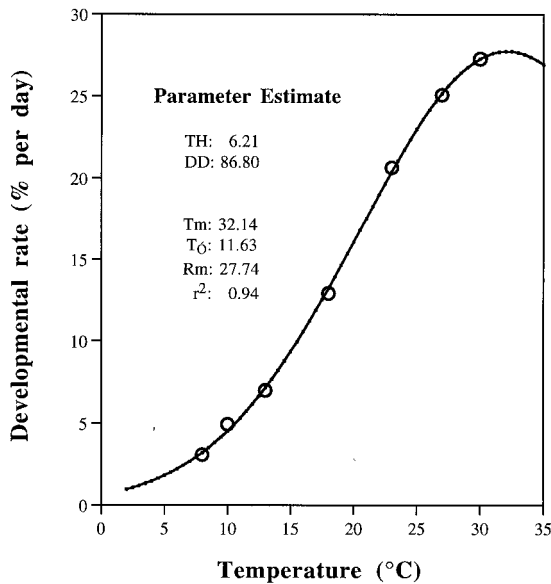


Fig. 2. Relationship between temperature and developmental rate for 1st-instar *C. fumiferana*. Observed mean values of inverse development time (open circles) were presented against values (solid line) estimated by a nonlinear regression model. Sample size at each temperature varied between 34 and 67.

rather narrow for any potential insecticide aimed at L1.

Although it took only a few more days for L1 to construct hibernacula at lower temperatures compared with insects reared at higher temperatures, it took a few additional weeks for the larvae to reach the 2nd stadium when they enter diapause. Therefore, temperature could have a significant impact on the timing of diapause initiation. Significant differences in timing of diapause initiation could arise between insects that oviposit in August and those that do so in September, or between different geographical locations where temperature conditions differ. As the timing of diapause could potentially influence the overwintering process in the spruce budworm (Han and Bauce 1997, 1998), the results from this work provide useful information for future studies on overwintering ecology of this species.

Data fitting and estimates of development temperature threshold (TH) and degree-day requirements (DD) are shown in Fig. 2. Although the development threshold was low ($\approx 6^\circ\text{C}$) and the estimated temperature for maximum development rate (T_m) was high ($\approx 32^\circ\text{C}$), significantly high L1 mortality at extreme temperatures (Table 1) suggests that this model would be most useful within the optimal temperature range between 13°C and 27°C . Because temperature-dependent development has been studied for all developmental stages of spruce budworm except L1, the estimated TH and DD for L1 development fill a gap in the data in studies on development of this insect.

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