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Modeling cold tolerance in the mountain pine beetle, *Dendroctonus ponderosae*

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Abstract

Cold-induced mortality is a key factor driving mountain pine beetle, *Dendroctonus ponderosae*, population dynamics. In this species, the supercooling point (SCP) is representative of mortality induced by acute cold exposure. Mountain pine beetle SCP and associated cold-induced mortality fluctuate throughout a generation, with the highest SCPs prior to and following winter. Using observed SCPs of field-collected *D. ponderosae* larvae throughout the developmental season and associated phloem temperatures, we developed a mechanistic model that describes the SCP distribution of a population as a function of daily changes in the temperature-dependent processes leading to gain and loss of cold tolerance. It is based on the changing proportion of individuals in three states: (1) a non cold-hardened, feeding state, (2) an intermediate state in which insects have ceased feeding, voided their gut content and eliminated as many ice-nucleating agents as possible from the body, and (3) a fully cold-hardened state where insects have accumulated a maximum concentration of cryoprotectants (e.g. glycerol). Shifts in the proportion of individuals in each state occur in response to the driving variables influencing the opposite rates of gain and loss of cold hardening. The level of cold-induced mortality predicted by the model and its relation to extreme winter temperature is in good agreement with a range of field and laboratory observations. Our model predicts that cold tolerance of *D. ponderosae* varies within a season, among seasons, and among geographic locations depending on local climate. This variability is an emergent property of the model, and has important implications for understanding the insect's response to seasonal fluctuations in temperature, as well as population response to climate change. Because cold-induced mortality is but one of several major influences of climate on *D. ponderosae* population dynamics, we suggest that this model be integrated with others simulating the insect's biology.
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Keywords: Cold tolerance; Cold resistance; Cold hardiness; Mountain pine beetle; Winter mortality; Model; Supercooling point

1. Introduction

As a species with a distribution spanning 25° of latitude and 3000 m of elevation, the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae, Scolytinae) occupies a diverse array of habitats, attacking and reproducing in all *Pinus* species located throughout the western USA and Canada. Except for a short adult dispersal period in summer, the insect's entire life cycle is spent within the phloem. Although voltinism and synchrony can vary geographically, larvae are the typical overwintering stage.

Mortality from cold exposure is one of the key factors in the population dynamics of *D. ponderosae*, (Yuill, 1941; Reid, 1963; Amman, 1973; Safranyik, 1978; Cole, 1981). Early investigations recognized that adequate acclimation to low temperatures could significantly increase this insect's cold hardiness and survival (Wygant, 1940; Yuill, 1941; Sømme, 1964). The physiological processes of cold hardening, particularly synthesis of cryoprotectants, are relatively well understood for insects (see Storey and Storey, 1988). In many xylophagous insects, cold tolerance changes considerably over the winter (Merivee, 1978; Sømme, 1982), and because of its apparent lack of diapause, the cold hardiness of *D. ponderosae* is probably less dependent on hormonal controls than in many diapausing insects

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(Merivee, 1978; Sømme, 1982; Hodkova and Hodek, 1994). In addition, because in this species development occurs entirely under the bark, photoperiod is probably not a significant cue in the development of cold hardiness. It is likely that the cold-hardening process is controlled mainly by temperature (Sømme, 1982), although Horwath and Duman (1986) found that thermoperiod was involved in antifreeze protein production in *Dendroides canadensis* as a surrogate to photoperiod. Laboratory evidence (BB unpublished data) suggests that daily temperature fluctuations may be needed to stimulate acclimation to cold in *D. ponderosae*.

Many insect species can withstand the formation of ice in the extracellular body fluid and have been labeled freeze tolerant (Salt, 1961). Others, including *D. ponderosae* (Bentz and Mullins, 1999) and other scolytine bark beetles (Ring, 1977; Gehrken, 1984; Miller and Werner, 1987; Lombardero et al., 2000), are freeze intolerant and must avoid freezing of body tissues. In these species, the supercooling point (SCP) refers to the temperature at which spontaneous nucleation of body water occurs and lethal ice crystals form in the insect tissue. The SCP can often be 20 °C or more below the freezing point of body fluids, and is often considered a measure of maximum cold hardiness (Lee, 1989, 1991). In some species, SCP also represents the lower lethal temperature (Lombardero et al., 2000; Tran et al., 2007; Hodkova and Hodek, 1994). It is widely recognized, however, that pre-freeze mortality at temperatures above the SCP can result in substantial mortality in freeze-avoiding species (Baust and Rojas, 1985; Knight et al., 1986; Lee, 1991; Sinclair, 1999; Bale, 2002; Renault et al., 2002).

The bark of host trees, in particular lodgepole pine (*Pinus contorta*), provides little buffer from cold to *D. ponderosae* living within the phloem (e.g. inner bark). Microhabitat temperatures can drop below −35 °C in the middle of winter and *D. ponderosae* has no specific behaviorial traits that allow it to escape from cold. Instead, it supercools to temperatures well below microhabitat temperatures (Bentz and Mullins, 1999), suggesting that SCPs are indeed a measure of maximum cold hardiness in this species. Supercooling capacity fluctuates seasonally and annually, and is highly variable within and among populations (Yuill, 1941; Bentz and Mullins, 1999). While no mortality was observed in larvae exposed for short time periods to temperatures slightly above the measured SCP, increased duration at subzero temperatures can cause mortality in *D. ponderosae* (Safranyik and Linton 1998), and further investigation into the ecological role of SCPs in *D. ponderosae* is needed.

In freeze-avoiding insects, Bale (2002) recognizes two distinct processes during cold hardening: (1) ridding the body of ice-nucleating agents (such as food in the gut and large proteins in the insect's hemolymph and tissues) and (2) accumulating cryoprotectant substances such as polyhydric alcohols (polyols) and antifreeze proteins (Lee and Denlinger, 1991; Duman, 2001). Removal of ice-nucleating substrates can depress an insect's SCP by 10–20 °C, and accumulation of colligative polyols and antifreeze proteins can depress the SCP by an additional 10 °C (Sømme, 1982; Duman et al., 1991). Glycerol is the most abundant antifreeze polyol in *D. ponderosae* (Sømme, 1964; Bentz and Mullins, 1999). Cessation of feeding and voiding the gut in the fall is a seasonal response that may be mostly irreversible in the short term. However, the remaining processes such as control of the concentration of intra- and extra-cellular ice-nucleating substances or the synthesis and catalysis of antifreeze substances seem to be under immediate control of temperature and thus may vary on a much shorter time scale (Kelleher et al., 1987; Lee et al., 1987; Bale, 2002; Danks, 2005).

Using data from early studies on *D. ponderosae* cold tolerance (Wygant, 1940; Yuill, 1941; Sømme, 1964), Safranyik et al. (1975) developed a model to predict the probability of outbreaks of this insect in British Columbia. In this model, −40 °C is the lethal temperature below which outbreaks come to an abrupt end due to extensive winter mortality. Because cold tolerance and cryoprotectant accumulation are both functions of low-temperature acclimation, a more realistic model of survival would describe the dynamic effect of temperature on the insect's supercooling capacity.

While it is clear that modeling the detailed processes of cold hardening would require far more physiological data than are currently available for *D. ponderosae*, our objective was to develop a process-based model of its cold tolerance that follows the general physiological principles of cold hardiness outlined above.

2. Methods

This cold tolerance model was developed from the data set of Bentz and Mullins (1999), consisting of time series of hourly phloem temperatures and measurements of the SCP of individual larvae from six locations sampled throughout the year in 1992–93 and 1994–95. Individual SCP measurements of 1216 larvae from the four *D. ponderosae* instars were available for analysis. One sample (14 January 1993, Logan, UT) contained only 2 individuals and was dropped, leaving 41 samples. The median SCP in each sample (LT_{50}) was determined and one frequency histogram of the SCP data, pooled over sites, was constructed for each month in which some sampling took place.

2.1. The model

The monthly distributions of SCPs observed by Bentz and Mullins (1999) (left column, Fig. 1) suggest that *D. ponderosae* larvae spend the year in one of three states on the basis of their SCP, and that the proportion of the population in each state shifts during the course of the year:

State 1: summer state, feeding larvae that we refer to as non-cold-hardened.

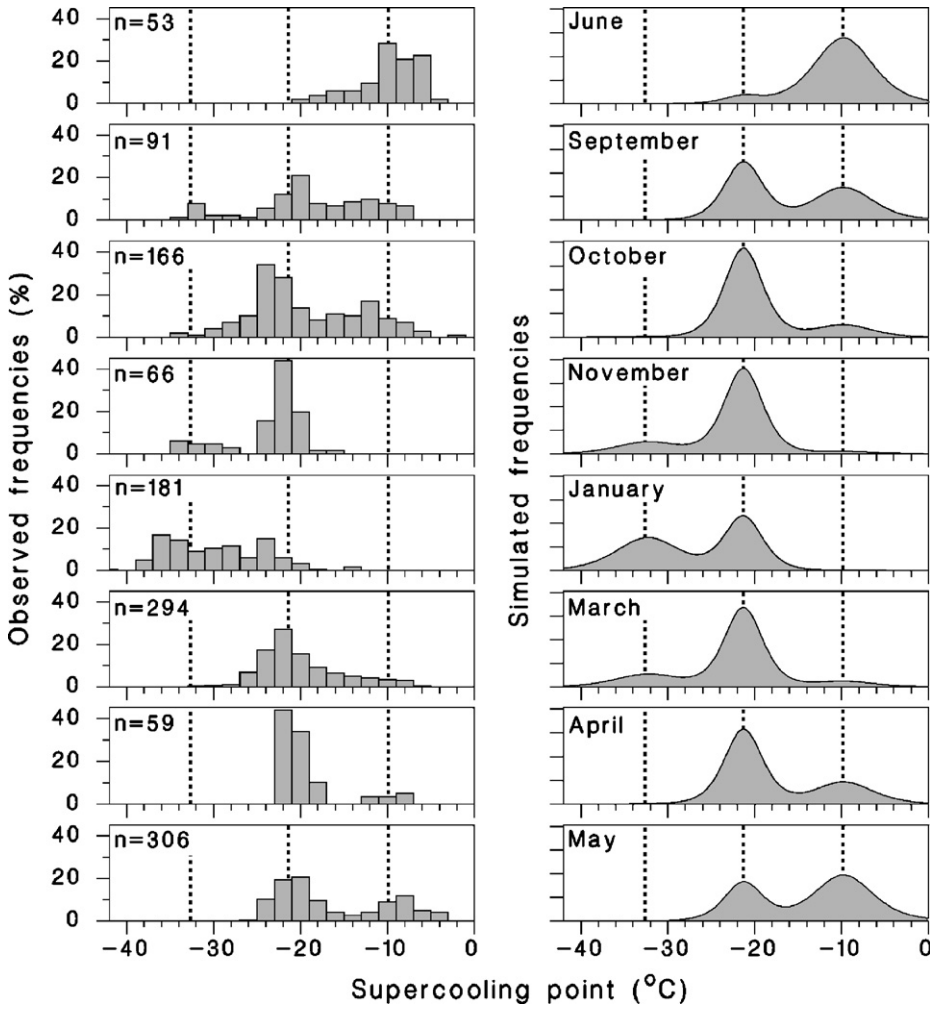

Fig. 1. Left column: frequency histograms of observed supercooling points (2 °C classes) among mountain pine beetle larvae collected at various times of the year from 7 locations in Utah and Wyoming in 1992–93 and 1994–95 (data of Bentz and Mullins, 1999). Right column: average monthly simulated distribution of SCPs at the same locations (from 1971 to 2000 normals). Vertical dotted lines indicate the median SCP in each of three different cold-tolerance states distinguished by the model.

State 2: fall and spring state, non-feeding larvae that have eliminated ice-nucleating substances (the first phase of cold hardening).
State 3: deep winter state, larvae containing high concentrations of polyols and antifreeze proteins.

In June, the vast majority of larvae are in State 1. In late September and into October the larvae void their gut and enter State 2. In October, larvae in States 1–3 may be present simultaneously in the population. In November, no larvae are left in State 1, and a higher proportion is in State 3. In January, the majority of larvae are in State 3. In March and April, most of the population returns to State 2, then to State 1 that represents more than 50% of individuals collected in May.

A family of logistic probability distribution functions was used to describe the distribution of SCP in each state:

$$X_i = \frac{\exp(-(T-\alpha_i)/\beta_i)}{\beta_i\{1+\exp[-(T-\alpha_i)/\beta_i]\}^2}, \quad (1)$$

where i is the state (1, 2 or 3), T is supercooling temperature (°C), α_i is the mean SCP in State i (or its median because the logistic distribution is symmetrical), and β_i is the distribution's spread in State i. We fitted the three distributions simultaneously to the pooled frequency histogram of individual SCP data by non-linear logistic regression (maximum likelihood estimation).

Transition of the population between states is controlled by a cascade of cold-hardening processes that determine the population's state of cold hardening C, conveniently defined here as a quantity ranging from 0 (no hardening) to 1 (maximum hardening). Also for convenience but without loss of generality, we chose $C = 0.5$ as representing the point at which 100% of individuals in the population are in State 2. Individuals remain in State 1 as long as $C < \lambda_0$. Once C exceeds λ_0 an increasing proportion enter State 2 (up to 100% at $C = 0.5$), and then State 3, up to 100% when $C \geqslant \lambda_1$ (Fig. 2a). Thresholds λ_0 and λ_1 are parameters to be estimated.

In the model, daily changes in C, the population's cold-hardening status, result from two simultaneous antagonistic processes. For simplicity, we hereafter refer to these as gain (G) and loss (L) of cold tolerance resulting from a series of behavioral and physiological processes such as cessation and resumption of feeding, elimination and accumulation of nucleating agents and of cryoprotectants (including glycerol). We assume that, under conditions that lead to gain of cold hardening, C approaches 1 monotonically and asymptotically, i.e. $dC/dt \propto (1-C)$. Similarly, under conditions that lead to loss of cold hardening, C drops asymptotically and monotonically to 0, i.e. $dC/dt \propto -C$. Thus:

$$dC/dt = (1 - C)G - CL. \tag{2}$$

Although in some insects gain and loss of cold tolerance take place at relatively warm temperatures, in the range of 0 to 5 °C (Storey and Storey, 1986), in others they occur at temperatures well below 0 °C (Kelleher et al., 1987; Muise and Storey, 1999). In our model, the effects of temperature on processes G and L are modeled as bell-shaped temperature responses, much the same as used to describe other developmental responses to temperature in poikilotherms (Régnière and Logan, 2003). To maintain parsimony in terms of numbers of parameters, and because we had no basis on which to choose more complex (e.g. asymmetrical) response functions, we again chose logistic probability distribution functions:

$$G = R\rho_G \frac{\exp[-(\tau - T_G)/\sigma_G]}{\sigma_G\{1 + \exp[-(\tau - T_G)/\sigma_G]\}^2} \tag{3}$$

and

$$L = R\rho_L \frac{\exp[-(\tau - T_L)/\sigma_L]}{\sigma_L\{1 + \exp[-(\tau - T_L)/\sigma_L]\}^2}, \tag{4}$$

where τ and R are daily mean and range of phloem temperature (°C). Parameters σ_G and σ_L are spread factors, ρ_G and ρ_L are the maximum rates at optimum temperatures T_G and T_L (Fig. 2b). We included daily temperature range R as predictor in Eqs. (3) and (4) to reflect the results of laboratory experiments suggesting that constant temperatures are not conducive to cold acclimation in *D. ponderosae* (BB unpublished data).

It has been shown that, at least in some species, the temperature range at which gain and loss occur drops as cold hardening intensifies (Merivee, 1978; Baust et al., 1982). In the model, optima T_G and T_L are allowed to shift with C, so the population's response to temperature varies during the year (Fig. 2c):

$$T_G = \mu_G + \kappa_G C \tag{5}$$

and

$$T_L = \mu_L + \kappa_L C. \tag{6}$$

To model the irreversibility of passage from States 1 to 2 in the fall due mostly to seasonal feeding cessation, we assume that loss of cold hardening prior to December 31st only occurs when $C > 0.5$. Thus, the bulk of the population remains in States 2 or 3 during that period and does not revert to the summer feeding state until the end of winter. The state of cold-hardening processes C_t on day t is the daily summation:

$$C_{t+1} = \begin{cases} \sum_{t_0}^{t}(1 - C_t)G_t & \text{while } t < t_1 \text{ and } C < 0.5, \\ \sum_{t_0}^{t}[(1 - C_t)G_t - C_t L_t] & \text{while } t \geqslant t_1 \text{ or } C \geqslant 0.5, \end{cases} \tag{7}$$

where t_0 is August 1st (when $c = 0$) and t_1 is December 31st.

Because of the symmetry of the SCP distributions in the three states, the median SCP of the population (LT_{50}) is a linear combination of the median of each distribution multiplied by the proportion p_i of the population in each State i:

$$LT_{50} = \sum_i p_i \alpha_i, \tag{8}$$

where α_i are as defined in Eq. (1), and the proportions p_i are:

$$\begin{aligned} p_1 &= \max\{0, \min[(0.5 - C)/(\lambda_0 - C)]\}, \\ p_3 &= \max\{0, \min[(C - 0.5)/(C - \lambda_1)]\}, \\ p_2 &= 1 - (p_1 + p_3). \end{aligned} \tag{9}$$

To calculate cold-injury mortality, we assume that individuals in a population are more or less sensitive to cold relative to their con-specifics, and that their relative position on the cold susceptibility scale remains the same throughout their life. Thus, individuals that survive a given cold event are not redistributed over the entire distribution of cold resistance (survival on successive days is not independent). Insects surviving to day t are those that are most cold resistant. Therefore, rather than computing survival as a serial product as if the daily survival probabilities were independent, this model computes it as the minimum probability of survival from the onset of simulation t_0 to day t. Thus mortality is simulated as a selective rather than a cumulative process. There is evidence that such selective mortality occurs in the southern pine beetle *Dendroctonus frontalis* (Tran et al., 2007) and in the red mite *Panonychus ulmi* (MacPhee, 1961). For any given minimum daily temperature T_t, on day t, survival is the lowest of the previous day's survival and probability of survival in all three cold-hardening states, weighted by the proportion of the population in each state:

$$p(survival)_t = \min\left(p(survival)_{t-1}, \sum_i \frac{p_i}{1 + e^{-(T_t - \alpha_i)/\beta_i}}\right), \tag{10}$$

where p_i are calculated with Eq. (9) and α_i and β_i, are as defined in Eq. (1). With this approach, it is simple to

determine the proportion of individuals that die in each of the three states.

2.2. Parameter estimation

A simulated annealing iterative optimization algorithm was used to minimize the sum of squared deviations (weighted for sample size) between observed and predicted median SCP by varying parameters (Goffe et al., 1994). The simulated annealing method is better at finding the absolute optimum than ordinary iterative non-linear algorithms (Chuine et al., 1998). Two logical constraints were used to restrict parameter search space. The first ensured that gain always proceeds at lower temperatures than loss, for physiological consistency (i.e. $\mu_G+\kappa_G<\mu_L+\kappa_L$). The second limited the rate of change dC/dt to stabilize the cold-hardening process, particularly in the fall when temperatures often warm up considerably for brief periods. While some limit was necessary to keep parameters within a reasonable range, the estimation process was not sensitive to the exact value of this criterion. We set $|dC/dt|<0.2$, which implies that the fastest that cold tolerance can be totally lost or gained is 5 days.

Model bias was tested by weighted linear regression between observed and predicted values of LT_{50}, and model precision was judged by goodness-of-fit (R^2) and root mean square error (RMSE). In addition, a jackknife cross-validation procedure was used to determine the predictive power of the model and to estimate the standard error of parameter values (Efron and Gong, 1983). Potential model performance differences due to site or generation year were tested by analysis of variance of model residuals (using site as a random factor and generation year as a fixed factor, weighted by sample size).

2.3. Model behavior

The model was run for the location of the six study sites in Bentz and Mullins (1999). Each run was replicated 30 times, each replicate using a stochastically different daily minimum and maximum air temperature time series generated by the method of Régnière and St-Amant (2007) from normals (1971–2000) of the nearest NCDC Coop Summary of the Day TD3200 (http://www.ncdc.noaa.gov/) weather station, after compensation for local thermal gradients (Régnière, 1996). Phloem temperatures (τ_{min}, τ_{max} in °C) were obtained by modifying daily minimum and maximum air temperatures (T_{max}, T_{min}) according to Bolstad et al. (1997):

$$\tau_{max} = T_{max} + \Delta_{max}\frac{(T_{max}-T_{min})}{24.4}, \quad (11)$$

where $\Delta_{max} = 3.25$ is an average between north and south sides of the bole, and

$$\tau_{min} = T_{min} + 1.8. \quad (12)$$

Daily minimum phloem temperature, LT_{50}, the daily probability of mortality, and the overall probability of survival from cold exposure were computed for each site.

We also obtained daily minimum and maximum air temperature records from 10 stations in the Sawtooth National Recreational Area in central Idaho for the period 1980–2006: 5 from the NCDC Coop Summary of the Day TD3200 database for the period 1980–2006, and 5 from the SNOTEL high-elevation weather network (http://www.wcc.nrcs.usda.gov/snow/) covering the period 1988–2006 (Table 1). The cold-tolerance model was run on data from each weather station. *D. ponderosae* generations typically occur from mid-summer to mid-summer. Therefore, for each year from August in calendar year $t-1$ to August in year t, minimum winter phloem temperature estimated by Eq. (12) was recorded, and minimum LT_{50} and overwinter survival were computed with the model. Trends over time for the three variables were tested with a General Linear Models procedure using site as a factor, year as a covariate, and testing the site × year interaction to detect differences in slope. The $\sin^{-1}(\sqrt{p})$ transformation was applied to the survival probabilities.

Table 1
Sources of daily minimum and maximum air temperature data from the Sawtooth National Recreational Area of central Idaho, and predicted average survival from cold injury

Station name	Latitude °N	Longitude °E	Elevation (m)	Years		Survival ±SE
				First	Last	
NCDC sites						
Lowman	44.083	−115.619	1194	1981	2005	0.529±0.023
Fairfield Ranger Station	43.342	−114.790	1543	1980	2006	0.353±0.043
Ketchum Ranger Station	43.684	−114.360	1795	1980	2001	0.556±0.034
Chilly Barton Flat	43.977	−113.829	1908	1980	2006	0.513±0.027
Stanley	44.217	−114.931	1911	1980	2006	0.184±0.034
SNOTEL sites						
Banner Summit	44.300	−115.233	2145	1988	2006	0.510±0.034
Galena	43.883	−114.667	2267	1988	2006	0.547±0.023
Dollarhide Summit	43.600	−114.667	2566	1988	2006	0.698±0.044
Galena Summit	43.850	−114.717	2576	1988	2006	0.613±0.042
Vienna Mine	43.800	−114.850	2731	1988	2006	0.610±0.036

Table 2
Final model parameter values and their standard errors

Equation	Symbol	Definition	Value	SE
(1)	α_1	Mean SCP in State 1	−9.8	0.3
	β_1	Spread of SCP in State 1	2.26	0.22
	α_2	Mean SCP in State 2	−21.2	0.1
	β_2	Spread of SCP in State 2	1.47	0.09
	α_3	Mean SCP in State 3	−32.3	0.6
	β_3	Spread of SCP in State 3	2.42	0.37
(3)	ρ_G	Maximum gain rate	0.311	0.023
	σ_G	Spread of the gain temperature response	8.716	0.324
(5)	μ_G	Optimum gain temperature at $C = 0$	−5.0	0.7
	κ_G	Optimal gain temperature vs. C	−39.3	1.9
(4)	ρ_L	Maximum loss rate	0.791	0.218
	σ_L	Spread of the loss temperature response	3.251	0.207
(6)	μ_L	Optimum loss temperature at $C = 0$	33.9	0.7
	κ_L	Optimal loss temperature vs C	−32.7	2.6
(9)	λ_0	Threshold C for State 1–2 transition	0.254	0.011
	λ_1	Threshold C for State 2–3 transition	0.764	0.018

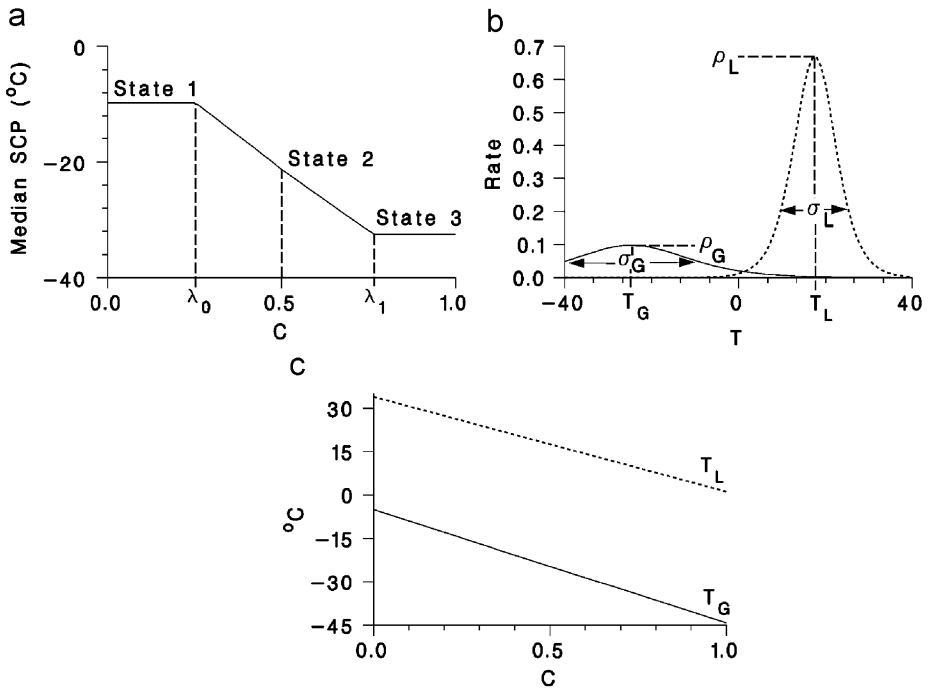

Fig. 2. (a) Change in median SCP and the degree of cold hardening C as the population shifts from State 1 in summer to State 3 in mid winter (Eqs. (8) and (9)). (b) Rate of gain (G) and loss (L) of cold tolerance during the cold hardening process, with $C = 0.5$ and a 15 °C daily temperature range (Eqs. (3) and (4)). (c) Relationship between optimal temperatures for gain (T_G) and loss (T_L) and C, where $C = 1$ is the fully cold hardened state (Eqs. (5) and (6)).

3. Results

3.1. Parameter estimation

The maximum likelihood estimates of the parameters of Eq. (1) describing the frequency distribution of SCP are listed in Table 2 (Fig. 3; goodness-of-fit $R^2 = 0.97$). The remaining 10 model parameters were estimated by simulated annealing from the median SCP observations of Bentz and Mullins (1999): four (ρ_G, ρ_L, σ_G and σ_L) that describe the cold-hardening temperature responses in Eqs. (3) and (4), four (μ_G, μ_L, κ_G and κ_L) that relate optimum temperatures to the state of the cold-hardening processes C in Eqs. (5) and (6) and two (λ_0 and λ_1) that control the shift between States 1–3 as a function of C in Eq. (9). Final parameter estimates and their standard

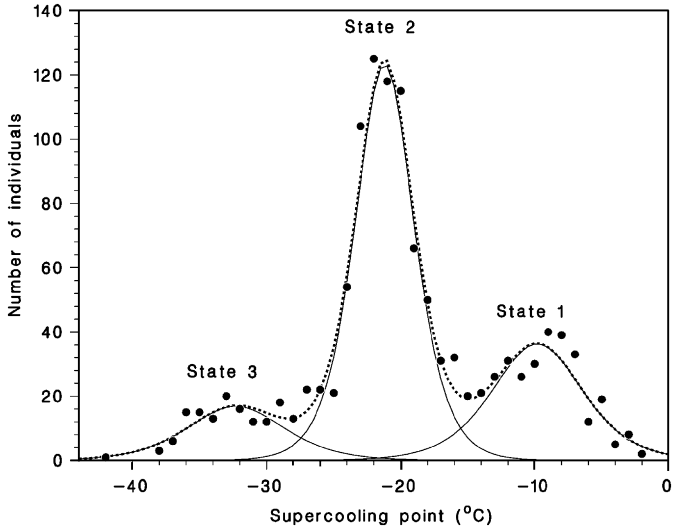

Fig. 3. Pooled frequency of all larval SCP (1 °C classes). Eq. (1) (solid lines: for each state; dotted line: combined) fitted by non-linear logistic regression to pooled observations of SCP among *Dendroctonus ponderosae* larvae (●).

deviations computed from cross-validation are presented in Table 2 (Fig. 2).

Predicted LT_{50} values varied considerably throughout the year, among sites and between generation years (i.e. 1992–93 and 1994–95), in response to input daily temperatures, and mimicked accurately the observed LT_{50} (Fig. 4). The correlation (unweighted) between observed and predicted LT_{50}'s was 0.904 (goodness-of-fit $R^2 = 0.817$; RMSE = 3.4 °C). The intercept of the weighted regression between observed and predicted values was 0.86 ± 1.97, not significantly different from zero ($t = 0.44$; $P = 0.663$) and the slope was 1.04 ± 0.10, not significantly different from unity ($t = 0.45$; $P = 0.659$). There was no significant effect of site ($F_{3,36} = 0.62$, $P = 0.609$) or generation ($F_{1,36} = 1.89$, $P = 0.178$) on deviations between model and observations. In cross validation, the model retained high predictive capacity ($R^2 = 0.744$, RMSE = 3.99 °C), and no bias (mean ± SEM observed and predicted LT_{50}: -18.3 ± 1.2 °C vs. -19.0 ± 0.9 °C; $t = 0.46$, $P = 0.646$). Thus, the model describes accurately the spatial and temporal patterns of variation in SCP among *D. ponderosae* larvae collected by Bentz and Mullins (1999).

3.2. Model behavior

Predicted average LT_{50} curves remained 10–15 °C under the average minimum temperature throughout the year at the 6 sites from the Bentz and Mullins (1999) study (based on 30-year normals 1971–2000) (Fig. 5). However, average annual survival varied among locations from 0.4 to 0.7 because daily minimum temperatures often dropped below the median SCP of the populations, even at the warmest location (Cedar). Most of the mortality predicted by the model occurred from early November to late February (Fig. 5). The partition of mortality between the three cold-tolerance states distinguished by the model varied considerably between sites. Mortality in State 1 was most important in the four coldest sites, and always occurred before November. Mortality in State 3 (the most cold tolerant) was negligible at Cedar, the warmest site, and highest at Galena, the coldest. In all cases, the bulk of mortality occurred in intermediate State 2. The model predicts little mortality later than March. These mortality patterns are consistent with the seasonal changes in the proportion of each site's population in the three cold-tolerance states (Fig. 5). At Cedar, the proportion reaching State 3 peaked at 0.3 in January. At Galena, the maximum was near 0.9 as compared with 0.65–0.7 at the other four sites. The model predicted much susceptibility to cold injury from September to November, as a result of the population hovering between States 1 and 2 during that time. The predicted distribution of SCPs in the populations, averaged over the six study sites for the middle of each month, corresponds well with the observed SCP distributions (right column, Fig. 1).

Daily observed maximum and minimum temperatures from the past 18–25 years at 10 sites in the Sawtooth National Recreation Area of central Idaho were used to predict annual *D. ponderosae* cold tolerance and survival. Extreme annual minimum phloem temperature among the 10 stations increased significantly from 1980 to 2006, by 0.34 °C year^{-1} (Fig. 6a; $F_{1,197} = 42.07$, $P < 0.001$). There were no significant site differences in either average extreme minimum temperature ($F_{9,197} = 0.46$, $P = 0.902$) or slope of the time trend ($F_{9,197} = 0.46$, $P = 0.898$). In response, the value of minimum LT_{50} predicted by the model also increased significantly over time, by 0.14 °C year^{-1} (Fig. 6a; $F_{1,197} = 79.73$, $P < 0.001$), and there were significant differences among weather stations in both average minimum LT_{50} ($F_{9,197} = 4.35$, $P < 0.001$) and slope of the time-trend ($F_{9,197} = 4.40$, $P < 0.001$). There was no significant change of minimum LT_{50} over time at Lowman, Chilly Barton Flat or Stanley. As a consequence of the slow and site-specific rise in minimum LT_{50} compared with the fast and generalized rise observed in minimum temperatures during this time period, the probability of survival from cold increased significantly over time, by 0.013 year^{-1} on average (Fig. 6b; $F_{1,197} = 50.68$, $P < 0.001$). The average probability of survival varied significantly among sites ($F_{9,197} = 3.56$, $P < 0.001$). Survival was particularly low at Stanley and high at Dollarhide Summit (Table 1). Survival changed at significantly different rates at a few sites (site × year interaction: $F_{9,197} = 3.64$, $P < 0.001$): there was a slight decrease at Lowman ($T = -2.25$, $P = 0.026$), but nearly twice the average increase in survival at Stanley ($T = 3.12$, $P = 0.002$).

The distribution of survival through the fall, winter and spring months, and the timing of peak daily mortality were compiled for the model runs using observed daily minimum and maximum temperature records from the 10 SNRA sites in Idaho. Survival ranged from 0.004 to 0.89,

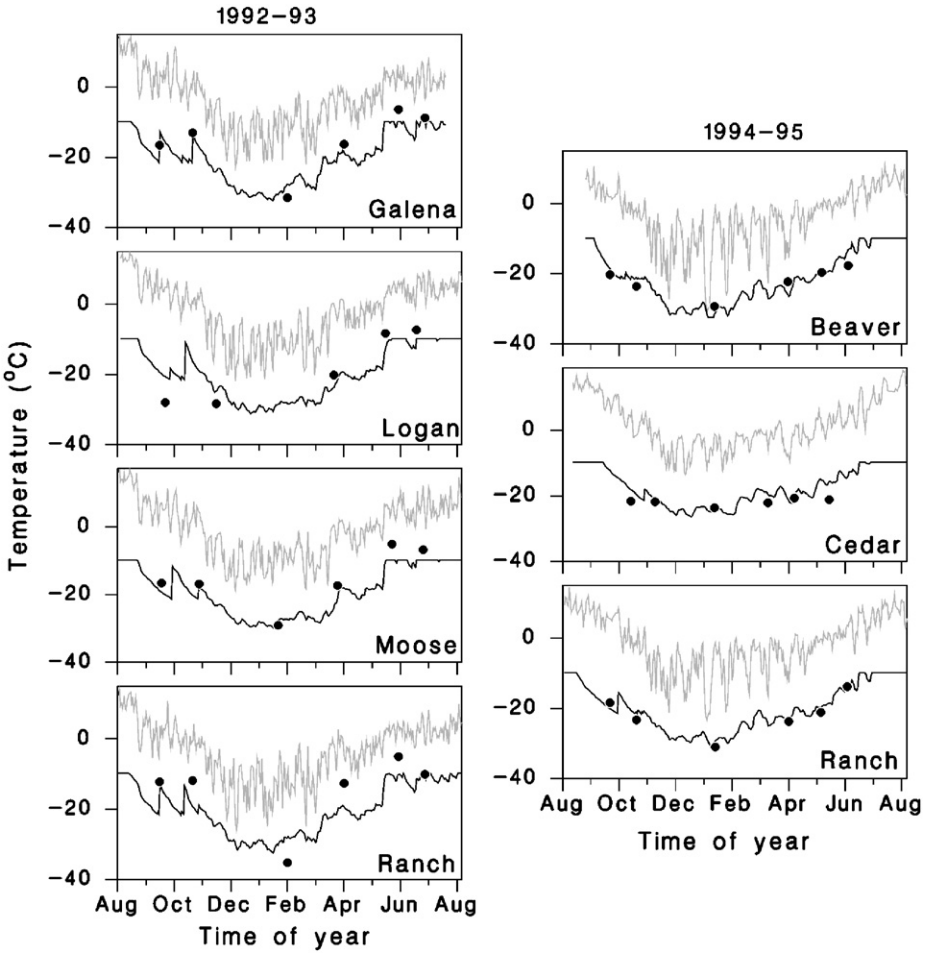

Fig. 4. Observed daily minimum phloem temperature (pale lines), observed median super-cooling points (●), and predicted median lethal temperature (LT_{50}, dark lines). Left column: 1992–93 generation. Right column: 1994–95 generation. Observations from Bentz and Mullins, 1999.

averaging 0.49 ± 0.22 (SD, $n = 217$; Fig. 7a). Most mortality occurred in November–February, with a sharp peak in December (Fig. 7b). The predicted minimum LT_{50} remained well below the extreme minimum winter temperature, down to about $-32\,^{\circ}C$ (solid line in Fig. 7c). On average, the relationship between low minimum temperatures and survival was nearly linear, with 0 survival at $-40\,^{\circ}C$, the low lethal temperature used by Safranyik et al. (1975), and 1.0 survival at $-10\,^{\circ}C$ (solid line in Fig. 7d). However, the model predicts a wide scatter around this average survival trend, which is due to details of the timing of extreme temperature relative to the daily fluctuations of LT_{50}. Laboratory observations of low temperature-induced mortality of small (1st and 2nd instar) and large (3rd and 4th instar) *D. ponderosae* larvae at several constant temperatures are illustrated in Fig. 7d for comparison (from Table 4 in Safranyik and Linton, 1998).

4. Discussion

When all mortality factors are considered, *D. ponderosae* survival within a generation varies with population phase and tree diameter, and ranges between 1.4 and 2.5% (Amman and Cole, 1983; Safranyik and Carroll, 2006). While many factors are known to influence this insect's survival including food quantity and quality, resinosis, parasites and predators, inter- and intra-specific competition, and host drying (Amman, 1972; Amman and Cole, 1983; Langor, 1989), cold-induced mortality has been considered the most important factor driving *D. ponderosae* population dynamics (Safranyik, 1978; Cole, 1981; Langor, 1989).

The highly dynamic nature of cold tolerance in this species has been known for a long time (Wygant, 1940; Yuill, 1941) and is in direct contrast with two other *Dendroctonus* species that show little change in cold hardiness despite alterations in acclimation conditions (Yuill, 1941; Lombardero et al., 2000). Mountain pine beetle SCP and associated cold-induced mortality fluctuate throughout a generation, with the highest SCPs prior to and following winter (Yuill, 1941; Bentz and Mullins, 1999). *D. ponderosae* cold hardiness is dictated by the local temperature regime, and because of this direct connection between cold tolerance and temperature, both across geographic locations and within a developmental season

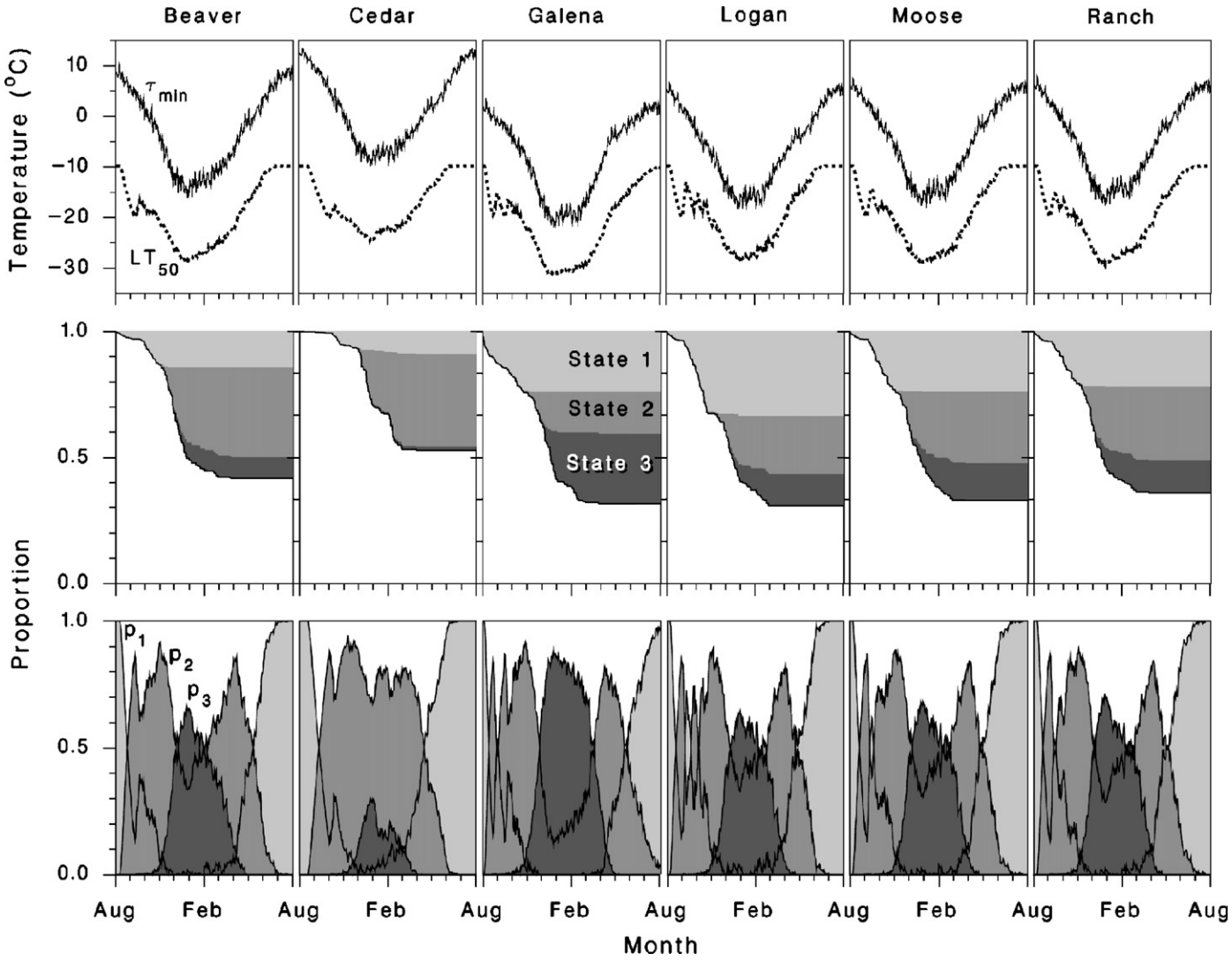

Fig. 5. Results of simulations from normals at the 6 sites of Bentz and Mullins (1999). Top row: average daily minimum phloem temperature and LT_{50}. Middle row: average daily probability of survival, with shades representing mortality in each state (State 1: pale, State 2: intermediate, State 3: dark). Bottom row: average proportion of the populations in States 1, 2 and 3 (same shading as middle row).

at the same location, merely assigning a constant mortality inducing temperature for all populations at all times is an oversimplification. Our mechanistic cold tolerance model describes the insect's larval survival as the result of a dynamic and reversible cold-acclimation process driven by fluctuations of daily temperature (mean and range). Further experimental work is needed to verify that daily range is actually an important determinant of acclimation in *D. ponderosae*. However, inclusion of this variable in the model did significantly improve fit.

This cold-tolerance model predicts the SCP distribution of a population as a function of daily changes in the temperature-dependent processes leading to gain and loss of cold tolerance. It is based on the changing proportion of individuals in a population in each of three distinct states: (1) a non cold-hardened feeding state (i.e. no elimination of ice-nucleating substances), (2) an intermediate state in which insects have ceased feeding, voided their gut content and eliminated as many ice-nucleating agents as possible from the body, and (3) a fully cold-hardened state where insects have accumulated a maximum concentration of cryoprotectants (e.g. glycerol). Shifts in the proportion of individuals in each state occur in response to the driving variables influencing the opposite rates of gain and loss of cold hardening, in part through synthesis and catalysis of cryoprotectants.

Eight of the 10 model parameters describe the complex and dynamic relationship between temperature and the rates of cold tolerance gain and loss. The optimal temperatures predicted by Eqs. (5) and (6) may seem extreme, with maximum gains at $-45\,^{\circ}C$ and maximum losses at $>30\,^{\circ}C$ (Fig. 2c). However, it must be kept in mind that these rates are also modulated by the state of cold hardening C through Eq. (2). The resulting net rate of change of cold tolerance (dC/dt) is thus a function of both temperature and C. With the parameter estimates obtained in this study, maximum gain of cold tolerance actually occurs at about $-10\,^{\circ}C$, when the state of cold tolerance

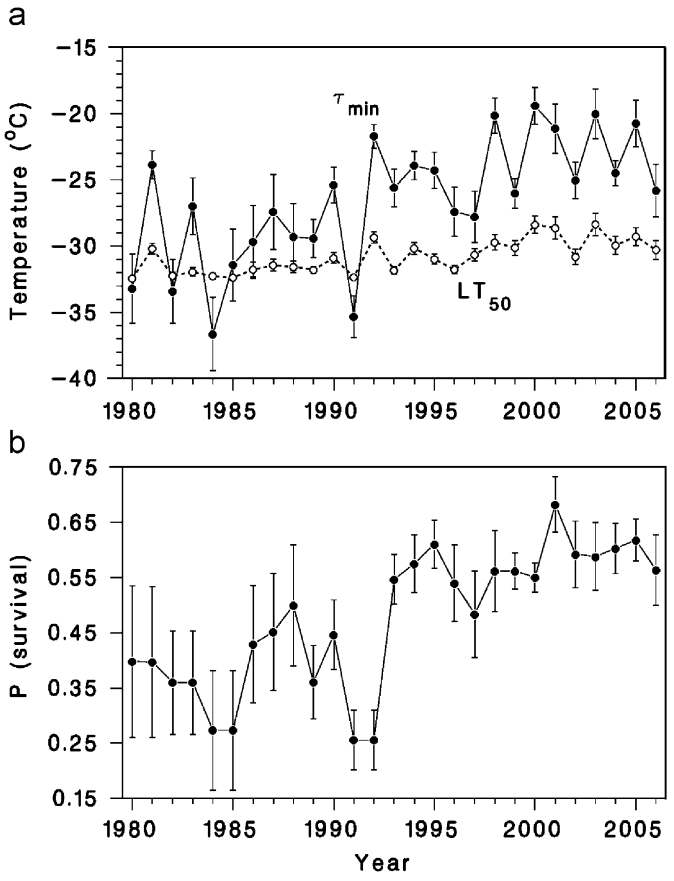

Fig. 6. Results of model runs from daily 1980–2006 temperature records at 10 stations in the Sawtooth National Recreation Area of central Idaho (see Table 1 for locations). Annual average (±SEM) (a) extreme minimum phloem temperature, lowest LT_{50}, and (b) *D. ponderosae* larval survival from cold exposure.

$C \rightarrow 0$, and maximum loss occurs at about 5 °C, when $C \rightarrow 1$ (Fig. 8). In addition, gain rates are much lower than loss rates and the optimum temperature of gain always remains lower than that of loss while the optimum temperatures of the two processes drop by more than 30 °C with increasing *C*. The temperature range of the gain component is broad, while that of the loss component is narrow (Fig. 8). Low temperature exposure has indeed been found to initiate and maintain cryoprotectant pools (Storey and Storey, 1983; Rojas et al., 1983; Rickards et al., 1987), while the transfer from cold to warm temperatures rapidly stimulates cryoprotecant loss in many insects (Storey and Storey, 1983; Pio and Baust, 1988).

The level of cold-induced mortality predicted by the model and its relation to extreme winter temperature is in good agreement with a range of field and laboratory observations for *D. ponderosae* (Yuill, 1941; Cole, 1981; Safranyik and Linton, 1998; Bentz and Mullins, 1999). Recent comparisons of cold tolerance of populations from different geographical areas suggest that selection for increased cold hardiness in northern and high elevation zones has occurred in a variety of insect species (Kukal and Duman, 1989; Tanaka, 1996; Shintani and Ishikawa, 1999; Chen and Kang, 2004). Genetic differences in *D. ponderosae* developmental parameters among geographically separated populations have been found (Bentz et al., 2001). Similar regional adaptations of cold tolerance may also exist in this species (BB unpublished data). At this point it is not clear whether our model can describe the full range of responses, or if its parameter values are limited to the part of the insect's range covered by the Bentz and Mullins (1999) study. The southernmost location in that study was Cedar, UT (37°37′N, 2620 m), and the northernmost was Ranch, ID (44°7′N, 2061 m), representing only 6°30′ in the center of the 25° latitudinal range of the insect's current distribution in western North America.

Our model predicts that cold tolerance of *D. ponderosae* varies within a season, among seasons, and among geographic locations depending on local climate. This variability is an emergent property of the model, and has important implications for understanding the insect's response to seasonal fluctuations in temperature, as well as population response to climate change. Cold hardening can be a metabolically expensive process (Storey and Storey, 1991). Model predictions suggest that while the capacity for increased cold hardening is inherent, the current temperature profile may dictate the physiological effort expended to supercool. This was observed in the predicted increase in LT_{50} over the recent decade in the Sawtooth National Recreation Area in response to an even more pronounced increase in extreme minimum temperatures as a result of recent climate warming. This warming trend has been correlated with insect survivorship and northward range expansion in a variety of species (Crozier, 2004; Battisti et al., 2005). Increases in minimum winter temperatures have been greater with global warming than increases in maximum summer temperatures (Easterling et al., 1997), and this trend is expected to continue. However, while an increase in minimum temperatures is undoubtedly influencing survival and expansion of *D. ponderosae* northward in British Columbia (Carroll et al., 2004), there are additional effects of climate on populations that are likely also playing a significant role.

Cold-induced mortality is but one of several major influences of climate on mountain pine beetle population dynamics. Temperature-driven development time is also an important contributor to population fitness. An intricate relationship between life-stage specific thresholds for development and seasonal temperature fluctuations are believed to maintain an adaptive seasonal synchrony in this non-diapausing insect (Bentz et al., 1991; Logan and Bentz, 1999). In particular, high temperature thresholds in the later life stages (e.g. 4th instar and pupa) are hypothesized as being one mechanism for maintaining synchrony and minimizing winter mortality (Powell et al., 2000; Jenkins et al., 2001).

Safranyik et al. (1975) proposed a risk model that includes developmental as well as winter-survival responses to temperature. The Safranyik model was used by Carroll et al. (2004) to study the impact of recent climatic warming

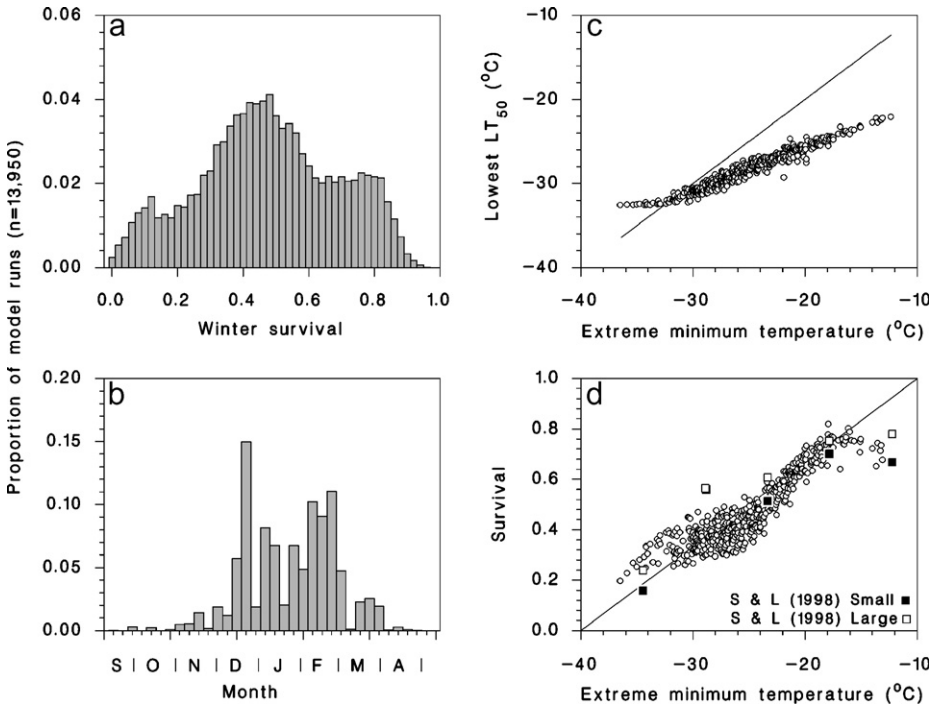

Fig. 7. Results of model runs from daily 1980 to 2006 temperature records at 10 stations in the Sawtooth National Recreation Area of central Idaho (see Table 1 for locations). (a) Distribution of p(survival) from individual runs ($n = 217$). (b) Distribution of timing of peak daily mortality (weekly classes). Relationship between extreme minimum temperature and (c) the lowest point reached by LT_{50} over the winter (solid line: equality), and (d) cold-exposure survival (Solid line: linear relationship from 0% survival at −40 °C and 100% at −10 °C; observations from Safranyik and Linton, 1998: ■, small larvae; □, large larvae).

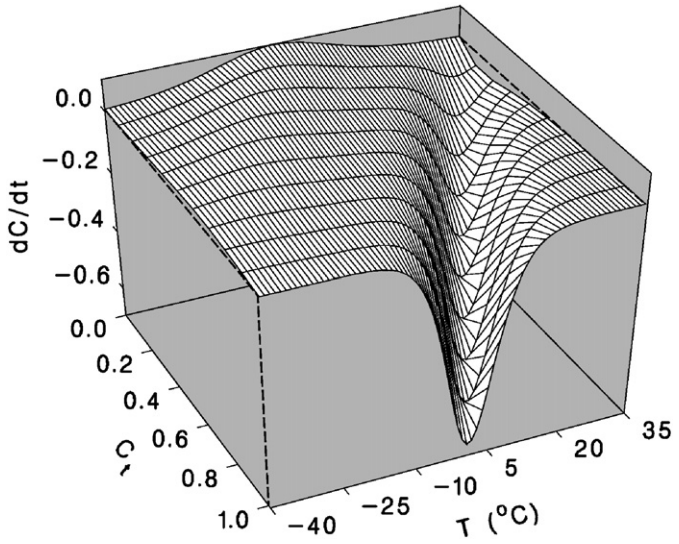

Fig. 8. Relationship between the rate of cold hardening as a function of temperature (°C) and the state of the cold hardening process (C_t). Eq. (2), with a 15 °C daily temperature range.

on *D. ponderosae* outbreaks in British Columbia, Canada. A detailed model of the insect's phenology was developed (Logan and Amman, 1986; Bentz et al., 1991) and applied to study the impact of temperature regimes on voltinism and synchrony (Logan et al., 1995, 1998; Logan and Bentz, 1999). This model, which does not include cold-induced mortality, has been used to interpret the influence of climate change on developmental timing, adaptive seasonality, population success and subsequent distribution in the USA (Logan and Powell, 2001; Logan et al., 2003; Hicke et al., 2006). Our simulations using daily temperature records from the Sawtooth National Recreation Area in central Idaho support the hypothesis that a warming trend since the early 1990s has led to increased *D. ponderosae* winter survival, perhaps contributing to the recent outbreak in this area (http://www.fs.fed.us/r1-r4/spf/fhp/aerial/index.html). In addition to a general warming trend, the annual pattern and range of temperature have also changed. A model analysis of mountain pine beetle phenology in the same area over the same years suggests that temperature influences on the insect's seasonality may also have contributed to the recent outbreak (Powell and Logan, 2005). Undoubtedly, both are important, and coupling the cold tolerance and phenology models will provide a powerful tool for evaluating climate change effects on *D. ponderosae* population success and range expansion.

Because Bentz and Mullins (1999) did not find significant differences in cold tolerance among the four larval instars of *D. ponderosae* on a given sample date, our model does not distinguish instars. However, Safranyik and Linton (1998) report finding a larger proportion of dead individuals in instars 1 and 2 than in instars 3 and 4. Shifts in activity induced by climate warming, both northward and

towards higher elevations, can lead to shifts in developmental timing (seasonality) and a concomitant diversification of the distribution of life stages exposed to low temperatures. To anticipate additional impacts of global warming on population success, experiments are needed to better quantify stage-specific cold tolerance, including lifestages not previously studied, with temperatures at or above SCPs. A better understanding of the cold-acclimation process, in particular the potential role of temperature fluctuations and the distinction between different processes of cold-hardening (cessation of feeding, elimination of nucleating agents, accumulation of polyols) is also needed.

It would be useful to develop a better microclimatic component to translate daily air minimum and maximum temperatures to phloem temperatures. Many *D. ponderosae* host tree species such as lodgepole pine provide little buffering to extreme winter temperatures, and minimum phloem temperatures are often the same as ambient air temperature in the middle of winter (Bentz, 1995). However, data of Tran et al. (2007) suggest that the buffering of minimum temperature provided by Eq. (12) from Bolstad et al. (1997) is probably too small.

Insect physiological processes are mediated by temperature in complex ways affecting a variety of important life history traits. Modeling the temperature-dependent processes involved in population overwintering success can be difficult due to the need for integration of climate effects through time. Recent attempts have focused on statistical fits of distributional models to survival and temperature data (Nedved et al., 1998; Carrillo et al., 2005) and the use of various combinations of survival, demographic and SCP data for estimating large scale patterns in insect population success (Bale, 1991; Virtanen et al., 1998; Ungerer et al., 1999; Tran et al., 2007). However, ours is the first mechanistic model that captures the dynamic relationship between temperature and cold hardiness. In a scenario of continued climate warming, novel situations will be presented to managers as insect populations respond to shifts in temperature. Model frameworks that integrate the effects of local climate through time will be particularly important for predicting within generation survival across the broad range of many ecologically and economically important insect species. Additionally, mechanistic models such as the one described here provide important tools for evaluating hypotheses concerning temperature-based physiological processes. In conjunction with the mountain pine beetle phenology model and the BioSIM landscape simulation system (Régnière, 1996; Régnière and St-Amant, 2007), our model will provide an important tool for hypothesis testing as well as forecasting *D. ponderosae* population survival under current and climate-change scenarios.

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References

Amman, G.D., 1972. Some factors affecting oviposition behavior of the mountain pine beetle. Environmental Entomology 1, 691–695.

Amman, G.D., 1973. Population changes of the mountain pine beetle in relation to elevation. Environmental Entomology 2, 541–546.

Amman, G.D., Cole, W.E., 1983. Mountain pine beetle dynamics in lodgepole pine forests. Part II: population dynamics. Gen. Tech. Rep. INT-145. USDA Forest Service, Intermountain Forest and Range Experiment Station Ogden, UT, 59p.

Bale, J.S., 1991. Implications of cold hardiness for pest management. In: Lee, Jr., R.E., Denlinger, D.L. (Eds.), Insects at Low Temperature. Chapman & Hall, London, pp. 461–498.

Bale, J.S., 2002. Insects and low temperatures: from molecular biology to distributions and abundance. Philosophical Transactions of the Royal Society of London B 357, 849–862.

Battisti, A., Stastny, M., Netherer, S., Robinet, C., Schopf, A., Roques, A., Larsson, S., 2005. Expansion of geographic range in the pine processionary moth caused by increased winter temperatures. Ecological Applications 15, 2084–2096.

Baust, J.G., Rojas, R.R., 1985. Review—insect cold hardiness: facts and fancy. Journal of Insect Physiology 31, 755–759.

Baust, J.G., Lee, R.E., Ring, R.A., 1982. The physiology and biochemistry of low temperature tolerance in insects and other terrestrial arthropods: a bibliography. Cryo Letters 3, 191–212.

Bentz, B.J., 1995. Ecological adaptations of the mountain pine beetle to cold temperatures. In: Hain, F.P., Salom, S.M., Ravlin, W.F., Payne, T.L., Raffa, K.F. (Eds.), Proceedings of the Joint IUFRO Conference: Behavior, Population Dynamics and Control of Forest Insects, Maui, HI, pp. 67–85.

Bentz, B.J., Mullins, D.E., 1999. Ecology of mountain pine beetle (Coleoptera: Scolytidae) cold hardening in the intermountain West. Environmental Entomology 28, 577–587.

Bentz, B.J., Logan, J.A., Amman, G.D., 1991. Temperature-dependent development of the mountain pine beetle (Coleoptera: Scolytidae) and simulation of its phenology. The Canadian Entomologist 123, 1083–1094.

Bentz, B.J., Logan, J.A., Vandygriff, J.C., 2001. Latitudinal variation in *Dendroctonus ponderosae* (Coleoptera: Scolytidae) development time and adult size. The Canadian Entomologist 133, 375–387.

Bolstad, P.V., Bentz, B.J., Logan, J.A., 1997. Modelling micro-habitat temperature for *Dendroctonus ponderosae* (Coleoptera: Scolytidae). Ecological Modelling 94, 287–297.

Carrillo, M.A., Koch, R.L., Burkness, E.C., Bennett, K., Ragsdale, D.W., Hutchison, W.D., 2005. Supercooling point of bean leaf beetle (Coleoptera: Chrysomelidae) in Minnesota and a revised predictive model for survival at low temperatures. Environmental Entomology 34, 1395–1401.

Carroll, A.L., Taylor, S.W., Régnière, J., Safranyik, L., 2004. Effects of climate change on range expansion by the mountain pine beetle in British Columbia. In: Shore, T.L., Brooks, J.E., Stone, J.E. (Eds.), Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre Information Report BC-X-399, Victoria, BC, pp. 223–232.

Chen, B., Kang, L., 2004. Variation in cold hardiness of *Liriomyza huidobrensis* (Diptera: Agromyzidae) along latitudinal gradients. Environmental Entomology 33, 155–164.

Cole, W.E., 1981. Some risks and causes of mortality in mountain pine beetle populations: a long-term analysis. Researches on Population Ecology 23, 116–144.

Chuine, I., Cour, P., Rousseau, D.D., 1998. Fitting models predicting dates of flowering of temperate-zone trees using simulated annealing. Plant, Cell and Environment 21, 455–466.

Crozier, L., 2004. Warmer winters drive butterfly range expansion by increasing survivorship. Ecology 85, 231–241.

Danks, H.V., 2005. Key themes in the study of seasonal adaptations in insects: patterns of cold hardiness. Applied Entomology and Zoology 40, 199–211.

Duman, J.G., 2001. Antifreeze and ice nucleator proteins in terrestrial arthropods. Annual Review of Physiology 63, 327–357.

Duman, J.G., Wu, D.W., Xu, L., Tursman, D., Olsen, M.T., 1991. Adaptations of insects to subzero temperatures. The Quarterly Review of Biology 66, 387–410.

Easterling, D.R., Horton, B., Jones, P.D., Peterson, T.C., Karl, T.R., Parker, D.E., Salinger, M.J., Razuvayev, V., Plummer, N., Jamason, P., Folland, C.K., 1997. Maximum and minimum temperature trends for the globe. Science 277, 364–367.

Efron, B., Gong, G., 1983. A leisurely look at the bootstrap, the jackknife, and cross-validation. American Statistician 37, 36–48.

Gehrken, U., 1984. Winter survival of an adult bark beetle *Ips acuminatus* Gyll. Journal of Insect Physiology 30, 421–429.

Goffe, W.L., Ferrier, G.D., Rogers, J., 1994. Global optimization of statistical functions with simulated annealing. Journal of Economic Entomology 60, 65–100.

Hicke, J.A., Logan, J.A., Powell, J., Ojima, D.S., 2006. Changing temperatures influence suitability for modeled mountain pine beetle outbreaks in the western United States. Journal of Geophysical Research 111, G02019.

Hodkova, M., Hodek, I., 1994. Control of diapause and supercooling by the retrocerebral complex in *Pyrrhocoris apterus*. Entomologia Experimentalis et Applicata 70, 237–245.

Horwath, K.L., Duman, J.G., 1986. Thermoperiodic involvement in antifreeze protein production in the cold hardy beetle *Dendroides canadensis*: implications for photoperiodic time measurement. Journal of Insect Physiology 32, 799–806.

Jenkins, J.L., Powell, J.A., Logan, J.A., Bentz, B.J., 2001. Low seasonal temperatures promote life cycle synchronization. Bulletin of Mathematical Biology 63, 573–595.

Kelleher, M.J., Rickards, J., Storey, K.B., 1987. Strategies of freeze avoidance in larvae of the goldenrod gall moth, *Epiblema scudderiana*: laboratory investigations of temperature cues in the regulation of cold hardiness. Journal of Insect Physiology 33, 581–586.

Knight, J.D., Bale, J.S., Franks, F., Mathias, S.F., Baust, J.G., 1986. Insect cold hardiness: SCPs and pre-freeze mortality. Cryo Letters 7, 194–203.

Kukal, O., Duman, J.G., 1989. Switch in the overwintering strategy of two insect species and latitudinal differences in cold hardiness. Canadian Journal of Zoology 67, 825–827.

Langor, D.W., 1989. Host effects on the phenology, development, and mortality of field populations of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae). The Canadian Entomologist 121, 149–157.

Lee Jr., R.E., 1989. Insect cold-hardiness: to freeze or not to freeze. BioScience 39, 308–313.

Lee Jr., R.E., 1991. Principles of insect low temperature tolerance. In: Lee, Jr., R.E., Denlinger, D.L. (Eds.), Insects at Low Temperature. Chapman & Hall, London, pp. 17–46.

Lee, Jr., R.E., Denlinger, D.L. (Eds.), 1991. Insects at low temperature. Chapman & Hall, London.

Lee Jr., R.E., Chen, C.-P., Denlinger, D.L., 1987. A rapid cold-hardening process in insects. Science 238, 1415–1417.

Logan, J.A., Amman, G.D., 1986. A distribution model for egg development in mountain pine beetle. The Canadian Entomologist 118, 361–372.

Logan, J.A., Bentz, B.J., 1999. Model analysis of mountain pine beetle (Coleoptera: Scolytidae) seasonality. Environmental Entomology 28, 924–934.

Logan, J.A., Powell, J.A., 2001. Ghost forests, global warming, and the mountain pine beetle (Coleoptera: Scolytidae). American Entomologist 47, 160–173.

Logan, J.A., Bolstad, P.V., Bentz, B.J., Perkins, D.L., 1995. Assessing the effects of changing climate on mountain pine beetle dynamics. In: Tinus, R.W. (Ed.), Interior West Global Change Workshop, Rocky Mountain Forest and Range Experiment Station, USDA Forest Service General Technical Report RM-GTR-262, pp. 92–105.

Logan, J.A., White, P., Bentz, B.J., Powell, J.A., 1998. Model analysis of spatial patterns in mountain pine beetle outbreaks. Theoretical Population Biology 53, 236–255.

Logan, J.A., Régnière, J., Powell, J.A., 2003. Assessing the impacts of global warming on forest pest dynamics. Frontiers in Ecology and the Environment 1, 130–137.

Lombardero, M.J., Ayres, M.P., Ayres, B.D., Reeve, J.D., 2000. Cold tolerance of four species of bark beetle (Coleoptera: Scolytidae) in North America. Environmental Entomology 29, 421–432.

MacPhee, A.W., 1961. Mortality of winter eggs of the European red mite *Panonychus ulmi* (Koch), at low temperatures, and its ecological significance. Canadian Journal of Zoology 39, 229–243.

Merivee, E., 1978. Cold-hardiness in Insects. Estonian SSR Academy of Science, Tallin, 188p.

Miller, L.K., Werner, R.A., 1987. Cold-hardiness of adult and larval spruce beetles *Dendroctonus rufipennis* (Kirby) in interior Alaska. Canadian Journal of Zoology 65, 2927–2930.

Muise, A.M., Storey, R.A., 1999. Regulation of glycogen synthetase in a freeze-avoiding insect: role in cryoprotectant glycerol metabolism. Cryo Letters 20, 223–228.

Nedved, O., Lavy, D., Verhoef, H.A., 1998. Modeling the time–-temperature relationship in cold injury and effect of high-temperature interruptions on survival in a chill-sensitive collembolan. Functional Ecology 12, 816–824.

Pio, C.J., Baust, J.G., 1988. Effects of temperature cycling on cryoprotectant profiles in the goldenrod gall fly, *Eurosta solidaginis* (Fitch). Journal of Insect Physiology 34, 767–771.

Powell, J.A., Logan, J.A., 2005. Insect seasonality: circle map analysis of temperature-driven life cycles. Theoretical Population Biology 67, 161–179.

Powell, J.A., Jenkins, J.L., Logan, J.A., Bentz, B.J., 2000. Seasonal temperature alone can synchronize life cycles. Bulletin of Mathematical Biology 62, 977–998.

Régnière, J., 1996. Generalized approach to landscape-wide seasonal forecasting with temperature-driven simulation models. Environmental Entomology 25, 869–881.

Régnière, J., Logan, J.A., 2003. Animal life cycle models. In: Schwartz, M. (Ed.), Phenology: An Integrative Environmental Science. Kluwer Academic Publishers, The Netherlands, pp. 237–254.

Régnière, J., St-Amant, R., 2007. Stochastic simulation of daily air temperature and precipitation from monthly normals in North America north of Mexico. International Journal of Biometeorology, in press, doi:10.1007/s00484-006-0078-z.

Reid, R.W., 1963. Biology of the mountain pine beetle, *Dendroctonus monticolae* Hopkins, in the East Kootenay region of British Columbia. III. Interaction between the beetle and its host, with emphasis on brood mortality and survival. The Canadian Entomologist 95, 225–238.

Renault, D., Salin, C., Vannier, G., Vernon, P., 2002. Survival at low temperatures in insects: what is the ecological significance of the supercooling point? Cryo Letters 23, 217–228.

Rickards, J., Kelleher, M.J., Storey, K.B., 1987. Strategies of freeze avoidance in larvae of the goldenrod gall moth, *Epiblema scudderiana*: winter profiles of a natural population. Journal of Insect Physiology 33, 443–450.

Ring, R.A., 1977. Cold-hardiness of the bark beetle, *Scolytus ratzeburgi* Jans. (Coleoptera: Scolytidae). Norwegian Journal of Entomology 24, 125–136.

Rojas, R.R., Lee Jr., R.E., Luu, T.A., Baust, J.G., 1983. Temperature dependence-independence of antifreeze turnover in *Eurosta solidaginis* (Fitch). Journal of Insect Physiology 29, 865–869.

Safranyik, L., 1978. Effect of climate and weather on mountain pine beetle populations. In: Kibbee, D.L., Berryman, A.A., Amman, G.D., Stark, R.W. (Eds.), Symposium Proceedings: Theory and Practice of Mountain Pine Beetle Management in Lodgepole Pine Forests. Washington State University, Pullman, Washington, University of Idaho, Moscow, Idaho, pp. 77–86.

Safranyik, L., Carroll, A., 2006. The biology and epidemiology of the mountain pine beetle in lodgepole pine forests. In: Safranyik, L., Wilson, B. (Eds.), The mountain pine beetle, a synthesis of biology, management and impacts on lodgepole pine. Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre, Victoria, BC, pp. 3–66.

Safranyik, L., Linton, D.A., 1998. Mortality of mountain pine beetle larvae, *Dendroctonus ponderosae* (Coleoptera: Scolytidae) in logs of lodgepole pine (*Pinus contorta* var. *latifolia*) at constant low temperatures. Journal of the Entomological Society of British Columbia 95, 81–87.

Safranyik, L., Shrimpton, D.M., Whitney, H.S., 1975. An interpretation of the interaction between lodgepole pine, the mountain pine beetle and its associated blue stain fungi in western Canada. In: Baumgartner, D.M. (Ed.), Management of Lodgepole Pine Ecosystems. Proceedings of a Symposium, Washington State University Cooperative Extension Service, 9–11 October 1973, pp. 406–428.

Salt, R.W., 1961. Principles of insect cold-hardiness. Annual Review of Entomology 6, 55–74.

Shintani, Y., Ishikawa, Y., 1999. Geographic variation in cold hardiness of eggs and neonate larvae of the yellow-spotted longicorn beetle *Psacothea hilaris*. Physiological Entomology 24, 158–164.

Sinclair, B.J., 1999. Insect cold tolerance: how many kinds of frozen? European Journal of Entomology 96, 157–164.

Sømme, L., 1964. Effects of glycerol on cold-hardiness in insects. Canadian Journal of Zoology 42, 89–101.

Sømme, L., 1982. Supercooling and winter survival in terrestrial arthropods. Comparative Biochemistry and Physiology 73A, 519–543.

Storey, J.M., Storey, K.B., 1983. Regulation of cryoprotectant metabolism in the overwintering gall fly larvae, *Eurostata solidaginis*: temperature control of glycerol and sorbitol levels. Journal of Comparative Physiology 149, 495–502.

Storey, J.M., Storey, K.B., 1986. Winter survival of the gall fly larva, *Eurosta solidaginis*: profiles of fuel reserves and cyroprotectants in a natural population. Journal of Insect Physiology 32, 549–556.

Storey, K.B., Storey, J.M., 1988. Freeze tolerance in animals. Physiological Reviews 68, 27–84.

Storey, K.B., Storey, J.M., 1991. Biochemistry of Cryoprotectants. In: Denlinger, D.L. (Ed.), Insects at Low Temperature. Chapman & Hall, London, pp. 64–93.

Tanaka, K., 1996. Seasonal and latitudinal variation in supercooling ability of the House Spider, *Achaearanea tepidariorum* (Araneae: Theridiidae). Functional Ecology 10, 185–192.

Tran, K., Ylioja, T., Billings, R., Régnière, J., Ayres, M.P., 2007. Testing a climatic model to predict population dynamics of a forest pest, *Dendroctonus frontalis* (Coleoptera: Scolytidae). Ecological Applications, in press.

Ungerer, M.J., Ayres, M.P., Lombardero, M.J., 1999. Climate and the northern distribution limits of *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytidae). Journal of Biogeography 26, 1133–1145.

Virtanen, T., Neuvonen, S., Nikula, A., 1998. Modelling topoclimatic patterns of egg mortality of *Epirrita autumnata* (Lepidoptera: Geometridae) with a geographical information system: predictions for current climate and warmer climate scenarios. Journal of Applied Ecology 35 (2), 311–322.

Wygant, N.D., 1940. Effects of low temperatures on the Black Hills beetle (*Dendroctonus ponderosae* Hopk.). Ph.D. Thesis, New York State College of Forestry, Syracuse, NY.

Yuill, J.S., 1941. Cold hardiness of two species of bark beetles in California forests. Journal of Economic Entomology 34, 702–709.