

# Ecology of Mountain Pine Beetle (Coleoptera: Scolytidae) Cold Hardening in the Intermountain West

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Environ. Entomol. 28(4): 577-587 (1999)

**ABSTRACT** The mountain pine beetle, *Dendroctonus ponderosae* Hopkins, spends the majority of its life cycle within the phloem of pine trees, experiencing exposure to temperatures below  $-30^{\circ}\text{C}$  in many parts of their expansive range. To better understand cold tolerance capabilities of this insect, seasonal patterns of cold-hardiness, as measured by supercooling points in the laboratory, were compared with seasonal patterns of host tree phloem temperatures at several geographic sites for 2 beetle generations. Larvae were found to be intolerant of tissue freezing, and supercooling points measured appear to be a reasonable estimate of the lower limit for survival. Of the compounds analyzed, glycerol was found to be the major cryoprotectant. No differences in supercooling points were found among instars or between larvae collected from the north and south aspect of tree boles. Both phloem temperatures and supercooling points of larvae collected from within the phloem were found to be different among the geographic sites sampled. Mountain pine beetle larvae appear to respond to seasonal and yearly fluctuations in microhabitat temperatures by adjusting levels of cold hardening.

**KEY WORDS** *Dendroctonus ponderosae*, bark beetle, supercooling point, freeze intolerant

THE MOUNTAIN PINE beetle, *Dendroctonus ponderosae* Hopkins, which overwinters under the bark of pine trees in a nonmobile stage, is unable to escape low temperature exposure. When escape from low temperatures is unavoidable, many insect species respond by adjusting physiological and biochemical processes that enhance their tolerance to freezing temperatures. Freeze tolerant species are able to withstand the formation of ice in the extracellular body fluid, whereas freeze intolerant species must avoid freezing of body tissues (Salt 1961). Freeze avoidance is accomplished through cold-hardening. The supercooling point refers to the temperature at which spontaneous nucleation of body water occurs and ice crystals begin to form in the insect tissue (Lee 1989). For those species that cannot survive tissue freezing, the supercooling point represents a lethal temperature threshold, although death may also result as a consequence of exposure to temperatures above the supercooling point (Lee 1991). The cold-hardening capacity of an insect may vary with the developmental stage, nutritional status, and duration of exposure to specific low temperatures.

Many physiological mechanisms involved in the cold-hardening process have been identified including ice-nucleating proteins, lipoproteins and anti-freeze proteins, evacuation of the gut to remove potential ice nucleating agents, ice nucleating bacteria, and accumulation of low molecular weight cryopro-

tectants such as polyhydric alcohols (polyols) and sugars (Hamilton et al. 1985, Lee and Denlinger 1991). Polyol synthesis is known to be triggered by low-temperature exposure, with increasing rates at lower temperatures (Baust 1982, Storey and Storey 1983). Most likely, thermoperiodic cues, represented by some threshold length of time at or below a particular temperature, promote accumulation of an adequate concentration of cryoprotectants before the time they are needed (Storey and Storey 1991). To more fully understand the role of cold-hardening in mountain pine beetle population dynamics, therefore, it is necessary to relate supercooling capacities as determined in the laboratory to the thermal history of the microhabitat where larvae reside (Bale 1991).

The microhabitat of the mountain pine beetle is the phloem of living pines, wherein the majority of the life cycle is spent typically overwintering as larvae. In the northernmost part of their range, winter temperatures below  $-30^{\circ}\text{C}$  are not uncommon. Mountain pine beetle eggs and pupae are considered the least cold tolerant life-stages (Reid 1963, Reid and Gates 1970, Amman 1973), whereas the large larvae are thought to be the most cold tolerant (Yuill 1941, Wygant 1942, Somme 1964). Given that effects of temperature on developmental rate are stage-specific (Bentz et al. 1991), cold-hardening capabilities may also depend on life-stage, with one stage more adapted for overwintering than another. Although temperature has previously been assigned as the most important mortality factor of the mountain pine beetle (Safranyik 1978, Cole 1981), little is understood about the timing of low temperatures and their effect on the population dynamics of this nondiapausing species. Specific objec-

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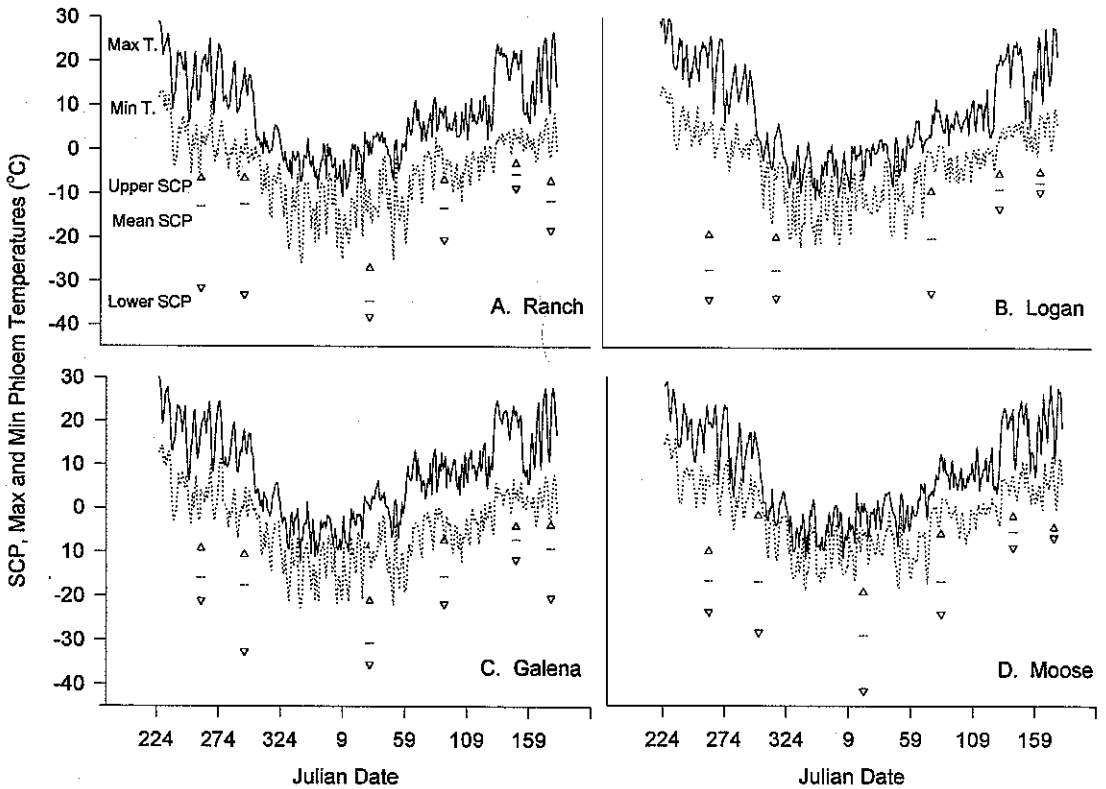


Fig. 1. Maximum and minimum phloem temperatures ( $T$ ,  $^{\circ}\text{C}$ ) at 4 sites (A–D) in 1992–1993 with the mean (—) and range ( $\Delta$ ,  $\nabla$ ) of associated larval supercooling points (SCP) ( $^{\circ}\text{C}$ ).

ferent from the Ranch and Beaver sites ( $df = 517$ ,  $P < 0.05$ ). No significant differences in supercooling points were observed among instars nor among individuals collected from the north and south bole aspect in either generation (Table 6). Although the proportion of 1<sup>st</sup> and 2<sup>nd</sup> instars in winter samples of lodgepole pine hosts were lower than proportions of 3<sup>rd</sup> and 4<sup>th</sup> instars at the same time, all instars were observed in samples throughout the winter and into May and June in both hosts.

There was no response from any individual allowed to warm to room temperature after supercooling point determination. Observed survival was 100% for the 16 larvae that were cooled to temperatures just above the supercooling point.

A similar seasonal trend was observed in mean supercooling points in both generations, for all instars, at all sites except Logan. Values were approximately  $-10$  to  $-20^{\circ}\text{C}$  in early fall, dropped to  $-25$  to  $-35^{\circ}\text{C}$  in January, and increased again in the spring and early summer (Figs. 1 and 2). At all sites except Beaver, the associated daily minimum phloem temperatures, a representation of the microhabitat of larvae, were above the average supercooling point for larvae on each sample date (Figs. 1 and 2). Around Julian day 1 at the Beaver site, minimum phloem temperatures were slightly below

the average supercooling point measured for individuals 8 d later. No mortality was observed at this time. Assigning mortality factors in the field was difficult, however, because of the rapid desiccation and decline of larval carcasses. Consequently, in both generations we observed very little mortality during field sampling. During the spring especially, many individual supercooling points were warmer than minimum phloem temperatures experienced at that time (Figs. 1 and 2). Supercooling points in the fall were always lower than  $-10^{\circ}\text{C}$  despite the absence of any temperatures below a threshold of 0 or  $5^{\circ}\text{C}$  (Figs. 3B and 4B). In the middle of February (around Julian day 45), small increases in temperature units above  $0^{\circ}\text{C}$  were associated with a raising of the supercooling point. Although cooling units were still being accumulated, the rate of accumulation during this time was decreasing, whereas heat units were constant or only slightly increasing.

The major polyol found in larval mountain pine beetles was glycerol, with negligible concentrations of sorbitol or dulcitol. There were no significant differences found in glycerol levels of larvae collected from the north and south aspects of tree boles. Glycerol levels (mean  $\pm$  SE) in the 3 samples collected in November ( $6.8 \pm 3.0 \mu\text{g/g}$ ), January ( $8.6 \pm 4.0 \mu\text{g/g}$ ),

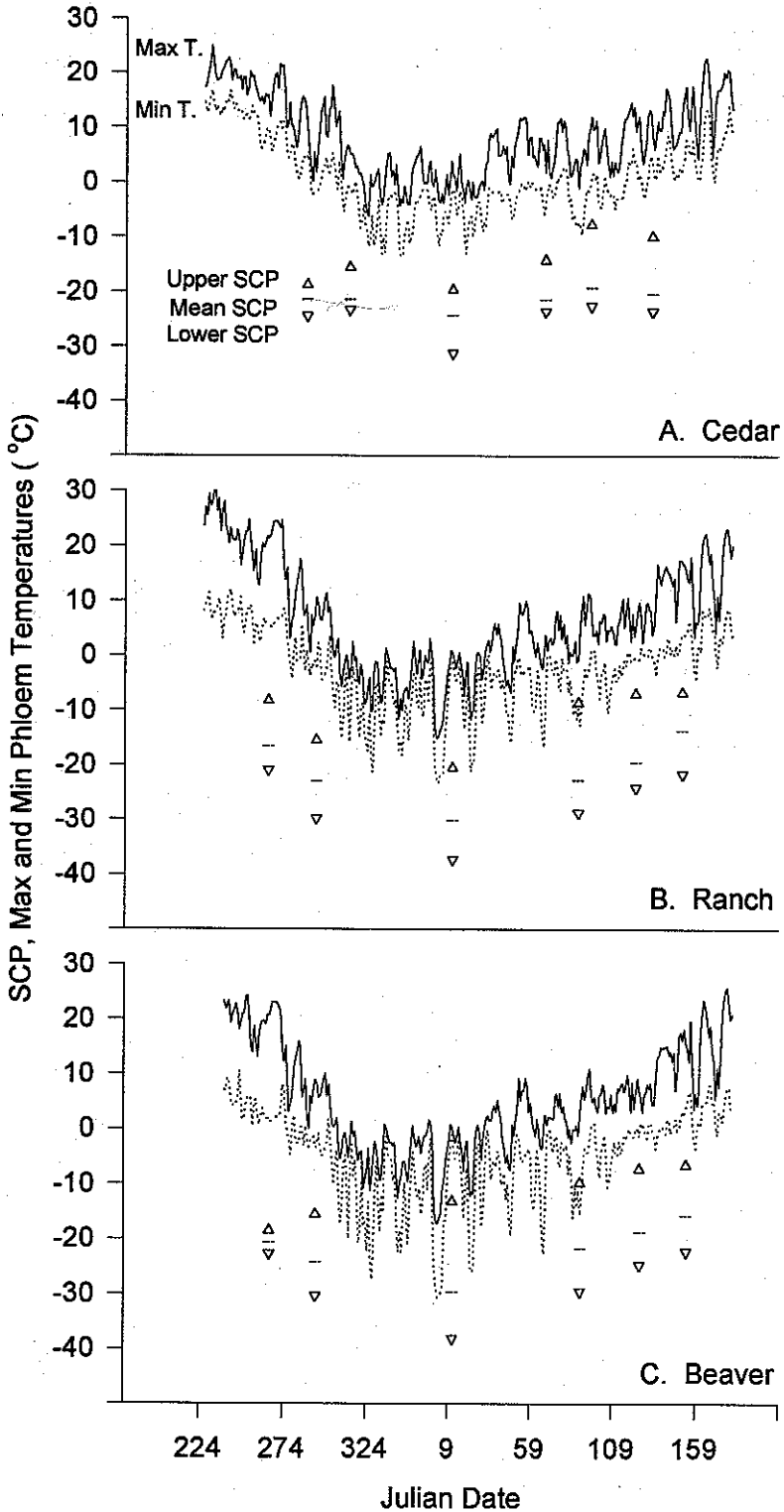


Fig. 2. Maximum and minimum phloem temperatures (T, °C) at 3 sites (A-C) in 1994-1995 with the mean (—) and range (Δ, ∇) of associated larval supercooling points (SCP) (°C).

Table 3. Results of ANOVA used to determine differences in cooling and heating units among sites and sample months

Source	Among sites		Among months	
	F (df = 3, 457)	P	F (df = 6, 457)	P
1992-1993				
Cooling units	254.40	0.001	12,609.15	0.001
Heating units	394.61	0.001	6,988.80	0.001
1994-1995				
Cooling units	3,124.54	0.001	1,517.73	0.001
Heating units	2,319.71	0.001	1,229.38	0.001

and March ( $10.6 \pm 6.4 \mu\text{g/g}$ ) were not significantly different.

### Discussion

Larvae that were cooled to temperatures just above the measured supercooling point survived, suggesting that mortality above the measured supercooling point is minimal, and that the supercooling point is a reasonable measure of cold tolerance for this insect. To truly understand mortality above the supercooling point however, long-term effects will need to be assessed. Additional tests wherein larvae are kept at temperatures just above the supercooling point for extended periods will need to be performed to conclusively determine if larvae can indeed survive temperatures above the supercooling point. The lack of response from any individual allowed to warm to room temperature after supercooling point determination suggests that the mountain pine beetle is freeze intolerant and cannot survive tissue freezing. Several other scolytid bark beetles have been found to be freeze intolerant (Ring 1977, Gehrken 1984, Miller and Werner 1987).

All mountain pine beetle instars were found to overwinter, although higher proportions of 3rd and 4th instars were observed during winter months. Although Amman (1973) and Langor (1989) observed higher mortality in smaller larvae (e.g., 1st and 2nd instars) during the winter months, and mountain pine beetle instars have been found to exhibit stage-specific development rates (Bentz et al. 1991), we found no significant differences among instars in their capacity to cold-harden. This is in contrast to a study with the weevil *Hypera punctata* (F.) where a positive correlation between supercooling points and larval body weight was found, suggesting that larger instars have less capacity to cold-harden (Watanabe and Tanaka 1997). Although we did not measure size of larvae within an instar, adult Douglas fir beetles, *Dendroctonus pseudotsugae* Hopkins, and mountain pine beetles emerging from bolts kept at cool, as compared with warm, temperatures were larger (Atkins 1967, Amman and Cole 1983). The Douglas fir beetles reared at the colder temperatures also had proportionately greater lipid content.

Although others have observed differences in cold-hardening capacity between aspects (Gehrken and Zachariassen 1978), supercooling points of mountain pine beetle larvae collected from the north and south

aspects of tree boles were not significantly different. Minimum phloem temperatures within the lodgepole pine from which larvae were collected also were not significantly different between the aspects. Southern exposures of tree boles had significantly warmer temperatures, but this had no consistent effect on supercooling capacity of the larvae. Minimum phloem temperatures, which typically occur around sunrise, appear to have more of an effect on larval cold-hardening than daily maximum temperatures. Supercooling points of larvae may indeed be influenced by changing temperatures within a 24-h period, although this effect was not observed in our monthly samples. Previous laboratory research with other insects has shown that short-term exposure to high temperatures may decrease the capacity to supercool (Lee et al. 1987). If this physiological phenomena occurs in the mountain pine beetle, winters with periodic warming trends could result in lower survival rates than winters with consistent cold temperatures, especially so on southern bole aspects.

Daily maximum, minimum, and average phloem temperatures were significantly different among the sites both years (Table 2). Accumulated temperature units, both above and below a 0 and 5°C threshold, were also significantly different among all sites (Table 3). These differences imply that larvae from at least some geographic sites were exposed to different microhabitat temperature regimes. Observed differences in temperature regimes among the sites may be contributing to the differences observed in larval supercooling points among the sites, and could also imply differential mortality. The most striking differences were observed between the Cedar and Beaver and Ranch sites in 1994-1995. In the middle of winter, larval supercooling points at Cedar were as much as 6°C higher than supercooling points at either Beaver or Ranch (Fig. 4C). The Cedar site, which was the only site composed of all ponderosa pine hosts, was located at the highest elevation (2,622 m), lowest latitude (37°C), and had the fewest accumulated cooling units. Cold units accumulated at the Cedar site were only a 3rd of the cold units accumulated in lodgepole pine at the other sites monitored that year (Fig. 4B). The Cedar site also had the highest mean supercooling points at all sample times except in April (Fig. 2A). Because of a high metabolic cost (Danks 1978), maintaining elevated levels of cold-hardiness (low supercooling points) is not advantageous at times when it is not required. Differences observed between the Cedar and Ranch and Beaver sites indicate that mountain pine beetle larvae may have the capacity to metabolize only amounts of cryoprotectants needed, given a particular thermal history. The thresholds required to trigger this metabolism, as well as other cold-hardening mechanisms, is unclear. Although supercooling points of eggs were not analyzed, we observed viable eggs in ponderosa pine at the Cedar site on all sample dates, whereas viable eggs were found no later than October in any samples from lodgepole pine. Observed differences in cold-hardening capacity between the Cedar and both Ranch and Beaver sites

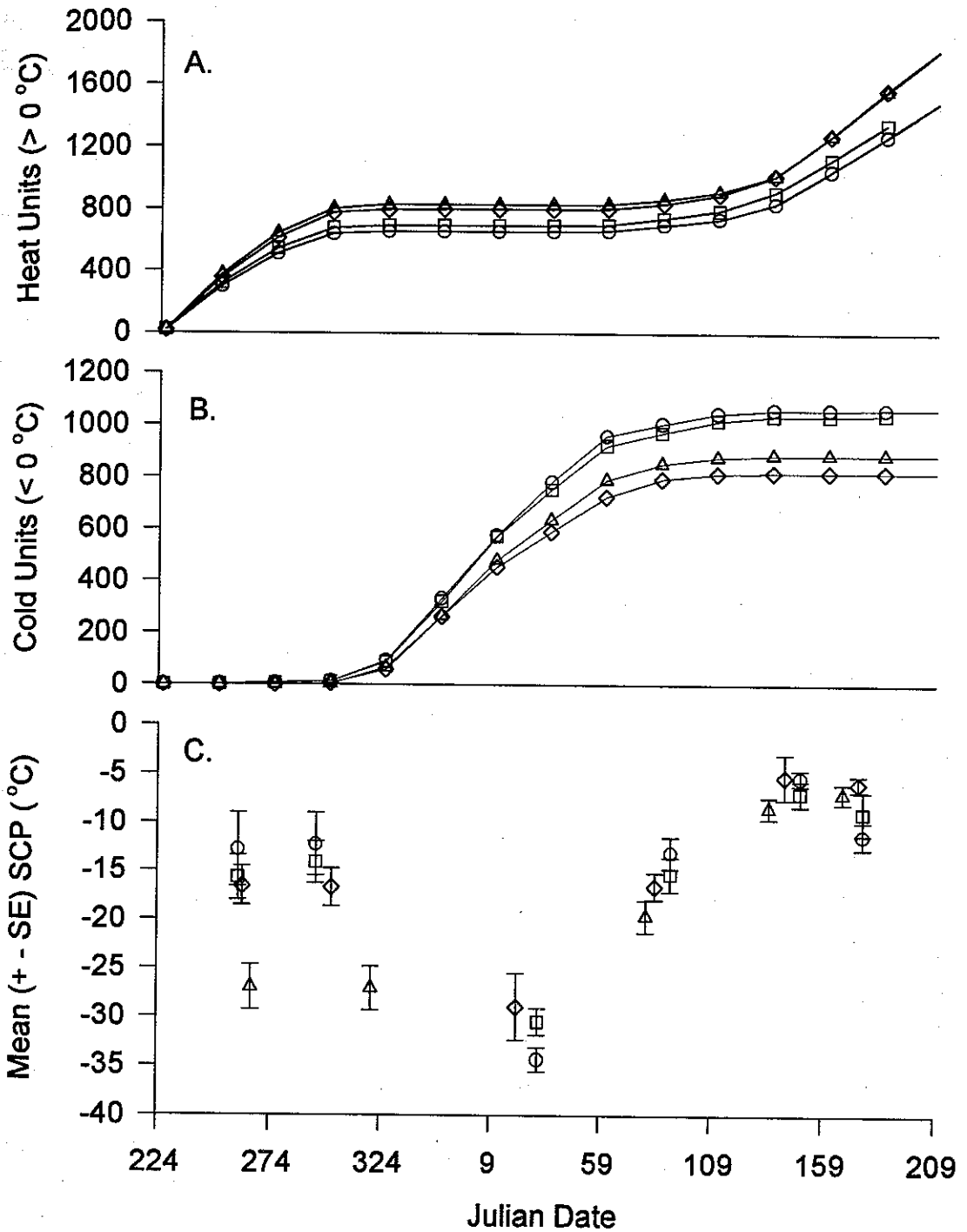


Fig. 3. (A) Accumulated heat units (>0°C) and (B) accumulated cold units (<0°C) at 4 sites in 1992–1993 and (C) associated mean supercooling points (SCP) (°C) and standard errors. Sites: ○, Ranchi; □, Galena; △, Logan; ◇, Moose.

may also be influenced, at least in part, by selective pressures on larvae because of tree host nutrition and other host-specific factors which differ between ponderosa and lodgepole pines.

Observed seasonality in the cold-hardening response suggests that mountain pine beetle larvae respond to fluctuating temperatures in the microhabitat (Figs. 1 and 2), as described for several scolytid bark

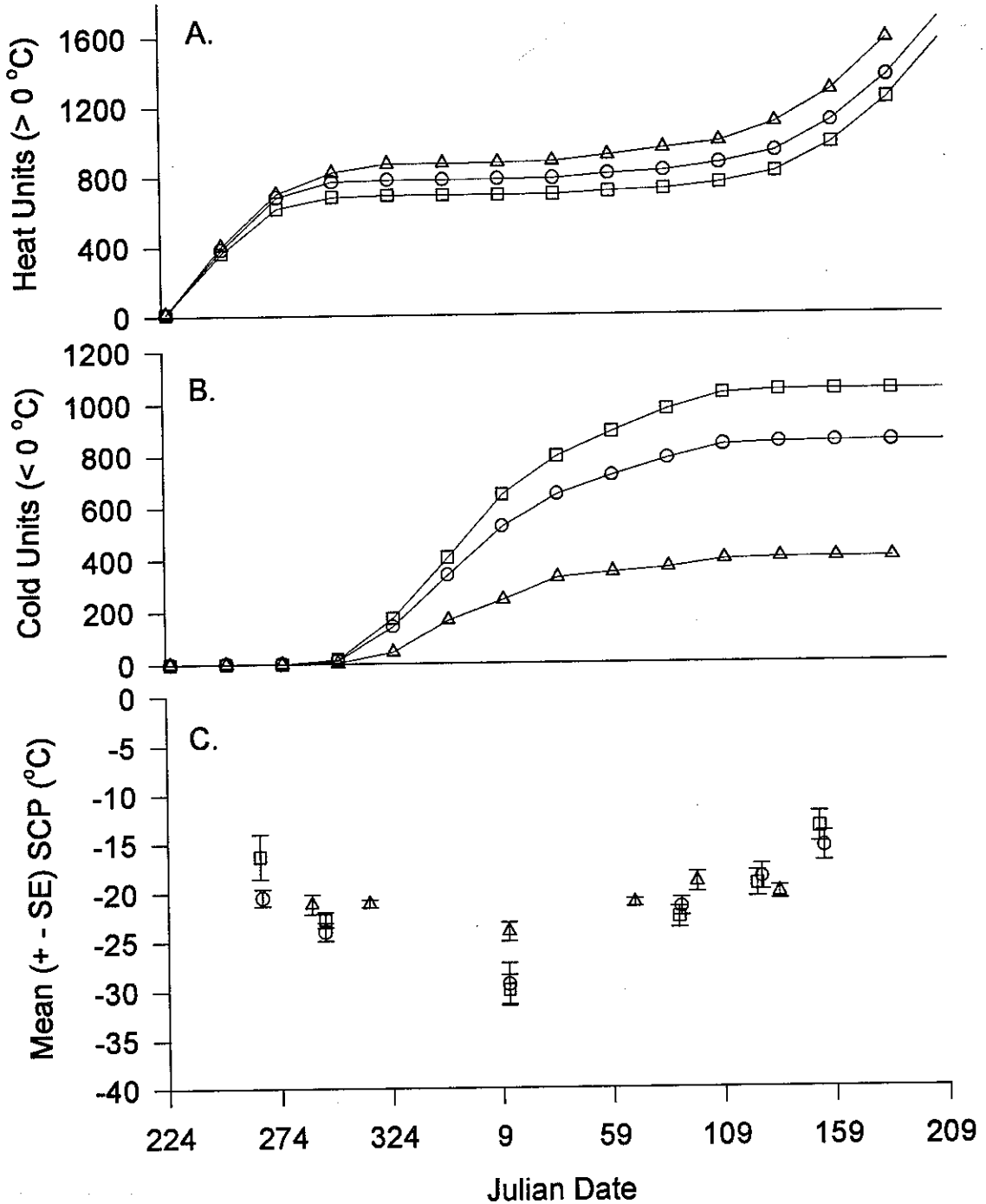


Fig. 4. (A) Accumulated heat units (>0°C) and (B) accumulated cold units (<0°C) at 3 sites in 1994–1995 and (C) associated mean supercooling points (SCP) (°C) and standard errors. Sites: ○, Ranch; □, Beaver; △, Cedar.

beetles (Hansen et al. 1980, Gehrken 1984, Miller and Werner 1987, Luik and Voolma 1990). A year-to-year fluctuation in cold-hardiness at the same site in response to temperature change was also observed. Phloem temperatures of infested trees at the Ranch

site that were <100 m apart were significantly colder in 1992–1993 than 1994–1995. Larvae responded with a mean supercooling point which was  $-5^{\circ}\text{C}$  colder in 1992–1993. A considerable amount of variation among supercooling points of individual larvae on any day, as

Table 4. 1992-1993 mean supercooling points for each instar, by site and sample date

Site	Instar	Sept.	Nov.	Jan.	March	May	June
Ranch	1st	-31.1 (1, 0.0)	-6.8 (1, 0.0)	N/A	-9.6 (1, 0.0)	N/A	N/A
	2nd	-14.5 (6, 3.5)	-12.9 (8, 3.0)	-35.0 (4, 1.5)	-15.2 (9, 1.3)	-3.3 (2, 0.0)	N/A
	3rd	-9.7 (9, 0.9)	-12.0 (4, 2.9)	-33.9 (11, 0.9)	-13.2 (13, 1.1)	-6.6 (7, 0.8)	-12.0 (11, 0.8)
	4th	N/A	-12.6 (3, 0.9)	-35.0 (3, 0.4)	-7.8 (3, 0.5)	-5.1 (15, 0.5)	-10.6 (10, 1.2)
Galena	1st	-14.5 (2, 5.0)	N/A	-21.2 (1, 0.0)	N/A	-7.2 (1, 0.0)	N/A
	2nd	-15.2 (7, 2.9)	-12.7 (7, 1.6)	-30.8 (15, 1.0)	-14.0 (9, 1.6)	-8.2 (3, 1.7)	N/A
	3rd	-17.8 (3, 0.7)	-16.1 (15, 1.8)	-31.0 (12, 1.1)	-16.3 (16, 1.0)	-7.3 (5, 1.2)	-9.1 (9, 1.7)
	4th	N/A	-11.7 (8, 1.8)	-30.2 (6, 1.6)	-14.9 (4, 3.5)	-5.3 (3, 0.6)	-8.9 (6, 1.4)
Logan	1st	-27.4 (2, 3.6)	N/A	-13.9 (1, 0.0)	N/A	N/A	N/A
	2nd	-27.4 (7, 2.0)	-24.4 (4, 2.9)	N/A	-21.8 (7, 0.8)	-10.0 (1, 0.0)	-8.9 (1, 0.0)
	3rd	-25.9 (6, 2.2)	-27.8 (16, 1.2)	-32.0 (1, 0.0)	-19.5 (16, 1.3)	-8.9 (10, 0.6)	-6.4 (2, 1.3)
	4th	-28.1 (2, 2.4)	N/A	N/A	-17.7 (6, 1.4)	-6.5 (3, 0.6)	-7.0 (7, 0.6)
Moose	1st	N/A	-16.2 (1, 0.0)	-29.9 (4, 3.7)	-14.3 (4, 2.9)	N/A	N/A
	2nd	-16.2 (11, 1.4)	-15.6 (10, 2.4)	-27.5 (2, 1.2)	-18.0 (11, 1.2)	-2.0 (1, 0.0)	N/A
	3rd	-17.1 (6, 1.8)	-15.9 (22, 1.1)	-30.1 (6, 3.4)	-15.6 (10, 1.3)	-9.1 (1, 0.0)	-6.8 (3, 0.2)
	4th	-17.1 (3, 3.2)	-20.5 (8, 2.4)	-26.5 (3, 3.5)	-17.1 (8, 0.8)	-5.2 (4, 1.1)	-5.4 (4, 0.5)

Total sample size = 467. N/A indicates no larvae were sampled at that time. Supercooling points are in °C. Numbers in parentheses beneath means indicate values for (sample size, ± standard error).

has been found with other insects (Block 1982, Watanabe and Tanaka 1997), was also observed.

We observed no cold-induced mortality in the small

sample of larvae cooled to temperatures just above the measured supercooling point, suggesting a selection for lower supercooling points than the minimum tem-

Table 5. 1994-1995 mean supercooling points for each instar, by site and sample date

Site	Instar	Sept.	Oct.	Nov.	Jan.	March	April	May	June
Ranch	1st	N/A	-21.8 (4, 0.6)	N/A	-22.9 (1, 0.0)	N/A	-22.5 (2, 3.1)	-24.1 (1, 0.0)	-19.7 (1, 0.0)
	2nd	N/A	-22.9 (3, 0.8)	N/A	-28.4 (9, 1.7)	N/A	-23.3 (6, 0.5)	-19.3 (14, 1.3)	-14.5 (4, 1.9)
	3rd	-13.1 (2, 4.6)	-20.4 (5, 1.3)	N/A	-28.8 (14, 1.4)	N/A	-22.7 (22, 0.8)	-17.4 (19, 1.3)	-14.3 (11, 1.7)
	4th	-16.8 (14, 1.2)	-23.5 (20, 0.4)	N/A	-32.2 (17, 0.8)	N/A	-22.6 (22, 1.0)	-20.8 (21, 0.9)	-12.7 (22, 0.9)
Beaver	1st	N/A	-21.7 (3, 0.7)	N/A	-26.1 (1, 0.0)	N/A	-24.2 (1, 0.0)	N/A	N/A
	2nd	N/A	-23.0 (7, 0.5)	N/A	-25.5 (2, 12.4)	N/A	-20.0 (9, 0.6)	-20.0 (5, 1.3)	-20.7 (2, 1.5)
	3rd	-20.5 (10, 0.4)	-22.4 (7, 0.2)	N/A	-30.1 (21, 1.2)	N/A	-21.6 (29, 0.6)	-18.2 (25, 1.1)	-14.2 (10, 1.8)
	4th	N/A	-25.2 (21, 0.7)	N/A	-29.1 (15, 1.7)	N/A	-22.3 (15, 1.0)	-18.6 (26, 0.9)	-15.5 (27, 0.8)
Cedar	1st	N/A	-22.4 (3, 0.9)	-20.8 (11, 0.4)	-23.2 (4, 1.60)	-21.4 (38, 0.3)	-19.7 (14, 1.2)	-20.4 (11, 0.6)	N/A
	2nd	N/A	-20.2 (4, 0.6)	-20.8 (14, 0.5)	-23.6 (21, 0.6)	-20.6 (23, 0.5)	-18.5 (30, 0.8)	-20.1 (44, 0.3)	N/A
	3rd	N/A	-21.1 (2, 0.2)	-21.6 (12, 0.4)	-22.9 (2, 0.4)	-21.5 (6, 0.6)	-19.6 (8, 0.4)	-19.9 (6, 0.5)	N/A
	4th	N/A	N/A	-21.5 (9, 0.4)	-26.9 (5, 1.9)	-21.7 (4, 0.4)	-19.6 (7, 0.5)	-21.4 (1, 0.0)	N/A

Total sample size = 750. N/N indicates no larvae were sampled at that time. Supercooling points are in °C. Numbers in parentheses beneath means indicate values for (sample size, ± standard error).

**Table 6.** Results of NNOVN used to determine factor effects on supercooling points in 1992–1993 and 1994–1995

Source	1992–1993			1994–1995		
	F	df	P	F	df	P
Site	2.80	3, 107	0.05	3.99	2, 89	0.05
Month	35.51	5, 107	0.001	50.87	7, 89	0.001
Aspect	0.18	1, 107	0.488	1.84	1, 89	0.098
Instar	0.11	3, 107	0.839	0.94	3, 89	0.172
Site × Month	3.36	15, 107	0.01	—	—	—

— interaction not analyzed because of missing cells.

perature occurring under the bark. However, additional research is needed in this area to ascertain if larvae can withstand long periods at these temperatures. Although the average supercooling point on a particular day was typically below the minimum phloem temperature, many individual supercooling points were often warmer than the minimum phloem temperature, especially in the spring, suggesting that mortality could be occurring (Figs. 1 and 2). Additionally, supercooling points in the spring were often higher than those in the fall at the same site. This could be due in part to the fact that maximum phloem temperatures in the spring were generally above lower developmental thresholds for 3rd and 4th instars (see Bentz et al. 1991), perhaps triggering a feeding response and concomitant decreased cold-hardening in the spring. Spring, therefore, may be the most susceptible time for cold-induced mortality in this insect.

It is well documented that individuals experience greater cold-hardening capabilities when exposed to cold temperatures (Baust and Miller 1970). However, the temperature at which acclimation for cold-hardening begins appears to be species-specific and mediated by climate. Acclimation for some insects is more effective at 3–5°C (Baust and Miller 1970), whereas in others polyol production was enhanced at subzero temperatures (–5 to –10°C) (Young and Block 1980). An increase in the rate of temperature acclimation below 0°C was correlated with a decrease in supercooling points, although we are unable to ascertain from our field study the appropriate threshold trigger for accumulation of cryoprotectants. Insects also respond to changes in the duration and temperature of daily cycles (Beck 1991). Observed maximum cold-hardiness occurred after the peak of daily cold units yet before the maximum accumulation of cold units (Figs. 3 and 4). Cold-hardening then decreased with a decrease in the rate of accumulation of cold units (below 0°C), whereas heat units remained relatively constant during this period. The rate of change in daily thermal units most likely play an important role in mountain pine beetle cold-hardening, and a better understanding of time lags and the decay effects of temperature history on cold-hardiness is needed.

Considering the diversity and complexity of the cold-hardening process, it is unlikely that any single environmental cue is responsible for the complex biochemical strategies that occur (Baust 1982). However,

for bark beetles, which spend the majority of time under the bark of host trees, temperature is the most reliable environmental cue for cold-hardening. Glycerol was found to be the predominant cryoprotectant in larval mountain pine beetle from central Idaho, whereas levels of sorbitol and dulcitol were negligible. Results on seasonality of this compound in larval mountain pine beetle and its direct relation to temperature are inconclusive given our results. However, glycerol content in adult and larval spruce beetles, *Dendroctonus rufipennis* Kirby, from Alaska (Miller and Werner 1987) and 4th-instar mountain pine beetle (Sømme 1964) from California appear to be regulated by temperature, as does the ethylene glycol concentration in *Ips acuminatus* Gyllenhal larvae (Gehrken 1984). Other mechanisms most likely also play a role, including other cryoprotectants and thermal hysteresis provided by high molecular weight particles such as proteins and glycoproteins.

We have investigated only 1 of the processes that may be responsible for cold-hardening and associated mortality in mountain pine beetle larval stages: freezing and the production of glycerol which plays a role in this process. Thermal history experienced by larvae beneath the bark of pine trees, as well as the daily change in thermal cycles are both important in the cold-hardening process for this insect. Larval instars were found to be freeze intolerant, with seasonal changes in supercooling capacity associated with phloem temperatures. All instars were found to overwinter in both lodgepole and ponderosa pine. Regional populations of mountain pine beetles can differ significantly in cold-hardiness, apparently because of local weather patterns. An important question that remains to be answered is if populations of mountain pine beetle at southern latitudes have adapted to the local climate, or if they are capable of surviving temperature regimes experienced by populations at more northern latitudes and colder sites. The amount of cold-hardening that occurs in mountain pine beetle populations appears to be dictated by temperature regimes at each particular site. It is unclear, however, if all populations in the intermountain region have the same capacity to cold-harden, and only do so if temperatures dictate it. This sensitivity of mountain pine beetles to local microclimate emphasizes their importance as biological indicators of regional climate change. Observed variation among individual supercooling points within an instar, flexibility in overwintering life stage, and the ability of larvae to respond seasonally to changing temperature conditions within the microhabitat represent important patterns of phenotypic plasticity which contribute to the success of this important outbreak species.

#### Acknowledgments

Keith Tignor and Karen Johnson were instrumental in supercooling point determination analysis, and Sandra Gabbert was responsible for GC/MS analysis of hemolymph samples. Jim Vandygriff and Bridget Clayton helped with the arduous winter sampling. We thank Rick Lee, Matt Ayres,

and several anonymous reviewers for valuable comments on the manuscript.

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