

Evidence for a Prepupal Diapause in the Mountain Pine Beetle (*Dendroctonus ponderosae*)

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Abstract

Dormancy strategies, including diapause and quiescence, enable insects to evade adverse conditions and ensure seasonally appropriate life stages. A mechanistic understanding of a species' dormancy is necessary to predict population response in a changing climate. Climate change is influencing distribution patterns and population success of many species, including *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae: Scolytinae), the most important mortality agent of pines in western North America. Diapause is considered absent in *D. ponderosae*, and quiescence in the final larval stage prior to pupation (i.e., prepupal) is considered the main dormancy strategy. We evaluated if a facultative diapause in the prepupal stage, rather than a pupation threshold ~15°C (i.e., quiescence), could describe pupation patterns in two latitudinally separated *D. ponderosae* populations in the western United States. We hypothesized that if pupation occurs at lower temperatures than previously described, and if significant prepupal developmental delays occur, diapause is a likely physiological mechanism. Although there was considerable variation within and between populations, pupation occurred below the previously established threshold suggesting a prepupal facultative diapause that is induced when late instars experience cool temperatures. Individuals that pupated at temperatures below 15°C also had developmental delays, relative to development at warmer temperatures, consistent with diapause development. Pupation patterns differed between populations wherein diapause was induced at cooler temperatures and diapause development was shorter in southern compared with northern *D. ponderosae*. Recognition of a facultative diapause that varies among and between populations is critical for making predictions about future population response and range expansion in a changing climate.

Key words: Bark beetle, phenology, diapause, latitudinal cline, mountain pine beetle

Dormancy, a period when development and metabolism are suppressed, is a ubiquitous strategy used by insects to evade adverse environmental conditions and to ensure that life stage advancement and reproduction are appropriately timed for annually fluctuating seasons (Tauber et al. 1986). Diapause and quiescence describe the physiological state during dormant periods when key energy-requiring processes are slowed and they can be considered either-or choices made along a developmental pathway (Danks 1987). Diapause is a neurohormonally mediated suppression of metabolism and morphological growth that can be induced by environmental conditions that are not by themselves limiting. Quiescence, conversely, occurs as a direct result of unfavorable conditions, is not hormonally regulated, and metabolic suppression is reversible upon the return of favorable conditions. Diapause in one life stage is often cued by conditions experienced in a previous life stage; yet, quiescence occurs as a direct effect, such as when habitat temperatures fall below a specific threshold (Danks 2007). Diapause and quiescence are integral components of complex life cycles that also include environmentally dependent rates and thresholds for morphogenesis.

Following induction, diapause development is a dynamic process that leads to the termination of diapause, a prerequisite for resumption of morphogenesis (Andrewartha 1952, Tauber et al. 1990, Honěk 1996). Diapause is a dormancy that persists despite subsequent favorable conditions. In some cases, it occurs in a specific life stage in every generation regardless of current conditions (obligatory), although more commonly environmental cues are used to program the diapause state (facultative) (Denlinger 2002). Multiple environmental factors can influence diapause, including temperature, photoperiod, nutrition, population density among others (Tauber et al. 1986, Danks 1987). Photoperiod and thermoperiod, the seasonally related pattern of temperature, are both daily cues with high reliability and are considered dominant controllers of diapause (Saunders 2014). In species with an overwintering diapause, however, low temperatures and changes in temperature from high to low, irrespective of photoperiod, can often be the main diapause inducer (Denlinger 1979, Valtonen et al. 2011, Saunders 2014). A facultative diapause may be realized in one generation and not in the next depending on environmental conditions, thereby resulting

in variable life cycle timing. An obligatory diapause, however, occurs in each generation and is expressed in climates where overwintering is a necessity because climatic conditions rarely allow for more than a single generation in a year (Williams and Sokolowski 1993). In some cases, rigorous laboratory experiments have shown that what was once considered an obligatory diapause was instead facultative (McKee and Aukema 2015), and the distinctions of “facultative” and “obligatory” diapause have been questioned (Tauber and Tauber 1981). Regardless, neurohormonal control of diapause suppresses metabolism and morphogenesis even during favorable conditions, whereas in quiescence, favorable conditions result in resumption of these processes.

Identification of the type of dormancy and environmental factors that initiate and inhibit its manifestation is critical for understanding life history events that influence the spatial and temporal patterns of insect abundance. Because environmentally controlled strategies are shaped by natural selection to provide optimal fitness in a given environment, dormancy and developmental strategies are highly evolved (Nylin and Gotthard 1998, Denlinger 2002). In species with large ranges across complex thermal habitats, adaptations specific to local climatic conditions result in intraspecific genetic variability in an insect's response to dormancy cues (Masaki 1961), thermal requirements for development (Honěk 1996), and, subsequently, voltinism (Bradshaw and Holzapfel 2008). Diapause incidence and duration, for example, tend to increase with latitude wherein northern populations require longer time for diapause development (Posledovich et al. 2015). In a changing climate, phenology and dormancy strategies that have evolved as a result of adaptation to local conditions may be disrupted and could result in an increase in population success, or alternatively, a developmental trap wherein maladaptive developmental pathways are pursued (Van Dyck et al. 2015). Of particular concern are herbivorous insect species with ecological and economic influences in agricultural and forest systems.

Dendroctonus ponderosae Hopkins (Coleoptera: Curculionidae: Scolytinae), the mountain pine beetle, is a native herbivore that causes widespread tree mortality in pine ecosystems of western North America, and its population dynamics and phenology are well studied (Bentz et al. 1991, Safranyik and Carroll 2006, Régnière et al. 2012). Successful reproduction during the epidemic population phase requires death of the host tree, and mass attacks of large numbers of adult beetles are required to overwhelm evolved tree defenses (Boone et al. 2011). Consequently, synchrony of adult emergence is critical. Moreover, because *D. ponderosae* inhabits environments with extreme cold, strategies that ensure survival during the coldest months are required. *Dendroctonus ponderosae* progresses through an egg stage, four instars, prepupa (i.e., the stage at the end of the last instar prior to pupation marked by the cessation of feeding following construction of a pupal chamber), pupa, and adult (Safranyik and Carroll 2006). Eggs and pupae are the least cold-tolerant life stages, fourth instars are the most cold tolerant, and adults are also capable of supercooling (Bentz and Mullins 1999, Lester and Irwin 2012, Bleiker et al. 2017, Rosenberger et al. 2017). A potential facultative diapause in the postreproductive adult stage was recently identified (Lester and Irwin 2012), although a winter diapause that serves to enhance synchrony and reduce the presence of the least cold-tolerant life stages has not been described (Logan and Bentz 1999, Safranyik and Carroll 2006). Instead, quiescence that is manifest through a relatively high temperature threshold for completion of the fourth instar/prepupae (i.e., 15–17°C) is considered to limit the presence of cold intolerant pupae during winter (Amman 1973) and to synchronize phenology and ultimately adult emergence (Powell et al. 2000, Jenkins et al. 2001). In comparison, the spruce beetle

(*Dendroctonus rufipennis* Kirby [Coleoptera: Curculionidae]) uses a diapause strategy in two life stages, the prepupal (Hansen et al. 2011) and reproductive adult (Schebeck et al. 2017). In spruce beetle, the fourth instar/prepupa can be completed (i.e., pupation) in 16–21 d at 15–21°C, although instar 4 larvae treated at temperatures below 15°C required 150–300 d to pupate. The discontinuous response is due to a facultative prepupal diapause (Hansen et al. 2001, 2011). *Dendroctonus ponderosae* can be reared successfully to the adult stage in the lab at temperatures of approximately ≥17°C (Safranyik and Whitney 1985, Bentz et al. 2001), and the relationship between fourth instar developmental rate and temperature is broadly similar to that for *D. rufipennis* (Hansen et al. 2001, Régnière et al. 2012). The potential for a prepupal diapause in *D. ponderosae* has not been rigorously investigated.

Our objective was to explore a potential prepupal diapause in *D. ponderosae*. We based our rationale and experimental design on previous work conducted on *D. rufipennis* (Hansen et al. 2001, Hansen et al. 2011). Because diapause strategies are known to vary latitudinally (Masaki 1961), in addition to the fact that *D. ponderosae* developmental parameters vary across latitudes (Bentz et al. 2001, 2011; Bracewell et al. 2013), we included northern (northern Utah or central Idaho) and southern (southeastern Arizona) U.S. *D. ponderosae* populations in our study. We hypothesized that if a facultative prepupal diapause was induced by a particular temperature treatment, pupation would be significantly delayed but eventually occur once diapause development was complete. Moreover, pupation observed at temperatures lower than previously reported threshold values (Régnière et al. 2012) following thermal treatments would suggest a prepupal diapause. We also evaluated diapause induction temperatures and proportions of individuals entering diapause in both populations.

Methods

Dendroctonus ponderosae Collection and Experimental Rationale

Dendroctonus ponderosae used in all experiments were collected from naturally infested *Pinus strobiformis* Engelm. on the Coronado National Forest, Pinaleno mountains, Arizona (~2,750 m) (hereafter southern population) and infested *Pinus contorta* Douglas from either the Uinta-Wasatch-Cache National Forest, Utah (UT) (~2,100 m) or Sawtooth National Recreation Area, Idaho (~2,000 m) (hereafter northern population). To collect adult beetles, infested trees were cut and bolts (43 cm long) brought to the laboratory in Logan, Utah. Trees infested with southern population beetles were cut in October 2008, October 2010, and September 2012, and trees with northern population beetles were cut in December 2009, October 2010, and October 2012. After transport to the Logan, UT laboratory, bolts from infested trees were kept at room temperature to allow for adult emergence. A random sample of adult beetles that emerged over a 3-wk period (on either side of peak emergence) was collected and gender determined based on the characteristics of the seventh abdominal tergite (Lyon 1958). Ten to 12 randomly selected male/female pairs that emerged from each population (i.e., southern or northern) were manually infested into bolts (43 cm long) taken from live *P. contorta*, cut from the Uinta-Wasatch-Cache National Forest, UT using methods described in Hansen et al. (2011). Bolts taken from live trees were stored at ~0°C for no longer than 2 mo prior to use. The same host tree species was used for both populations to standardize results. We acknowledge that use of *P. contorta* for southern individuals could have influenced aspects of development. Individuals were reared in *P. contorta* bolts for varying amounts of

time depending on the particular experiment, as described below, and were then placed in phloem sandwiches (i.e., phloem enclosed between a layer of glass and plastic; 30.5 × 30.5 cm). Phloem sandwiches enable visual inspection of life stage changes on a daily basis (see Bentz et al. 1991, Hansen et al. 2001 for details). Phloem for experimental sandwiches was taken from live *P. contorta* from the Uinta-Wasatch-Cache National Forest, Utah.

Our experimental designs were informed by previous research with *D. rufipennis* prepupal diapause wherein larvae were treated with either constant or thermocycle temperatures of diapause-averting and diapause-inducing conditions (Hansen et al. 2011). Bark beetle offspring development occurs within the subcortical environment beneath the bark, and photoperiod cues were not found to influence prepupal diapause induction in *D. rufipennis* (Hansen et al. 2011). We therefore did not include photoperiod in the current experiments with *D. ponderosae*. Although respiration rate is frequently used to evaluate diapause status (Tauber et al. 1986), our previous research with *D. rufipennis* prepupae found that prepupal stage respirometry was unable to distinguish diapause and non-diapause states, possibly due to low metabolism during histolysis (Hansen et al. 2011). Instead, delays in completion of the fourth instar were considered evidence of diapause induction. Developmental suppression is considered the most obvious phenotype associated with diapause (Danks 1987, Denlinger 2002) and has been used to identify prepupal diapause in other *Dendroctonus* species (Gent et al. 2017). We therefore based our evaluation of *D. ponderosae* diapause status on developmental delays. In all experiments, phloem sandwiches were monitored for up to ~350 d or until all individuals died or pupated.

Prepupal Diapause Versus Thermal Threshold for Pupation

The objective of this experiment was to determine whether *D. ponderosae* pupation can occur at temperatures below the previously described pupation threshold in the range from 15 to 17°C. This threshold was identified in multiple studies when a majority of individuals from northern populations reared at constant temperatures below ~15–17°C from egg through the fourth instar did not pupate (Safranyik and Whitney 1985, Règnière et al. 2012). We hypothesized that a prepupal diapause is induced when late instar larvae (i.e., third and fourth instars) experience temperatures of ≤15°C. If pupation can occur below 15–17°C when individuals are pretreated at warmer temperatures during the third and fourth instars, we suggest that a prepupal diapause is likely rather than a thermal threshold that must be surpassed for pupation. We compared pupation in individuals reared from the egg stage at several constant temperatures above and below 17°C to individuals that were reared from the egg stage to third and early fourth instars at 21°C then transferred to a range of cooler temperatures (pretreated). Differences in pupation rates among individuals from the northern and southern populations were also evaluated.

Constant temperature-treated individuals were produced by allowing offspring within lab-infested bolts to develop 9–10 d into the egg stage at 21°C. Bark was removed from bolts, and eggs were removed from the distal 4 cm of each gallery. Collected eggs were placed in a common Petri dish and no attempt was made to track maternal lineage. Eggs were then transferred to preformed niches in phloem sandwiches. Each phloem sandwich was initiated with nine eggs, and there were seven sandwiches per constant temperature treatment. The southern population was exposed to eight constant temperature treatments with seven sandwiches per treatment (5, 8, 10, 15, 17.5, 20, 25, and 27.5°C). The northern population was kept

at two constant temperatures (10 and 20°C) in the current experiment, and data from earlier experiments (Bentz et al. 1991, Règnière et al. 2012) collected at several constant temperatures (10, 15, 17.5, 20, and 25°C) were also used in the current analysis. Phloem sandwiches were monitored daily and development through each life stage noted as a change in head capsule width; unsclerotized head capsules or the presence of recent head capsule exuvia were also considered evidence of a molt within 24 h (Logan et al. 1998). Pupation was recorded as the first day when larvae began taking on pupal characteristics or a head capsule exuvia was observed.

To produce pretreated individuals, a second set of bolts was manually infested with adult beetles from either the southern or northern population as described above. Bolts were placed at 21°C until offspring were at least the third instar. Larvae were removed from the bolts and placed in niches in phloem sandwiches as described above. Five larvae were placed in each of seven phloem sandwiches for each temperature treatment and source population (i.e., northern or southern), then placed at constant temperatures, and monitored daily to record progression through the fourth instar and if pupation occurred. Larval instars and pupation were distinguished as described above. Experiments were conducted over multiple years between 2009 and 2013, and each population was not necessarily exposed to the same constant temperature. Southern population pretreated individuals were reared in phloem sandwiches at eight temperatures (5, 8, 10, 15, 17, 20, 25, and 27.5°C), and northern population pretreated individuals were reared in five temperatures (8, 12, 15, 17, and 20°C).

During phloem sandwich monitoring, some larvae submerged into the phloem and were not visible for several days. These individuals were not included in calculations of the number of days spent as fourth instar/prepupae prior to pupation. A portion of submerged larvae, however, later reappeared as pupae and were included in analyses describing the proportion that pupated. Therefore, sample sizes may differ for analyses of delayed development and pupation rates. Individuals that pupated were coded as 1, and 0 if they had not pupated after ~350 d. Logistic regression and a Wald test were used to test whether the likelihood of pupation differed between pretreated and constant temperature-treated individuals from each population, and between the northern and southern populations. Differences among temperatures and treatments in the number of days spent as fourth instar/prepupa prior to pupation were tested using a generalized linear model using a Poisson distribution, and a Tukey–Kramer adjustment for multiple comparisons. All analyses were conducted using SAS software (SAS Institute Inc., Cary, NC, v9.4).

Evaluation of Diapause and Induction Temperature Thresholds

In the first experiment, we observed that some individuals exposed to a warm temperature during the third or fourth instar pupated after being placed at a temperature of <15°C. These results suggest a prepupal diapause rather than quiescence in the form of a relatively high pupation threshold. Our objectives in this experiment were to further evaluate diapause potential and diapause induction threshold temperatures. We used a diapause-averting thermophase temperature (21°C) and several potential diapause-inducing cryophase temperatures in a thermocycle (Beck 1982, Hansen et al. 2011). At constant 21°C, the fourth instar is completed after about 10 d (Règnière et al. 2012), whereas at temperatures below 15°C, pupation is delayed in a large proportion of both populations (see Results). In the absence of diapause, a thermocycle with 12 h of 21°C and 12 h of 10, 12.5, and 15°C should result in completion

of the fourth instar after no more than twice the number of days as individuals kept at a constant 21°C (i.e., ~20 d). We consider evidence of diapause to be the fourth instar developmental times that are significantly longer than twice that required at constant 21°C.

Randomly selected female/male pairs from the northern and southern populations were manually infested into bolts as described above, and eggs were collected for placement into niches of phloem sandwiches, 9–10 per sandwich. Eclosion and development of all individuals in a sandwich to the second instar proceeded at 21°C. Each population was then exposed to six temperature treatments with five sandwiches per treatment: constant 21°C, constant 10°C, and 21:17.5°C, 21:15.0°C, 21:12.5°C, and 21:10°C thermocycles. Thermophase and cryophase durations were 12 h each in constant darkness, except for the short time periods when larvae were measured in light conditions. Phloem sandwiches were monitored daily, and development through each larval instar was denoted by changes in head capsule width and/or evidence of molting. Pupation was recorded as the first day when larvae began taking on pupal characteristics. The likelihood of pupation in each thermocycle treatment was compared between the two populations using logistic regression and a Wald chi-square test. A generalized linear mixed model with a Poisson distribution and Tukey–Kramer multiple comparison adjustment were used to test for differences in the number of days spent as fourth instar/prepupae prior to pupation in thermocycle treatments compared with a constant 21°C. All analyses were conducted using SAS software.

Results

Prepupal Diapause Versus Thermal Threshold for Pupation

Pupation occurred in both populations at temperatures below the previously hypothesized pupation threshold of ~15°C. A very

small proportion of constant temperature-treated individuals from both populations pupated at 10, 12, and 15°C, although the majority (>95%) remained prepupa after up to 350 d (Figs. 1a, 2a, and 3). Of those individuals that were pretreated, 41% (38/93) of southern and 12.5% (7/56) of northern individuals pupated at temperatures of ≤15°C. The likelihood of pupation differed by population and was also significantly influenced by the interaction of population, temperature, and treatment (Table 1). When southern population individuals were pretreated at 21°C into the third and early fourth instars before being placed at low constant temperatures, the likelihood of pupation was significantly greater than for individuals kept at a constant temperature from the egg stage (Figs. 2b and 3a; Table 1). By contrast, treatment did not influence the likelihood of pupation in northern individuals (Figs. 1b and 3a). Southern population individuals that were pretreated had higher likelihoods of pupation than northern individuals at all temperatures of <20°C (Fig. 3b). Southern population individuals kept at a constant 17°C were also more likely to pupate than northern individuals kept at the same constant temperature (Fig. 3b).

In addition to differences among temperatures and populations in the proportion of individuals that pupated, we observed significant developmental delays of fourth instar/prepupa prior to pupation at temperatures below 15°C, resulting in nonlinear trends in development across temperatures. At 8 and 10°C, southern individuals that pupated following pretreatment spent 232 ± 18.6 d ($N = 11$) and 247.5 ± 31.8 d ($N = 13$), respectively, as fourth instar/prepupa before pupation, significantly longer than southern pretreated individuals at 15°C ($N = 14$, mean = 24.1 ± 5.3 d; $z = 40.36$, Adj. $P < 0.0001$; $z = 39.45$, Adj. $P < 0.0001$) (Fig. 4b). A similar trend was seen in data from the northern population, although fewer pretreated individuals pupated at the coldest temperatures (Figs. 1b and 4a).

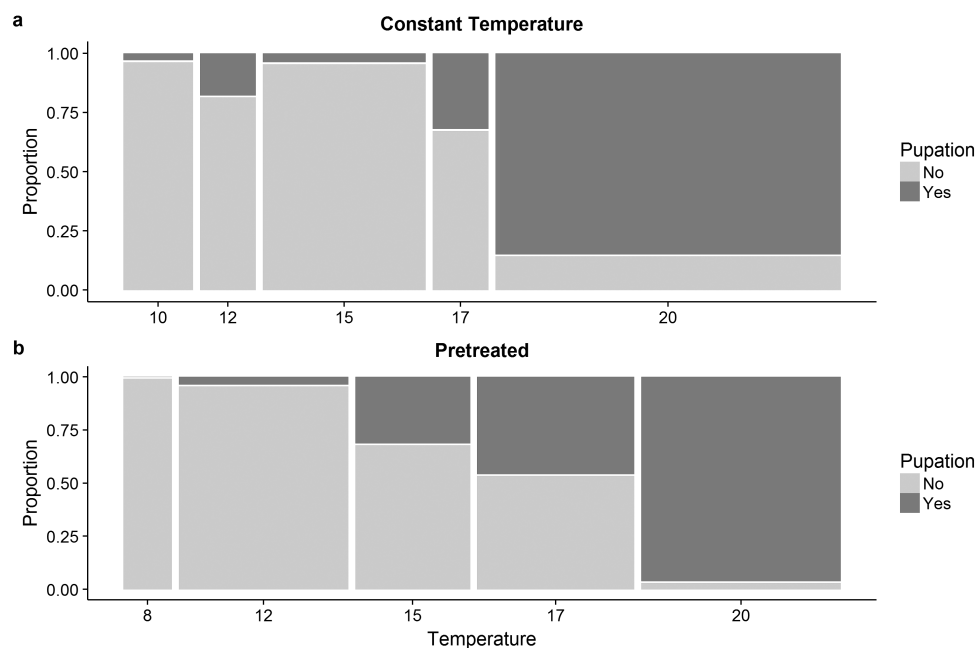


Fig. 1. Mosaic plots showing the proportion of northern population individuals that pupated at constant temperatures when (a) kept at the same constant temperature from egg through pupation and (b) pretreated at 21°C into the third and early fourth instar prior to being placed at a cooler temperature. The widths of each rectangle represents the proportion of individuals in each temperature treatment. Constant temperature sample sizes at 8°C: $N = 8$ (8 not pupated, 0 pupated); 10°C: $N = 35$ (34 not pupated, 1 pupated); 12°C: $N = 28$ (23 not pupated, 5 pupated); 15°C: $N = 82$ (79 not pupated, 1 pupated); 17°C: $N = 28$ (19 not pupated, 9 pupated); and 20°C: $N = 174$ (25 not pupated, 149 pupated). Pretreated sample sizes at 8°C: $N = 9$ (9 not pupated, 0 pupated); 12°C: $N = 28$ (27 not pupated, 1 pupated); 15°C: $N = 19$ (13 not pupated, 6 pupated); 17°C: $N = 26$ (14 not pupated, 12 pupated); and 20°C: $N = 33$ (1 not pupated, 19 pupated).

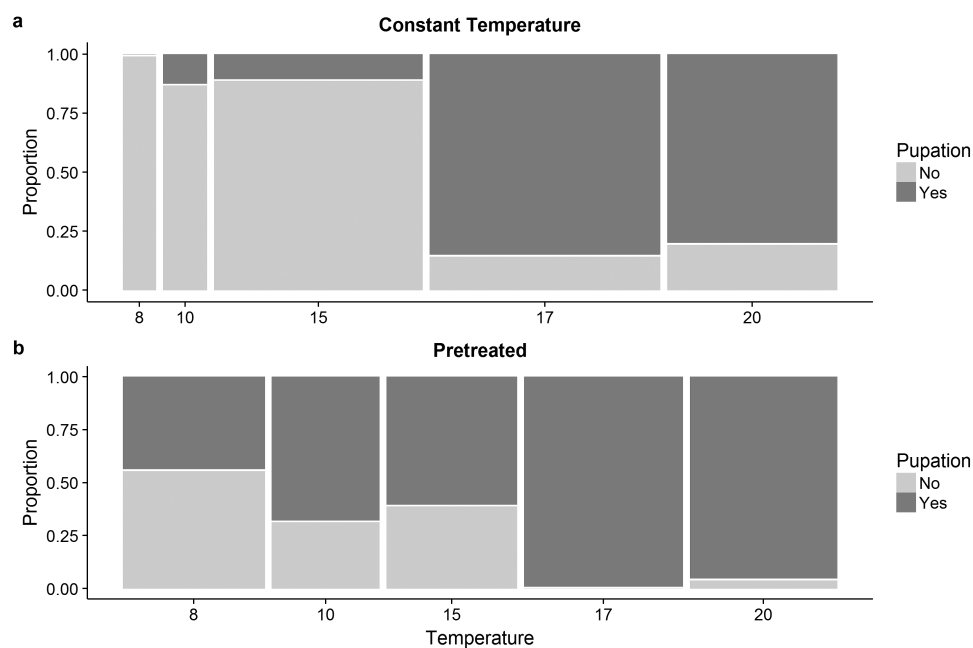


Fig. 2. Mosaic plots showing the proportion of southern population individuals that pupated at constant temperatures when (a) kept at the same constant temperature from egg through pupation and (b) pretreated at 21°C into the third and early fourth instar prior to being placed at a cooler temperature. No pretreated individuals pupated at 5°C (not shown). The widths of each rectangle represent the proportion of individuals in each temperature treatment. Constant temperature sample sizes at 8°C: $N = 6$ (6 not pupated, 0 pupated); 10°C: $N = 8$ (7 not pupated, 1 pupated); 15°C: $N = 38$ (34 not pupated, 4 pupated); 17°C: $N = 42$ (6 not pupated, 36 pupated); 20°C: $N = 31$ (6 not pupated, 25 pupated). Pretreated temperature sample sizes at 5°C: $N = 26$ (26 not pupated, 0 pupated); 8°C: $N = 25$ (14 not pupated, 11 pupated); 10°C: $N = 19$ (6 not pupated, 13 pupated); 15°C: $N = 23$ (9 not pupated, 14 pupated); 17°C: $N = 28$ (0 not pupated, 28 pupated); and 20°C: $N = 26$ (1 not pupated, 25 pupated).

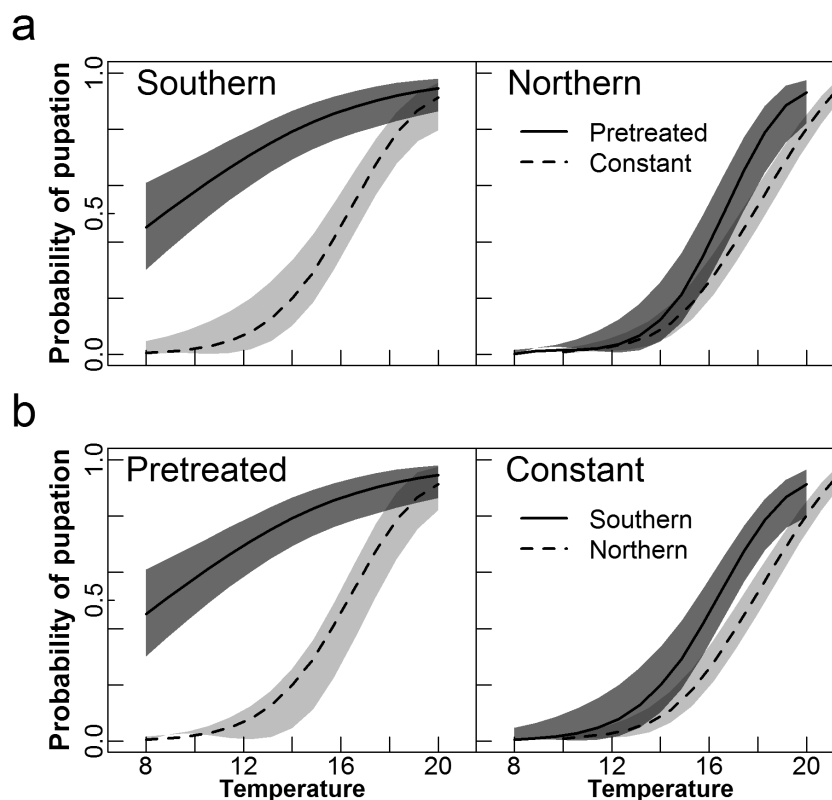


Fig. 3. Predicted probability of pupation for southern and northern population individuals when kept at the same constant temperature from egg through pupation (i.e., Constant) or pretreated at 21°C into the third and early fourth instars prior to being placed at a cooler temperature (i.e., Pretreated). Predicted probabilities across temperatures based on logistic regression (see Table 1). The same data were used for graphs shown in (a) and (b).

Evaluation of Diapause and Induction Temperature Thresholds

Results from our first experiment indicated a prepupal diapause in *D. ponderosae*. To evaluate potential diapause induction temperatures, we tested individuals in thermocycle treatments. We hypothesized that if diapause was not induced, the time required as a fourth instar/prepupa in a thermocycle treatment with 12 h at 21°C and 12 h at a cooler temperature would not differ from twice the time required at a constant 21°C (i.e., assuming no development would occur at low temperatures, and development at 21°C would not be affected). Within the southern population, time spent as a fourth

Table 1. Results from logistic regression testing for differences in the likelihood of pupation between constant temperature-treated and pretreated (i.e., treatment) larvae across a range of temperatures, and that came from either northern or southern population

Effect	Wald chi-square	<i>P</i> > chi-square
Temperature	125.08	<0.0001
Treatment	4.9954	0.0254
Population	12.7665	0.0004
Temperature × treatment	2.2283	0.1355
Temperature × population	7.3957	0.0065
Population × treatment	5.6364	0.0176
Temperature × population × treatment	4.3317	0.0374

Pretreated larvae were kept at 21°C until the third or fourth instar prior to being placed at a cooler temperature. Constant temperature-treated larvae were kept at the same temperature from the egg through pupation. We assumed that individuals that did not pupate were in diapause, and that diapause development was not completed during the time of the experiment. Number of pupated and not pupated individuals by population, treatment, and temperature are listed in Figs. 1 and 2.

instar/prepupa prior to pupation in any of the thermocycle treatments was not greater than twice the time at a constant 21°C, suggesting that diapause was not induced by 12 h at 10, 12.5, 15, or 17.5°C (Fig. 5b). Conversely, in the northern population individuals in the 21:12.5°C and 21:15°C thermocycle, treatments required significantly more than twice the number of days as a fourth instar/prepupa prior to pupation (mean = 73.8 and 48.7 d, respectively) than northern individuals at a constant 21°C (mean = 16.5 d; 21:12.5°C: $z = 7.86$, Adj. $P < 0.0001$; 21:15°C: $z = 3.77$, Adj. $P = 0.0016$), suggesting that diapause induction occurred with 12 h per day at 12.5 and 15°C (Fig. 5b). The likelihood of pupation at 12.5 and 15°C was also significantly greater in the southern compared with the northern population (Wald chi-square = 13.0804, $P = 0.0003$) (Fig. 5a). Three northern individuals pupated in the 21:10°C thermocycle treatment without a substantial delay in development. An additional six individuals died before pupation in that treatment after as long as 156 d, suggesting possible diapause induction but without diapause completion.

Discussion

We observed *D. ponderosae* pupation below the previously described threshold within the range from 15° to 17°C (Safranyik and Whitney 1985, Bentz et al. 1991). This result is inconsistent with a hypothesis that quiescence, in the form of a relatively high temperature threshold for pupation, is the only explanation for *D. ponderosae* pupation timing (i.e., completion of fourth instar and prepupae) and seasonality (Logan and Bentz 1999). Our data provide evidence for a facultative prepupal diapause in *D. ponderosae* that is induced when late instars are exposed to cool temperatures and is averted by warm temperature exposure during these same instars. Southern population individuals that were pretreated as third and fourth instars at 21°C pupated at temperatures as low as 8°C (Figs. 2 and 3). We also observed a significant delay in

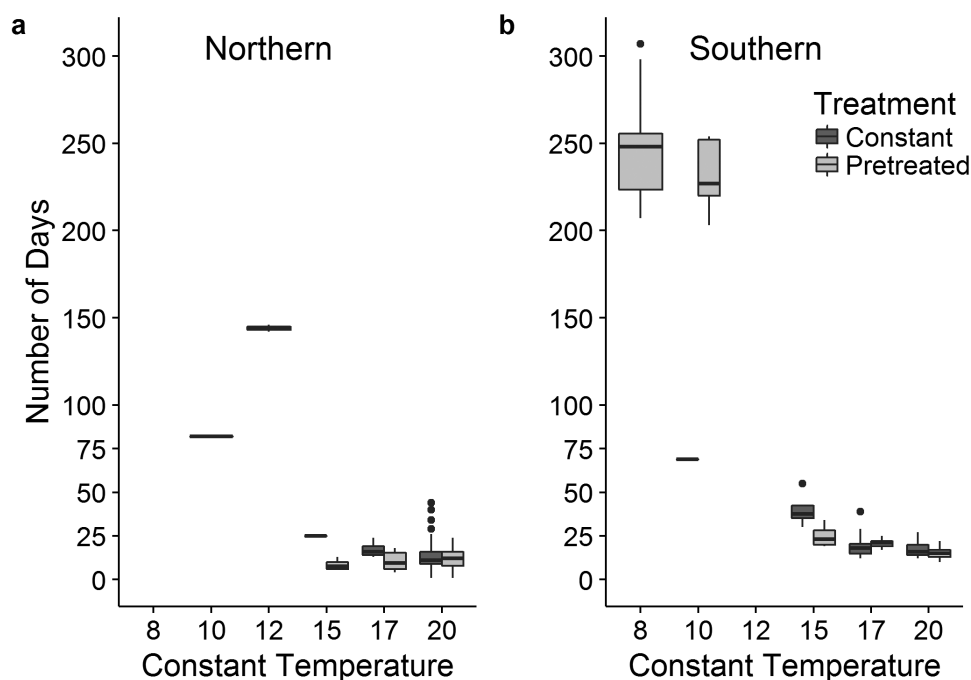


Fig. 4. Number of days spent as fourth instar/prepupae prior to pupation in (a) northern and (b) southern population individuals that were either kept at the same constant temperature from egg through pupation, or pretreated at 21°C prior to being placed at a cooler temperature. Sample size of northern individuals at a constant 10°C = 1, 12°C = 2, 15°C = 1, 17°C = 9, and 20°C = 81. Sample size of northern-pretreated individuals at 15°C = 4, 17°C = 12, 20°C = 13. Sample size of southern individuals at a constant 10°C = 1, 15°C = 4, 17°C = 35, and 20°C = 22. Sample size of southern-pretreated individuals at 8°C = 7, 10°C = 8, 15°C = 5, 17°C = 14, and 20°C = 14.

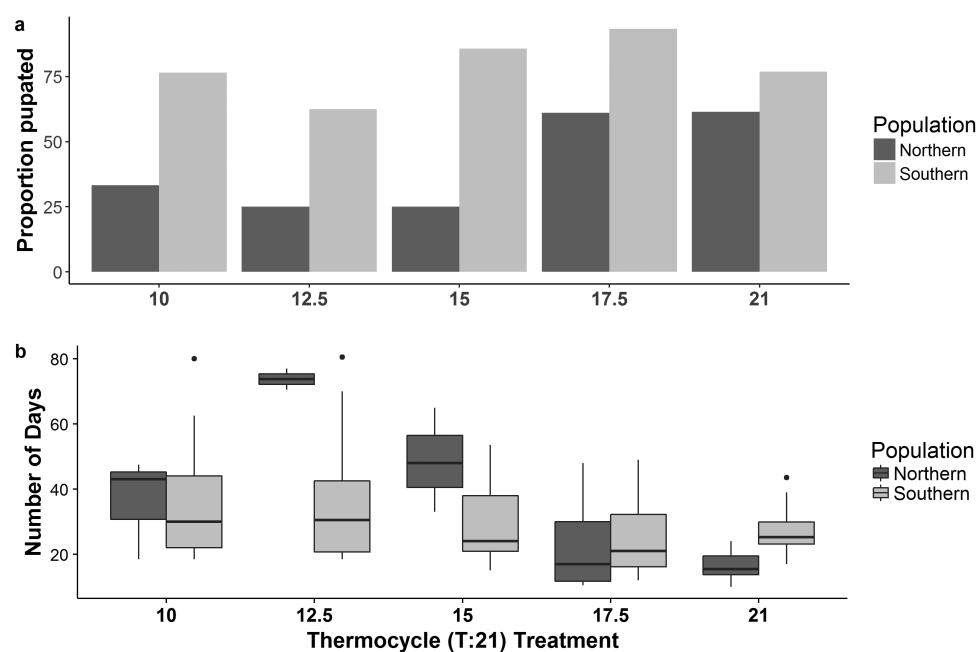


Fig. 5. Northern and southern *D. ponderosae* exposed to thermocycle treatments (12:12 h) of 21:10°C, 21:12.5°C, 21:15°C, 21:17.5°C, and a constant 21°C. Shown are the (a) proportion pupated and (b) the number of days as fourth instar/prepupae prior to pupation in each thermocycle treatment. Also shown are the median (solid line) 25th and 75th percentiles (lower and upper vertical lines), and outlying observations (dots). Northern individuals in 21:12.5°C and 21:15°C thermocycle treatments required significantly more than twice the number of days for pupation at a constant 21°C (21:12.5°C: $z = 7.86$, Adj. $P < 0.0001$; 21:15°C: $z = 3.77$, Adj. $P = 0.0016$). No individuals from either population pupated at a constant 10°C (not shown). Number of northern individuals that pupated: $N = 3$ (10°C), 2 (12°C), 3 (15°C), 11 (17.5°C), 8 (21°C). Number of southern individuals that pupated: $N = 13$ (10°C), 10 (12.5°C), 18 (15°C), 14 (17.5°C), 10 (21°C).

development of fourth instar/prepupae prior to pupation at temperatures below 15°C when compared with development of individuals at temperatures above 15°C (Fig. 4). These observations are similar to those from experiments with other phloem feeding beetles, including *D. rufipennis*, *D. micans* Kugelann, and *Hylobius abietis* Linnaeus wherein significant nonlinear and delayed prepupal development to pupation was observed at temperatures below ~15°C, strongly implying a facultative diapause that occurs when late stage larvae are exposed to cool temperatures (Hansen et al. 2001, 2011; Inward et al. 2012; Gent et al. 2017).

Several lines of evidence, based on temperature treatments and timing of pupation, indicate differences between the northern and southern *D. ponderosae* populations in diapause induction thresholds and diapause development times. Threshold temperatures for diapause induction occurred at warmer temperatures for the northern compared with southern population individuals. For example, a greater proportion of southern individuals exposed to constant temperatures pupated at 17°C (Fig. 2) compared with northern individuals at this same temperature (Fig. 1), indicating that more northern than southern individuals were induced into diapause (i.e., did not pupate) at this temperature. Moreover, a greater proportion of pretreated southern individuals pupated at 12.5 and 15°C than did northern pretreated individuals. In addition to differences in diapause induction thresholds, the time spent as fourth instar/prepupae prior to pupation (i.e., diapause development time) differed between the populations. At 8 and 10°C, pretreated southern individuals took ~8 times longer to pupate than did southern individuals at ≥15°C, suggesting that diapause was induced at these temperatures and that conditions were met for completion of diapause development for some individuals after ~245 d (Fig. 4). By contrast, northern pretreated individuals had not pupated at 8 and 10°C after up to 350 d when all prepupae either died or experiments were terminated. These results suggest that diapause was induced in both northern

and southern individuals at 8 and 10°C, and that diapause development was completed, under the experimental conditions, for the southern but not northern individuals. Diapause appears to be easier to induce in northern compared with southern individuals, and harder to terminate.

Given the large *D. ponderosae* geographic distribution across varying thermal habitats, it is not surprising to identify local adaptation in temperature-dependent life history strategies. Our finding of a greater diapause incidence and duration in the northern compared with southern population is similar to that found for other insects (Posledovich et al. 2015). In addition to the latitudinal differences in diapause induction cues, thresholds, and development times reported here, *D. ponderosae* morphological development time has been shown to be faster in northern compared with southern individuals in common garden experiments (Bentz et al. 2011, Bracewell et al. 2013). Despite these differences, life cycle duration under field conditions is similar across a wide latitudinal band, with the univoltine cycle predominant despite considerable variation in thermal input (Bentz et al. 2014). Univoltinism is considered optimal for this insect (Logan and Bentz 1999, Safranyk and Carroll 2006), and observed differences in diapause and morphogenesis traits suggest divergent selection in life history traits to maintain univoltinism across varying thermal environments.

Our results support a facultative prepupal diapause in *D. ponderosae* that occurs when late stage larvae are exposed to cool temperatures. It is unclear how often diapause is induced in current climates, although the fourth instar/prepupae are often found overwintering and are considered to be the most cold-tolerant life stage (Rosenberger et al. 2017). In our opinion, the previous lack of recognition of the diapause syndrome in *D. ponderosae* likely resulted from the facultative nature of the prepupal diapause and the large amount of variation within and between populations in induction cues and diapause developmental timing. Additionally,

previous laboratory experiments (e.g., [Safranyik and Whitney 1985](#), [Bentz et al. 1991](#)) focused on northern populations in which the easily induced and relatively hard to terminate prepupal diapause can appear as a simple high temperature threshold for pupation, masking a more complex physiology. We observed considerable variability in offspring phenotypes, suggesting roles for both quiescence and diapause ([Gardner et al. 2012](#)) that could serve to decrease the amount of variance in fitness returns (i.e., bet hedging) ([Sgró et al. 2016](#)) and be an important adaptation avenue for *D. ponderosae* in a changing climate. Although more specific information is needed on diapause induction cues, genetic and phenotypic drivers, and associated latitudinal variation, recognition of a prepupal diapause that varies among and between populations is critical for making predictions about future population response and range expansion in a changing climate.

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References Cited

- Addison, A. L., J. A. Powell, D. L. Six, M. Moore, and B. J. Bentz. 2013. The role of temperature variability in stabilizing the mountain pine beetle-fungus mutualism. *J. Theor. Biol.* 335: 40–50.
- Amman, G. D. 1973. Population changes of the mountain pine beetle in relation to elevation. *Environ. Entomol.* 2: 541–548.
- Amman, G. D., and W. E. Cole. 1983. Mountain pine beetle dynamics in lodgepole pine forests Part II: population dynamics. USDA Forest Service, GTR-INT-145: 59 p.
- Andrewartha, H. 1952. Diapause in relation to the ecology of insects. *Bio. Rev.* 27: 50–107.
- Beck, S. D. 1982. Thermoperiodic induction of larval diapause in the European corn borer, *Ostrinia nubilalis*. *J. Insect Physiol.* 28: 273–277.
- Bentz, B. J., and D. E. Mullins. 1999. Ecology of mountain pine beetle (Coleoptera: Scolytidae) cold hardening in the Intermountain West. *Environ. Entomol.* 28: 577–587.
- Bentz, B. J., J. A. Logan, and G. D. Amman. 1991. Temperature-dependent development of the mountain pine beetle (Coleoptera: Scolytidae) and simulation of its phenology. *Can. Entomol.* 123: 1083–1094.
- Bentz, B., J. Logan, and J. Vandygriff. 2001. Latitudinal variation in *Dendroctonus ponderosae* (Coleoptera: Scolytidae) development time and adult size. *Can. Entomol.* 133: 375–387.
- Bentz, B. J., R. R. Bracewell, K. E. Mock, and M. E. Pfrender. 2011. Genetic architecture and phenotypic plasticity of thermally-regulated traits in an eruptive species, *Dendroctonus ponderosae*. *Evol. Ecol.* 25: 1269–1288.
- Bentz, B. J., J. C. Vandygriff, C. Jensen, T. Coleman, P. Maloney, S. Smith, A. Grady, and G. Schen-Langenheim. 2014. Mountain pine beetle voltinism and life history characteristics across latitudinal and elevational gradients in the western United States. *For. Sci.* 60: 434–449.
- Bleiker, K. P., G. D. Smith, and L. M. Humble. 2017. Cold tolerance of mountain pine beetle (Coleoptera: Curculionidae) eggs from the historic and expanded ranges. *Env. Entomol.* 46: 1165–1170.
- Boone, C. K., B. H. Aukema, J. Bohlmann, A. L. Carroll, and K. F. Raffa. 2011. Efficacy of tree defense physiology varies with bark beetle population density: a basis for positive feedback in eruptive species. *Can. J. For. Res.* 41: 1174–1188.
- Bracewell, R. R., M. E. Pfrender, K. E. Mock, and B. J. Bentz. 2013. Contrasting geographic patterns of genetic differentiation in body size and development time with reproductive isolation in *Dendroctonus ponderosae* (Coleoptera: Curculionidae, Scolytinae). *Ann. Entomol. Soc. Am.* 106: 385–391.
- Bradshaw, W. E., and C. M. Holzapfel. 2008. Genetic response to rapid climate change: it's seasonal timing that matters. *Mol. Ecol.* 17: 157–166.
- Danks, H. V. 1987. Insect dormancy: an ecological perspective (Biological Survey of Canada Monograph Series, No. 1). *Entomol. Soc. Can.* 439, <http://www.biology.ualberta.ca/bsc/pdf/contents.pdf>.
- Danks, H. 2007. The elements of seasonal adaptations in insects. *Can. Entomol.* 139: 1–44.
- Denlinger, D. L. 1979. Pupal diapause in tropical flesh flies: environmental and endocrine regulation, metabolic rate and genetic selection. *Biol. Bull.* 156: 31–46.
- Denlinger, D. L. 2002. Regulation of diapause. *Annu. Rev. Entomol.* 47: 93–122.
- Gent, C. A., D. Wainhouse, K. R. Day, A. J. Peace, and D. J. G. Inward. 2017. Temperature-dependent development of the great European spruce bark beetle *Dendroctonus micans* (Kug.) (Coleoptera: Curculionidae: Scolytinae) and its predator *Rhizophagus grandis* Gyll. (Coleoptera: Monotomidae: Rhizophaginae). *Agricul. For. Entomol.* 19:321–331.
- Gardner, J., T. Yong, S. A. Pitcher, and M. P. Hoffmann. 2012. Overwintering of *Trichogramma ostrinae* (Hymenoptera: Trichogrammatidae) within target and not-target host eggs. *Biocont. Sci. Tech.* 23:367–380.
- Gurney, W., P. Crowley, and R. Nisbet. 1994. Stage-specific quiescence as a mechanism for synchronizing life cycles to seasons. *Theor. Pop. Biol.* 46: 319–343.
- Hansen, E. M., B. J. Bentz, and D. L. Turner. 2001. Physiological basis for flexible voltinism in the spruce beetle (Coleoptera: Scolytidae). *Can. Entomol.* 133: 805–817.
- Hansen, E. M., B. J. Bentz, J. A. Powell, D. R. Gray, and J. C. Vandygriff. 2011. Prepupal diapause and instar IV developmental rates of the spruce beetle, *Dendroctonus rufipennis* (Coleoptera: Curculionidae, Scolytinae). *J. Insect Physiol.* 57: 1347–1357.
- Hilbert, D., J. Logan, and D. Swift. 1985. A unifying hypothesis of temperature effects on egg development and diapause of the migratory grasshopper, *Melanoplus sanguinipes* (Orthoptera: Acrididae). *J. Theor. Biol.* 112: 827–838.
- Honek, A. 1996. The relationship between thermal constants for insect development: a verification. *Acta Soc. Zool. Bohem.* 60: 115–152.
- Inward, D. J., D. Wainhouse, and A. Peace. 2012. The effect of temperature on the development and life cycle regulation of the pine weevil *Hylobius abietis* and the potential impacts of climate change. *Agricul. For. Entomol.* 14: 348–357.
- Jenkins, J. L., J. A. Powell, J. A. Logan, and B. J. Bentz. 2001. Low seasonal temperatures promote life cycle synchronization. *Bull. Math. Biol.* 63: 573–595.
- Lester, J. D., and J. T. Irwin. 2012. Metabolism and cold tolerance of overwintering adult mountain pine beetles (*Dendroctonus ponderosae*): evidence of facultative diapause? *J. Insect Physiol.* 58: 808–815.
- Logan, J. A., and B. J. Bentz. 1999. Model analysis of mountain pine beetle (Coleoptera: Scolytidae) seasonality. *Env. Entomol.* 28: 924–934.
- Logan, J., B. Bentz, J. Vandygriff, and D. Turner. 1998. General program for determining instar distributions from headcapsule widths: example analysis of mountain pine beetle (Coleoptera: Scolytidae) data. *Env. Entomol.* 27: 555–563.
- Lyon, R. 1958. A useful secondary sex character in *Dendroctonus* bark beetles. *Can. Entomol.* 90: 582–584.
- Masaki, S. 1961. Geographic variation of diapause in insects. *Bull. Fac. Agric. Hirosaki Univ.* 7: 66–98.
- McKee, F. R., and B. H. Aukema. 2015. Successful reproduction by the eastern larch beetle (Coleoptera: Curculionidae) in the absence of an overwintering period. *Can. Entomol.* 147: 602–610.
- Nylin, S., and K. Gotthard. 1998. Plasticity in life-history traits. *Annu. Rev. Entomol.* 43: 63–83.
- Posledovich, D., T. Toftegaard, C. Wiklund, J. Ehrlén, and K. Gotthard. 2015. Latitudinal variation in diapause duration and post-winter development in two pierid butterflies in relation to phenological specialization. *Oecologia* 177: 181–190.
- Powell, J. A., J. L. Jenkins, J. A. Logan, and B. J. Bentz. 2000. Seasonal temperature alone can synchronize life cycles. *Bull. Math. Biol.* 62: 977–998.

- Régnière, J., J. Powell, B. Bentz, and V. Nealis. 2012. Effects of temperature on development, survival and reproduction of insects: experimental design, data analysis and modeling. *J. Insect Physiol.* 58: 634–647.
- Rosenberger, D.W., B.H. Aukema, and R.C. Venette. 2017. Cold tolerance of mountain pine beetle among novel eastern pines: A potential for trade-offs in an invaded range? *For. Ecol. Manag.* 400: 28–37.
- Safranyik, L., and H. Whitney. 1985. Development and survival of axenically reared mountain pine beetles, *Dendroctonus ponderosae* (Coleoptera: Scolytidae), at constant temperatures. *Can. Entomol.* 117: 185–192.
- Safranyik, L., and A. Carroll. 2006. The biology and epidemiology of the mountain pine beetle in lodgepole pine forests, pp. 3–66. In L. Safranyik and B. Wilson (eds.), *The mountain pine beetle A synthesis of biology, management, and impacts on lodgepole pine*. Natural Resources Canada, Canadian Forest Service, Victoria, BC, Canada.
- Saunders, D. S. 2014. Insect photoperiodism: effects of temperature on the induction of insect diapause and diverse roles for the circadian system in the photoperiodic response. *Entomol. Sci.* 17: 25–40.
- Schebeck, M., E. M. Hansen, A. Schopf, G. J. Ragland, C. Stauffer, and B. J. Bentz. 2017. Diapause and overwintering of two spruce bark beetle species. *Physiol. Entomol.* 42: 200–210.
- Sgrò, C. M., J. S. Terblanche, and A. A. Hoffmann. 2016. What can plasticity contribute to insect responses to climate change? *Annu. Rev. Entomol.* 61: 433–451.
- Tauber, C. A., and M. J. Tauber. 1981. Insect seasonal cycles: genetics and evolution. *Ann. Rev. Ecol. Syst.* 12: 281–308.
- Tauber, M. J., C. A. Tauber, and S. Masaki. 1986. *Seasonal adaptations of insects*, Oxford University Press, New York, 426 pp.
- Tauber, M. J., C. A. Tauber, J. R. Ruberson, A. J. Tauber, and L. P. Abrahamson. 1990. Dormancy in *Lymantria dispar* (Lepidoptera: Lymantriidae): analysis of photoperiodic and thermal responses. *Ann. Entomol. Soc. Am.* 83: 494–503.
- Valtonen, A., M. P. Ayres, H. Roininen, J. Pöyry, and R. Leinonen. 2011. Environmental controls on the phenology of moths: predicting plasticity and constraint under climate change. *Oecologia*. 165: 237–248.
- Van Dyck, H., D. Bonte, R. Puls, K. Gotthard, and D. Maes. 2015. The lost generation hypothesis: could climate change drive ectotherms into a developmental trap? *Oikos*. 124: 54–61.
- Venables, W. Ripley. BD, 2002. *Modern applied statistics with S*. New York: Springer Science & Business Media. 200: 183–206.
- Williams, K. D., and M. B. Sokolowski. 1993. Diapause in *Drosophila melanogaster* females: a genetic analysis. *Heredity (Edinb)*. 71 (Pt 3): 312–317.