Physiological basis for flexible voltinism in the spruce beetle (Coleoptera: Scolytidae)

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Abstract—The spruce beetle, *Dendroctonus rufipennis* (Kirby), has described life cycles of 1–3 years. Although temperature has been shown to be strongly associated with flexible voltinism in the spruce beetle, the physiological basis for this phenomenon is not clear. Two competing hypotheses were tested under laboratory conditions. First, we tested the hypothesis that larval diapause, induced by cool temperatures during or before instar III, initiates prolonged life cycles while larvae not diapausing complete development to adults before the first winter. We compared development times at constant temperature (12°C) and field-simulated thermoperiod treatments against development times in a reference (21°C) treatment for which there is no indication of diapause induction. The constant temperature treatment was not significantly different than the reference treatment, although there were a few outliers. The thermoperiod treatment was significantly longer than the reference treatment, but only by a few days. These results provide little support for the hypothesis of larval diapause induction during or before instar III. Second, we investigated the hypothesis of life-cycle regulation through life stage specific developmental temperature thresholds, particularly, a relatively high threshold for pupation that might prevent development beyond the prepupal life stage under cool conditions. We found little evidence of distinct differences in low-temperature thresholds between life stages. Instar-IV larvae held at ≤15°C, however, did not pupate for 125–300 days, a developmental arrest that suggests diapause. Based on all present and previous investigations, the induction-sensitive phase appears to be late in the instar-IV or early in the prepupal stages. For semivoltine spruce beetles, this life stage occurs late in the growing season, after most temperature-dependent development has been completed. It is our conclusion that spruce beetle voltinism is primarily under direct temperature control and that prepupal diapause is the default overwintering strategy for individuals not completing development to maturity by fall.

Résumé—On connaît, chez le Dendroctone de l'épinette, *Dendroctonus rufipennis* (Kirby), des cycles biologiques de 1 à 3 ans. Bien que la température soit étroitement associée au voltinisme flexible de cette espèce, les mécanismes physiologiques en cause restent mal connus. Deux hypothèses ont été éprouvées dans des conditions de laboratoire. D'abord, nous avons testé celle selon laquelle la diapause larvaire, déclenchée par des températures fraîches avant ou au cours du stade III, donne lieu à des cycles biologiques prolongés, alors que les larves qui ne font pas de diapause atteignent le stade adulte avant le premier hiver. Nous avons comparé la durée du développement, d'une part, à température constante (12°C) et lors de traitements sur le terrain à la thermopériode simulée, et, d'autre part, à une température témoin (21°C) qui ne semble pas déclencher de diapause. Le traitement à

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température constante ne donne pas de résultats significativement différents du traitement à la température témoin, bien qu'il y ait quelques données aberrantes. La durée du développement à la thermopériode simulée est significativement plus longue que celle à la température témoin, mais seulement de quelques jours. Ces résultats supportent mal l'hypothèse d'une diapause déclenchée avant ou durant le stade III. En second lieu, nous avons éprouvé l'hypothèse d'un contrôle du cycle par des seuils de température de développement spécifiques à chaque stade et, en particulier, par un seuil relativement élevé pour la nymphose qui peut potentiellement empêcher le développement au-delà du stade de prénymphe dans des conditions fraîches. Nous n'avons pas constaté de différences de seuils de basse température entre les stades. Cependant, les larves de stade IV gardées à 15°C ont retardé leur nymphose de 125 à 300 jours, un arrêt du développement qui indique peut-être l'existence d'une diapause. D'après ces résultats et ceux d'études antérieures, la phase sensible du déclenchement de la diapause semble se situer vers la fin du stade IV ou au début du stade de prénymphe. Chez les Dendroctones de l'épinette semivoltines, cette étape du cycle apparaît vers la fin de la saison de croissance, après que tout le développement régi par la température ait été complété. Nous croyons que le voltinisme du Dendroctone de l'épinette est surtout contrôlé de façon directe par la température et que la diapause au stade de prénymphe est une stratégie de rechange qui permet aux larves qui n'ont pas atteint leur maturité avant l'automne de survivre à l'hiver.

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Introduction

The spruce beetle, *Dendroctonus rufipennis* (Kirby) (Coleoptera: Scolytidae), is an important mortality agent of spruce trees, *Picea* spp. (Pinaceae), throughout North America. Because of the substantial impact on spruce forests, the number of spruce beetle investigations is significant (Linton and Safranyik 1988; Reynolds and Holsten 1997). Despite this, many key life-history strategies remain poorly understood. For example, life cycles of 1, 2, and 3 years have been reported (McCambridge and Knight 1972; Schmid and Frye 1977) but with limited understanding of the physiological processes or environmental conditions generating these profound differences. Efforts to understand spruce beetle life-history strategies and to predict population dynamics are confounded by the plasticity with which this insect responds to variations in environmental conditions (Knight 1961; Dyer 1969). This variability probably results from adaptations to the subalpine environment (Sota 1996). Temperature has been shown repeatedly to be strongly associated with spruce beetle voltinism (Massey and Wygant 1954; Dyer 1969, 1970; Dyer and Hall 1977; Werner and Holsten 1985), yet the physiological basis of these observations is not clear.

It has been hypothesized that a hibernal diapause, induced by temperatures below 15°C during instar III and manifest during instar IV, initiates the 2 (or 3) year life cycle (Dyer and Hall 1977). Alternatively, in the absence of diapause, larvae develop to adults by the end of the first growing season, resulting in the univoltine cycle (Dyer 1970). The evidence supporting larval diapause, however, is inconclusive because it does not eliminate an alternative explanation for larval overwintering and maintenance of seasonality and life-cycle synchronicity.

The competing hypothesis, wherein insects can maintain appropriate seasonality without diapause, requires at least one life stage to have a high developmental temperature threshold relative to those for other life stages (Tauber *et al.* 1986; Powell *et al.* 2000). A directly related example is the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, a species that shows no evidence of diapause. Instead, it has been demonstrated that life stage specific development thresholds result in quiescence,

promoting an adaptive seasonality (Bentz *et al.* 1991; Logan and Bentz 1999; Jenkins *et al.* 2001).

Our motivation for exploring the physiological basis for larval overwintering, and thus semivoltine generations, results from efforts to develop a temperature-based voltinism model for the spruce beetle. Such a model may need to consider the presence of diapause and its potential to confound predictions. Previous results are equivocal in determining a larval diapause for the spruce beetle. Dyer (1970) hypothesized a larval diapause based on laboratory results wherein development was arrested for about 25 days in cold-treated insects compared with reference insects that did not apparently diapause. For insects with a hibernal diapause, however, the diapause development threshold is typically reduced in the fall (Tauber *et al.* 1986). If this is true for the spruce beetle, the warm fall conditions effectually simulated in Dyer's experiment should have served to prolong dormancy because the conditions for completing diapause development would not be realized (see examples: Gerson *et al.* 1999; Togashi 1995; Tauber *et al.* 1990; Bakke 1970). Furthermore, some beetles in Dyer's cold-treated regimes developed beyond the prepupal stage before being placed at the reference temperature (21.1°C), suggesting that any diapause-inducing cues were, at best, marginally achieved by the treatments. Dyer and Hall (1977) present as evidence for larval diapause their observation that relatively cold treatments resulted in reduced pupation even though there had been "adequate heat accumulation" to produce adults. Dyer and Hall's calculations of degree-days, however, are questionable because they assume (*i*) equivalent developmental temperature thresholds for all life stages (see Bentz *et al.* 1991); (*ii*) a linear relationship between development rate and temperature (see Logan and Hilbert 1983); and (*iii*) instantaneous changes in subcortical habitat temperatures despite the thermal mass of experimental logs.

The objectives of this research were to (*i*) test the hypothesis of spruce beetle larval diapause, induced during or before instar III by temperatures below 15°C and manifested during instar IV and (*ii*) investigate the possibility of life-cycle synchrony through life stage specific developmental temperature thresholds. Although 3-year generations have also been reported from high elevation, north-aspect spruce stands (McCambridge and Knight 1972), we did not consider them because they occur infrequently, if ever (Schmid and Frye 1977), and such beetles are less likely to strongly influence population dynamics.

Materials and methods

Diapause

We tested for larval diapause by comparing the development times of insects exposed to constant low-temperature and thermoperiod (the daily temperature cycle) treatments with insects exposed to a reference treatment. Larvae were reared in their respective treatments until instar IV, and placed in equivalent (reference) temperatures while development to the teneral adult stage was timed. If a cold treatment induces diapause during instar III, broods in these regimes should have substantial delays in completing development to the teneral adult compared with broods in the reference treatments. Diapausing larvae, in fact, may not exhibit any development under these conditions. Conversely, if the cold-treated larvae are simply in quiescence, they should resume development without significant delays once returned to the favorable, reference temperature.

Dyer and Hall (1977) concluded that temperatures below 15°C before and during instar III induced a diapause in instar IV. A temperature below 16.5°C during instars I and II also has been correlated with 2-year generations (Werner and Holsten 1985).

Therefore, 12°C was chosen as the constant temperature treatment to accommodate the opposing concerns of inducing diapause and allowing a reasonable development rate. For the reference temperature, 21°C was chosen because spruce beetles can be reared to the teneral adult stage without an apparent delay at this temperature (Dyer 1970). For the laboratory experiments involving thermoperiod, we reproduced temperature regimes of phloem and air temperatures as recorded from a field site with no univoltine beetles (Dixie National Forest, Utah; altitude 3170 m; 37°38′N, 122°52′W) (Hansen *et al.* 2001).

Photoperiod was considered a possible diapause-inducing cue. Dyer (1970), however, was able to arrest development simply by altering the temperature regime, and beetles in his reference treatment developed without an apparent delay, although the experimental photophase was shorter (12L:12D) than the field photophase during late summer. Our field observations show that beetles with a 1- and 2-year life cycle occur within a single tree, correlating with microhabitat temperature influences (Hansen *et al.* 2001), whereas photoperiodic cues are clearly identical. On the other hand, thermoperiod is roughly comparable to photoperiod in providing seasonal cues for diapause induction, and there are examples of thermoperiodic diapause induction in the absence of photoperiodic input (Beck 1983). Thermoperiod is a plausible induction cue for the spruce beetle because all life stages develop under the bark of their host. Furthermore, Dyer and Hall (1977) did not include photoperiod in treatments that they concluded to have induced diapause. For these reasons, we concentrated on temperature as a diapause-inducing cue, although photoperiod was included in a portion of our experiments.

Experiment 1.1. Constant temperature effects on development time in infested bolts

Two live Engelmann spruce (*Picea engelmannii* Parry ex. Engelm.) were felled on the Cache National Forest, Utah, and cut into thirty-six 46 cm long bolts in late June 1998. Bolt ends were sealed with paraffin wax to reduce desiccation and each bolt was numbered sequentially from the ground up to a minimum diameter of about 25 cm for each tree. New adult spruce beetles were collected from baited funnel traps (Pherotech, Inc, Delta, British Columbia) on the Cache National Forest in early July 1998. Beetle sex was determined (Lyon 1958) and female–male pairs were inserted manually into the phloem of green bolts every 15 cm (circumference) on three rings spaced 15 cm vertically. Bolts from the two trees were systematically assigned to each treatment to reduce any confounding tree or upper–lower bole effects. Bolts were maintained at 21°C until the larvae had developed to instar II, about 19 days after infestation (broods in a bolt were considered to have reached a specific life stage if ≥50% of sampled insects had developed to that life stage). Eighteen of these bolts were placed in a constant temperature treatment of 12°C until instar IV was attained by the larvae. At this time, cold-treated bolts were returned to a constant 21°C. The second group of 18 bolts (reference treatment) were maintained at 21°C during the entire experiment. After the larvae developed to instar IV, each bolt was subsampled weekly to determine the proportion of teneral adults. Each subsample consisted of removing a randomly selected 15-cm^2 bark section, comprising one-half of two adjacent galleries, and determining the life stage of each beetle therein. Sampled sections were sealed with paraffin wax to minimize desiccation of the residual habitat. Sampling continued weekly on each bolt until the first subsample of 100% teneral adults or until sampling space was exhausted.

The measured response variable is the proportion of teneral adults per weekly sample under equivalent temperatures. For the 18 bolts in each treatment, time starts

($t = 0$ weeks) at the first observation of ≥50% instar IV. At each subsequent weekly subsample, individuals were tallied by life stage.

Experiment 1.2. Constant temperature effects on development in phloem sandwiches

Eighteen phloem sandwiches were constructed for each treatment using instar IV (five per sandwich) from the bolt samples in Experiment 1.1. Sandwiches consisted of a 15 cm^2 × 0.3 cm phloem section, shaved off a fresh bolt, and mounted between sterilized glass/Plexiglas plates. Larvae were placed into a niche in the phloem where they continued to feed and develop. Sandwiches from both treatments were housed in a single desiccator of constant temperature and humidity (about 95%). All insects in each sandwich were examined three times per week for metamorphosis. Time measurements started when each sandwich was constructed and ended when all surviving insects reached the teneral adult stage (mortality was about 15%). The response variable is the proportion of teneral adults per sample for each sandwich.

Experiment 2.1. Thermoperiod effects on development time in infested bolts

We compared three treatments: (1) reference (constant 21°C, constant darkness); (2) sine-wave thermoperiod based on field-recorded daily maximum and minimum phloem temperatures and constant darkness (T1); and (3) square-wave thermoperiod based on weekly averaged field-recorded maximum and minimum air temperatures and photoperiod (T2). Temperatures were based on recordings from the Dixie National Forest field site with no univoltine beetles. Daily maximum and minimum field-measured phloem temperatures were inputted into sine-wave programmable environmental cabinets (Gray *et al.* 1998) to create the conditions for T1. Temperatures within the cabinets were controlled by thermocouples inserted into the phloem of the experimental bolts, resulting in a satisfactory approximation of field phloem temperatures. Environmental chambers (Percival Scientific, Inc, Boone, Iowa), with square-wave or constant temperature capabilities, were used for the T2 and reference treatments. Because temperature controller thermocouples for the square-wave environmental chambers cannot be inserted into the bolts, field-measured air temperature was used for T2. These manual controllers were reset once per week using weekly averaged maximum and minimum field air temperatures. A sunrise–sunset table from a station close to the field site was used as a template for setting light timers in the square-wave chamber, mimicking photoperiod on the Dixie National Forest.

One live Engelmann spruce was felled on the Cache National Forest, cut into thirty-six 33 cm long bolts, and numbered sequentially from the ground up in October 1998. Bolt length was reduced from Experiment 1.1 to allow for all 36 experimental bolts to be obtained from a single tree, eliminating the possibility of a confounding tree effect. Bolts were girdled at their midpoints by extracting a 3 cm high phloem ring, resulting in two 15 cm high residual phloem bands. This protocol was added from Experiment 1 to reduce the possibility of adults colonizing vertically adjacent phloem sections where adult introductions were unsuccessful. Bolts from a field-infested Engelmann spruce were cut on the Dixie National Forest in November 1998 and new adult beetles were reared out at room temperature. Beetle sex was determined and pairs were manually inserted every 12 cm (circumference) into the fresh bolts along the lower surfaces of the residual phloem bands.

After 14 days at 21°C, the numbered bolts were systematically allocated into the three treatments. At this time, bolts were sampled and brood development was estimated to be equivalent to that found at the field site on Julian day 198. Field

temperatures from this date were used to initiate the thermoperiod portion of the treatments. Bolts were maintained in their respective treatments longer than in Experiment 1, from 14 days after infestation until 100% instar-IV larvae were observed. At the first subsample of 100% instar-IV larvae, bolts were returned to constant 21°C and weekly sampling was begun until the first observation of 100% teneral adults.

Experiment 2.2. Development time in phloem sandwiches

Phloem sandwiches were constructed and monitored (see Experiment 1.2) using instar-IV larvae (five per sandwich) from the bolt samples in Experiment 2.1.

Data analysis

Logistic regression was used to obtain parameter estimates for mixed models that predict the logit [or log odds, where logit = $\log_e(\pi/1 - \pi)$] of teneral adult proportion against time. The general form of the model is

$$\ell = \beta_0 + \beta_1 W + \beta_2 R + \beta_3 T + \beta_4 WR + \beta_5 WT + \varepsilon$$

where ℓ is the predicted logit; β_x is the parameter estimate; W is the sample time (weeks or days from $t = 0$); $R = 1$ for reference, 0 otherwise; $T = 1$ for treatment, 0 otherwise; and ε is the error term. Time (weeks or days from $t = 0$) was considered a covariate in the model, whereas treatment is a fixed effect. Where appropriate, bolt within tree and tree within treatment were included in the model as random effects. Error distribution was specified as binomial. GLIMMIX (a SAS macro for fitting generalized linear mixed models; 25 September 1998 release; SAS Institute Inc, Cary, North Carolina) was used to accommodate these random effects within a logistic regression model. Model residuals were checked for normality and were plotted against predicted values to detect unusual patterns. Only linear time effects were considered in the model because quadratic effects generate unrealistic predictions (*i.e.*, declining proportion of teneral adults as time advances). Parameters for each experiment were estimated separately and response functions were compared for significant ($\alpha = 0.05$) differences, using t statistics, between treatments and references within each experiment (as in ordinary least squares regression, the t statistic in logistic regression is used to test H_0: $\beta_x = 0$, where $t = b_x/\text{SE}(b_x)$ and b = estimated coefficient for the xth term (Hamilton 1992)).

Life stage specific developmental temperature thresholds

To estimate life stage specific development rates and thresholds, molting was monitored within phloem sandwiches at seven constant temperatures (5.5, 7, 9, 12, 15, 18, and 21°C), similar to the protocol of Bentz *et al.* (1991) for the mountain pine beetle. Development was first measured at 12, 15, 18, and 21°C. Female–male pairs of new adults, collected in infested bolts from the Dixie National Forest in November 1998, were inserted manually into green bolts in April 1999. After 1 week at room temperature, bark was removed from the bolts and eggs were collected from the most recently excavated 1 cm of gallery (*i.e.*, these eggs are presumed to be oviposited within a few hours of collection). Ten eggs were inserted into separate niches for each of seven phloem sandwiches (hereafter referred to as "stage-I" sandwiches) per temperature, resulting in a total of 70 eggs per temperature. Sandwiches were maintained in constant humidity desiccators within each respective environmental chamber. Individuals were monitored daily for eclosion or larval molting as determined from head capsule color and size (Hall and Dyer 1974). Some larvae were occasionally not visible, as they submerged into the phloem, and observations were omitted if the specific date of molting

could not be determined. A second set of phloem sandwiches (hereafter referred to as "stage-II" sandwiches) were constructed 3 weeks later. Six instar-III larvae were inserted into each of the seven sandwiches per temperature for a total of 42 instar-III larvae per temperature.

Construction and monitoring of stage-I and stage-II sandwiches were repeated for the 5.5, 7, and 9°C treatments in December 1999, using broods from adults collected in October 1999 on the Dixie National Forest. Because no eggs hatched at 5.5 or 7°C after more than 100 days, we constructed another set of stage-I sandwiches, leaving the desiccators at room temperature. After head capsules became visible within the amniotic sacs, we transferred the desiccators to their respective environmental chambers to monitor temperature-dependent development subsequent to eclosion at 5.5 or 7°C. Because development proceeds more slowly at these lower temperatures, we examined individuals for molting three times per week rather than daily. Also, because the growing season is rarely more than 3 months for spruce beetle, we reasoned that it was ecologically inconsequential if the time to complete a life stage was in excess of 100 days. Therefore, monitoring became irregular after that time and some temperature treatments were discontinued after about 125 days at the same life stage.

To detect the possibility that development of pupae can be completed at temperatures lower than for instar-IV larvae, six more sandwiches (hereafter referred to as "stage-III" sandwiches) were constructed, each with six larvae reared at room temperature to the prepupal life stage (nonfeeding instar-IV larvae in a pupal chamber), and monitored daily. On the day that the first individual was observed to pupate, two sandwiches each were transferred to environmental chambers held at 7, 9, and 12°C.

Results

Experiment 1.1. Constant temperature effects on development in bolts

There was no difference in development time between insects in the treatment and reference bolts ($t = -0.53$; $P|t| = 0.6514$). Only the sample week (indicating more adults with increasing time) and the intercept were significant in the model. In this experiment, therefore, there is no evidence for diapause induction from a constant temperature treatment of 12°C through 50% instar IV.

Experiment 1.2. Constant temperature effects on development in phloem sandwiches

There was a significant difference in development time between insects in the treatment and reference bolts ($t = 6.37$; $P|t| = 0.0237$). The model results, however, are highly influenced by six outliers in the 12°C treatment. Although the other 172 surviving beetles in both treatments had become teneral adults by $t = 25$ days, 4 of the 6 outliers eclosed on days 31, 33, 33, and 45. The other two larvae lived until days 52 and 95, respectively, without pupating. When these six outliers are omitted, the model results are similar to those from Experiment 1.1 (*i.e.*, no evidence of diapause induction).

Experiment 2.1. Thermoperiod and photoperiod effects on development in bolts

Development times of insects in the treatments (T1 and T2) were significantly longer than those of insects in the reference (T1: $t = -2.26$, $P|t| = 0.0316$; T2: $t = -3.28$, $P|t| = 0.0027$). Pupation and eclosion of insects in T1 and T2 treatments were delayed about 1 week compared with insects in the reference treatment (Table 1).

TABLE 1. Weekly sampled proportions (%) of adult *Dendroctonus rufipennis* in reference, T1, and T2 treatments (Exp. 2.1).

Week	Reference	T1	T2
0	0 (261)	0 (212)	0 (220)
2	40 (266)	0 (251)	0 (239)
3	69 (255)	17 (188)	19 (208)
4	90 (211)	57 (228)	62 (234)
5	92 (132)	86 (195)	90 (233)
6	97 (92)	95 (202)	99 (150)
7	100 (36)	96 (104)	82 (17)
8	—	97 (34)	100 (19)
9	—	95 (20)	—

NOTE: Values in parentheses are the sample sizes (*n*) and are combined from 12 bolts per treatment. Week 0 is when all the sampled broods were instar IV.

Because the thermoperiod treatments were based on fall field temperatures, temperatures became increasingly cold as the experiment progressed (*i.e.*, insects in the last bolts to be returned to the reference temperature experienced the coldest temperatures of the entire experiment). Insects exposed to the thermoperiod treatments for the longest duration, and also the coldest temperatures of the experiment, took longer to develop from instar-IV larvae to adults to after being returned to the reference temperature.

Experiment 2.2. Thermoperiod and photoperiod effects on development in phloem sandwiches

Development to teneral adults in the sine-wave treatment (T1) was significantly slower ($t = -2.14$; $P|t| = 0.0406$) than in the reference treatment. Development in the square-wave treatment (T2), however, was not significantly different ($t = -1.30$; $P|t| = 0.2051$) than in the reference treatment. Pupation and eclosion of insects in the T1 treatment were delayed about 1–3 days compared with insects in the reference treatment.

Life stage specific developmental temperature thresholds

There was little apparent variation in life stage specific development thresholds (Table 2, Fig. 1). Although no eclosion of eggs was observed at 5.5 or 7°C when freshly oviposited eggs were used, eclosion did occur at those temperatures if the eggs were first reared at room temperature until head capsules became visible within the amniotic sacs. Instars I, II, and III were completed by at least one individual at all temperatures, including 5.5°C. Completion of instar IV was not observed in stage-II sandwiches at 9 and 12°C after 124 days, at which time the experiment was ended for those temperatures. Individuals in the 5.5 and 7°C stage-II sandwiches also had not pupated by that time, although we left these sandwiches in their respective environmental chambers. Pupation was observed at 5.5 and 7°C after some 200–300 days as instar IV/prepupae. Also, three stage-I individuals pupated at 9°C after 160–210 days as instar IV/prepupae. At the cooler temperatures, most of the extended instar IV/prepupal span was during the nonfeeding prepupal stage (Table 3).

At 18 and 21°C, development proceeded to teneral adults for all surviving individuals in both stage-I and stage-II sandwiches (to detect possible differences between stage-I and stage-II individuals, we conducted analysis of variance but found no significant differences within identical life stages). At 15°C, all surviving individuals in stage-II sandwiches completed development of the instar-IV and pupal stages in 15–29 days, likewise for 2 of 15 survivors in stage-I sandwiches. The remaining

TABLE 2. **Mean and median time (days) required by** *Dendroctonus rufipennis* **to complete each life stage at seven constant temperatures.**

	Temperature (°C)						
	5.5	7	9	12	15	18	21
Egg							
Mean	*	*	46.6	27.5	18.1	12.6	10.0
Median			45	27	18	13	10
n			30	44	54	61	51
Instar I							
Mean	31	28.7	18.0	9.1	5.6	4.6	3.8
Median	31	26	16	9	6	4	4
n	2	16	34	20	47	42	35
Instar II							
Mean	†	56.0	20.7	10.6	7.0	4.6	3.1
Median		49	18	10	7	5	3
n		3	24	23	40	37	28
Instar III							
Mean	†	†	31.5	14.7	10.6	6.6	5.4
Median			27	13.5	11	6	5
n			15	17	15	30	20
Instar IV and prepupae							
Mean	283.5	219.5	186.3	‡	20.1§	17.6	16.9
Median	282.5	217	198		20	18	17
n	30	24	3		31	55	43
Pupae‖							
Mean	80.4	52.3	38.0	24.3	12.9	9.9	7.6
Median	78	53	38	23.5	13	10	8
n	20	34	4	6	38	59	56

NOTE: Stage-I insects held at treatment temperatures beginning with eggs; stage-II and stage-III insects reared at room temperature until instars III and IV, respectively.

* Eclosion only occurred when eggs were preconditioned at room temperature.

† At least one individual completed this life stage at this temperature, but there was no accurate measurement of time.

‡ Experiment ended after 124 days without pupation.

§ All stage-II larvae (29/29) pupated, while most stage-I larvae (13/15) did not pupate after as long as 142 days.

‖ Values for 9 and 12°C derived from stage-III phloem sandwiches.

survivors in the 15°C stage-I sandwiches continued as instar IV/prepupae for up to 142 days, at which time the experiment was ended.

Discussion

Our results offer little support for the hypothesis of diapause induction during or before instar III. Development times of insects treated at constant 12°C through 50% instar IV (*i.e.*, equal proportions of instars III and IV at the end of the cold treatment) were not significantly longer than development times of reference insects, although the phloem sandwiches produced a few outliers. When a field-simulated thermoperiod was used, development times of insects in the treatment bolts were significantly greater than those in the reference bolts, although only by about 1 week. In the phloem sandwiches, the contrast between the T1, T2, and reference treatments is even smaller and only marginally significant in the logistic model. These minor delays are not convincing
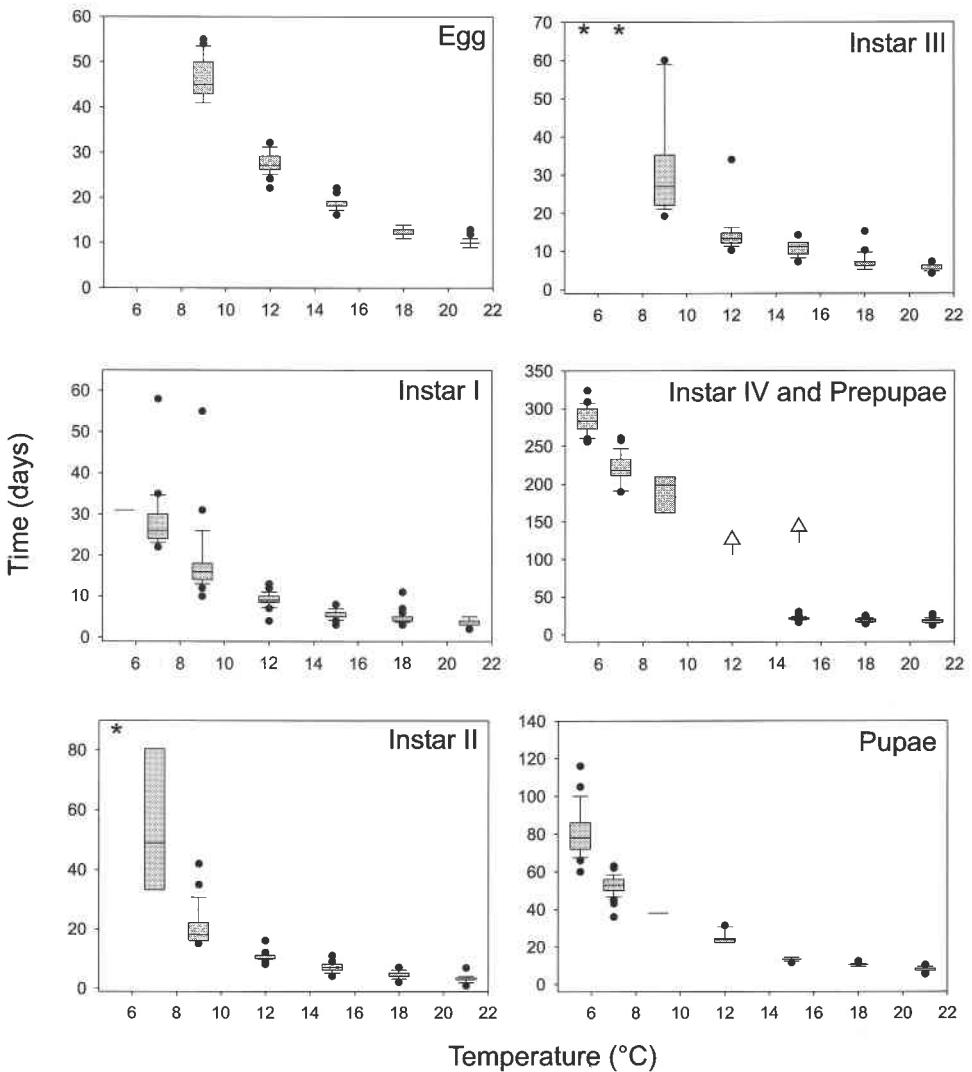
FIGURE 1. Time (days) for *Dendroctonus rufipennis* to complete each life stage at seven constant temperatures. An asterisk indicates that at least one individual completed the life stage at that temperature, but there was no accurate measurement of time. Arrows indicate that experiments were ended without pupation after 124 days at 12°C and after 142 days at 15°C for 13/15 stage-I larvae (held at 15°C through all life stages). Box plots show median (line), 25 and 75th percentiles (box), 10 and 90th percentiles (whiskers), and outliers (points).

evidence of diapause, although such results fit the continuum of possibilities under the diapause response (Zaslavski 1988).

Alternatively, if spruce beetle seasonality is regulated by differential development thresholds, we would expect to observe a relatively high threshold for pupation. This is because virtually all semivoltine beetles spend their first winter as prepupae, and such a life-history regulator would act to halt development in a prepupal life stage during fall as temperatures decline. Our results do not support this hypothesis. In fact, we observed minimal differences in development thresholds across all life stages. Furthermore, the

TABLE 3. Mean and median time (days) required by *Dendroctonus rufipennis* to complete instar-IV and prepupal stages at seven constant temperatures.

	Temperature (°C)						
	5.5	7	9	12	15	18	21
Instar IV							
Mean	57.8	60.5	68.4	56.3	14.3	11.7	12.8
Median	54	61	59	55	13	11.5	12
n	32	20	39	32	32	48	29
Prepupae							
Mean	226.4	160.3	48.0	*	6.8	5.9	5.3
Median	226	161.5	48		7	6	5
n	26	20	2		35	60	37

NOTE: Because there is no discrete molt between these life stages, these values are reported separately from the more accurate values in Table 2 in which the instar-IV and prepupal stages are combined. Timing of the prepupal stage began after excavation of an obvious pupal chamber and (or) the appearance of a pearly white cuticle, signaling the cessation of feeding.

* Experiment ended after 124 days (instar IV and prepupae combined) without pupation.

data for instar IV/prepupae cannot easily be reconciled into a single curve representing temperature-dependent development (Fig. 1). By comparison, a single curve can be fitted to the median data points for the other life stages. Also, the longest development delays at low temperatures occurred after excavation of pupal chambers (Table 3). Instead of a relatively high threshold for pupation, our results suggest a prepupal diapause. We suspect that after 150 or more days at 5.5 and 7°C, the diapause process had completed development, allowing morphological development to proceed.

We believe that our diapause experiments failed to produce stronger evidence because the induction-sensitive phase is later in the life cycle than we tested for, based on the hypothesis of Dyer and Hall (1977). Dyer and Hall rejected instar IV as the induction-sensitive phase because, in their trials, development proceeded to maturity when experimental temperatures were reduced from 16 to 11.7°C when most larvae (79 and 94%, in separate trials) were instar IV. Dyer and Hall (1977), however, do not distinguish the proportion of instar IV that more specifically could be classified as prepupae and the life stages of the remaining brood (21 and 6%) are not given. Therefore, Dyer and Hall's (1977) results could be interpreted to support diapause induction during instar IV or, even, early in the prepupal stage rather than during or before instar III. Therefore, all past and present evidence suggests diapause induction during the late instar-IV or early prepupal stages.

Although the evidence indicates an induction threshold near 15°C, it should be recognized that such a threshold is dynamic and could be influenced by preconditioning as evidenced by the different results from the stage-I and stage-II insects treated at 15°C. Ambiguity in identifying an induction threshold or, for that matter, the induction-sensitive life stage could be the result of overlapping temperature zones conducive to diapause and morphological development, as suggested for *Melanoplus sanguinipes* (F.) (Orthoptera: Acrididae) (Hilbert *et al.* 1985) and *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae) (Han and Bauce 1996).

Although prepupal diapause appears to be associated with semivoltine generations, we do not believe it appropriate to consider diapause as the causal agent of flexible voltinism. For semivoltine spruce beetles under field conditions, the prepupal stage is not found until near the end of the growing season (*i.e.*, September and October) (Hansen *et al.* 2001). In other words, prepupal diapause is manifest only if late summer and early fall temperatures (*i.e.*, July, August, and September) fail to drive development

from egg through adult. Therefore, for purposes of modeling voltinism, we believe that spruce beetle development may be considered under direct temperature control and that model predictions are unlikely to be confounded by prepupal diapause induction.

Conceivably, the main ecological significance of prepupal diapause is to synchronize semivoltine broods that must act en masse to overcome the defenses of a living host. Alternatively, prepupal diapause may serve to ensure that the insect does not overwinter as a pupa, hypothesized to be a cold-intolerant life stage of the closely related mountain pine beetle (Amman 1973). Regardless, we consider this phenomenon to be of little consequence to predicting voltinism relative to direct temperature control. Relatively warm summer conditions are required to initiate the univoltine life cycle. When such conditions are not met, prepupal overwintering is the default life-history course, and diapause merely prevents pupation during an unfavorable season. It is therefore more applicable to characterize the meteorological conditions that result in univoltine broods rather than those that might induce diapause. These results should be considered in developing predictive management tools, such as phenology models. Additionally, this knowledge can be used to more accurately assess the consequences of year-to-year climatic variability or global climate change on spruce beetle voltinism and concomitant population trends.

Acknowledgments

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