Cold Tolerance of Four Species of Bark Beetle (Coleoptera: Scolytidae) in North America

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ABSTRACT We investigated the overwintering biology of four temperate-latitude bark beetles: *Dendroctonus frontalis* Zimmermann, *Ips pini* (Say), *I. grandicollis* (Eichhoff), and *I. perroti* Swaine. All four species were freeze-susceptible. However, there was variation within and among species in overwintering biology that related to their geographic distribution. *D. frontalis* and southern populations of *I. grandicollis* continued to reproduce and develop under the bark of their host plants throughout the winter and did not show any seasonal adjustments in their lower lethal temperatures: mean supercooling point ± SD = −12.15 ± 4.02 and −12.25 ± 2.50°C. In contrast, northern populations of *I. grandicollis* and *I. pini* employ a behavioral strategy in which adults migrate to the forest soil, where they are insulated from temperature extremes by litter and snow. Furthermore, adult supercooling points of both northern populations declined from about −13°C in summer to about −17°C in early winter. A concomitant decline in lipid content suggests that lipid metabolism may be involved in seasonal adjustments of cold tolerance in *I. pini*. An assortment of temperature manipulations failed to provide any evidence of cold tolerance acclimation. Immatures, which remain in the inner bark of their host trees, have lower lethal temperatures of −5 to −12°C, and are especially vulnerable to mortality from freezing. *I. perroti*, a northerly distributed species, had similar cold tolerance and overwinter behavior as northern populations of the other two *Ips* species. Winter mortality from freezing could be an important determinant of population dynamics in all four species. Understanding variations in cold tolerance and overwinter behavior among insects species may help predict population dynamics and distribution of potential pests.

KEY WORDS *Dendroctonus frontalis*, *Ips pini*, *Ips grandicollis*, *Ips perroti*, Scolytidae, cold tolerance

TEMPERATURE HAS BROAD effects on the physiology and behavior of virtually all insects in all developmental stages. Temperature influences metabolic rate, flight activity, reproduction, nutrition, development, and survival. The ability to survive annual temperature minima can be a critical determinant of insect distribution limits (Ungerer et al. 1999, Virtanen and Neuvonen 1999). Winter climate can apparently exert strong selection for physiological and behavioral attributes that promote overwinter survival (Kukal et al. 1991; Gillyboeuf et al. 1997). Insects exhibit a range of physiological strategies that promote survival at low temperatures. One broad dichotomy distinguishes between freeze-susceptible and freeze-tolerant species (Salt 1961, Danks 1978, Bale 1987, Lee and Denlinger 1991). Freeze-susceptible species tend to (1) choose thermally buffered microsites for overwintering and (2) lower the temperature at which ice formation occurs (supercooling point) by producing antifreeze agents, minimizing internal nucleation sites, and avoiding contact with external ice. For these species, the supercooling point is usually the lower lethal temperature. Most insects are freeze-susceptible (Sømme 1982). In contrast, freeze-tolerant strategies tend to encourage the formation of extracellular ice at relatively warm temperatures. These species can survive freezing by virtue of adaptations that protect cell membranes from mechanical damage during crystallization of intercellular water and allow osmoregulation in cells that are strongly hypo-osmotic to their environment. Because some insect species suffer mortality even from low, nonfreezing temperatures, Bale (1993) expanded this physiological classification to recognize the distinction between species that are chill-tolerant and chill-susceptible.

Physiological ecology has a heritage of investigating adaptations to extreme environments, so a majority of the research on insect cold-tolerance has been conducted on species that inhabit high latitude environments characterized by very low winter temperatures (Ring 1981, Miller 1982, Danks et al. 1994, Strathdee and Bale 1998). Less is known about the ecological importance of winter climate for insect species that inhabit temperate latitudes. The objective of this study was to compare the physiological and behavioral adaptations for overwintering in four species of bark

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beetles (Coleoptera: Scolytidae) that inhabit mid-latitude forests of North America.

Bark beetles include some of the most important agents of disturbance in forest ecosystems (Raffa 1988, Price et al. 1997, Logan et al. 1998). The literature includes numerous suggestions that winter temperatures can influence the population dynamics of bark beetles (Beal 1933, 1934; Yuill 1941; Chansler 1966; Berryman 1970; Barson 1974; McClelland and Hain 1979; Ragenovich 1980; Lawson 1993). However, detailed knowledge of cold tolerance and overwintering biology is limited to relatively few species: *D. ponderosae* Hopkins (Sømme 1964, Bentz and Mullins 1999), *Scolytus ratzeburgi* Janson (Ring 1977), *D. rufipennis* Hopkins (Miller 1982, Miller and Werner 1987), *I. acuminatus* (Gyllenhall) (Gehrken 1989), *I. typographus* L. (Hansen et al. 1982, Schopf 1985), *D. micans* (Kugelann) (Luik and Voolma 1990), and *S. laevis* Chapuis (Hansen and Sømme 1994).

In this study, we investigated the winter biology of *D. frontalis* Zimmermann, *I. pini* (Say), *I. grandicollis* (Eichhoff), and *I. perroti* Swaine. All four species feed and reproduce within the phloem of pine trees, but their overwintering behavior and geographic distribution differ. *D. frontalis* occurs from Pennsylvania and West Texas to northern Nicaragua, *I. pini* from Alaska and Newfoundland to Chihuahua and North Carolina, *I. grandicollis* from southern Manitoba and Quebec to Honduras and Florida, and *I. perroti* from Alberta and New Brunswick to Minnesota and Michigan (Wood 1982). All developmental stages of *D. frontalis* spend the winter under the bark of their host trees; their development slows but does not stop. Southern populations of *I. grandicollis* have similar behavior. However, northern populations of all the three *Ips* species spend the winter as adults within the litter of the forest floor. In our study sites in Wisconsin, *Ips* spp. tend to cease oviposition in late summer, but not all of the progeny complete development before winter, so part of the population typically enters the winter as larvae and pupae within the phloem of their host tree. Within the northern distributions of all four species, air temperatures drop well below 0°C in most winters.

In addition to characterizing the winter biology of these four species, our research was designed to evaluate the ecological importance of winter climate for temperate-latitude bark beetles of North America. It could be that winter climates in this region are sufficiently benign relative to the cold tolerance of bark beetles that winter is a relatively minor factor in the behavior, physiology, and ecology of bark beetles. If so, temperatures that produce winter mortality should be rare and there may be no conspicuous differences in the cold tolerance of species and populations that inhabit different climatic regions. Alternatively, it could be that winter temperatures have exerted selection for behavioral and physiological attributes that promote overwinter survival. If so, potentially lethal winter temperatures should be relatively common, and adaptations to promote winter survival should be evident. Putative adaptations include higher cold tolerance in northern populations, seasonal adjustments of cold tolerance, short-term acclimatization of cold tolerance in response to temperature regime, higher cold tolerance in life stages that experience the lowest temperatures, and behaviors that minimize exposure to low temperatures.

Materials and Methods

Seasonal Patterns in Cold Tolerance. We conducted field and laboratory studies to test the ability of beetles to survive at low temperatures. Supercooling points were measured by cooling individual insects at 0.20°C/min using a programmable, low-temperature water bath and recording the temperature at which crystallization occurred (evident as an exotherm). Cooling rates can sometimes influence survival (Miller 1978). We chose 0.20°C/min because similar rates can occur in nature (Marchand 1996). We also included measurements to test the effects of cooling rate.

Specimens of *D. frontalis* and southern populations of *I. grandicollis* came from wild populations inhabiting loblolly pine, *Pinus taeda* L., within the Kisatchie National Forest in Louisiana (31° 25′ N, 92° 17′ W) and Bankhead National Forest in Alabama (34° 10′ N, 87° 50′ W). Logs were shipped overnight to our laboratory in New Hampshire and supercooling measurements taken after arrival. Some measurements also included *I. grandicollis* from a laboratory culture at the Southern Research Station in Pineville, LA, (originating within 1 yr of the study from wild populations in the Kisatchie National Forest); the laboratory culture allowed positive identification of *I. grandicollis* larvae, which cannot be easily distinguished from congeners when collected from the wild. The supercooling point of *I. grandicollis* adults from the field and laboratory populations did not differ. *Ips* specimens came from wild populations inhabiting *Pinus resinosa* Aiton in pine forests near Colfax, WI (45° 0′ N, 91° 43′ W). Logs infested by wild populations of *Ips* were collected in September 1997, transferred to our laboratory in New Hampshire, and held in environmental chambers for 1 mo at 10–15°C under natural photoperiods. As adults emerged from logs, they were introduced into screen boxes (10 by 10 by 5 cm) containing soil and litter and placed within the soil of a pine forest in Hanover, NH (43° 42′ N, 72° 17′ W). One box was removed from the soil each month, individuals were scored as dead or alive, and supercooling points were measured. One additional box containing 60 *I. pini* was placed in the forest at 1 m above ground where it was exposed to air temperatures without snow cover. Identical microcosms with beetles were placed inside an incubator at 0°C with photoperiods set to match outdoor treatments. Immature stages remained within logs inside an incubator at 0°C. In 1998, the experiment was repeated with the same methodology except using only the outdoor microcosms (same site as 1997) and the incubator logs at 0°C.

Freeze-Tolerance. We tested for freeze-tolerance in association with measurement of supercooling points. After we observed the exotherm that indicated

freezing, adults and immatures were warmed to ≈22°C and monitored for the ability to resume activity. To further test whether the supercooling point equaled the lower lethal temperature, we exposed adults of *D. frontalis*, *I. pini*, and *I. grandicollis* to temperatures near the average supercooling point (±1 SD) for 10 min, 48 h, and 72 h after cooling them with the same rate of 0.20°C/min and compared their survival to that predicted from a normal distribution with the mean and standard deviation of supercooling points measured on a matched set of the same population from the same acclimation regime. Finally, we tested for the ability of immature *Ips* to resume activity and continue development after freezing. Logs with first instars and others with third instars and pupa were acclimated for 1 d at 15°C, then 1 d at 8°C, then 1 d at 0°C before being exposed for 7 d to −17°C. After the −17°C treatment, logs containing larvae were gradually warmed (1 d at 0°C, then 1 d at 8°C and 1 d at 15°C before being moved to 22°C) and placed into boxes containing a fresh log. Logs with first instars were dissected after 7 d at 22°C to see whether early larvae had survived and resumed development. Remaining logs were examined after one month at 22°C to see whether late larvae or pupae had completed development and begun to reproduce within the new log.

Acclimation experiments. *Short-Term Acclimation.* We tested for short-term acclimation of *D. frontalis* adults (March 1998) and *I. pini* adults (December and January of 1997–1998 and 1998–1999) by exposing subsets of animals to an assortment of temperature regimes before supercooling measurements and by manipulating cooling rates during supercooling measurements. Treatments included warming a set of beetles from field microcosms to 20°C for 10 min to 3 d before measuring supercooling points. In other treatments, individuals were cooled slowly (0.003°C/min and 0.0008°C/min) during 48 h until −10°C or beetles were moved directly to −10°C; other animals were exposed to diurnal cycles from 5 to −7°C over 24 h or exposed to −5°C for 48 h. After these acclimation treatments, supercooling points were measured with the same technique and cooling rate used in the rest of the study (0.20°C/min).

Long-Term Acclimation. We tested for long-term acclimation of *D. frontalis* and *I. grandicollis* from Louisiana by exposing infested logs to 0°C in a growth chamber under natural photoperiods during 4 mo and 6 wk, respectively, before measurement of supercooling points. For comparison, we measured a subset of animals before acclimation and measured another set of animals from the same field population as acclimation treatments were completed. We compared the cold tolerance and behavior of northern and southern populations of *I. grandicollis* when exposed to reciprocal temperature regimes during winter. Replicated pine logs infested by *I. grandicollis* from Louisiana and Wisconsin were exposed to three different temperature regimes: greenhouse temperatures (minimum, maximum, average = 15.9, 30.9, 20.6°C), winter field temperatures in New Hampshire (minimum = −26.7, maximum = 21, average = −3.9°C for the studied period), and constant 0°C (in an incubator with photoperiods matching the other treatments). Infested logs were placed within large screened boxes containing a fresh pine log and 10 cm of soil and pine litter. Northern populations of *I. pini* were also included in this experiment. After 7 wk, we examined the microcosms, scored beetles for whether they had migrated to the soil or started colonizing the new log, recorded survival and measured supercooling points.

Water and Lipids Content. We evaluated seasonal and interspecific patterns in the water content and lipid content of adult beetles. Lipid content was quantified by extraction with three rotations (24 h each) of 1 ml of petroleum ether plus ethanol (65:35) at 38°C (Anderbrant et al. 1985). Water content was measured gravimetrically.

Overwintering Habitats. In October 1998, 975 recently emerged *I. grandicollis* and 284 *I. pini* were introduced into litter of a 40-yr-old *Pinus resinosa* forest at Colfax, WI, and allowed to choose their overwintering microhabitat. To aid in locating the animals later, beetles were released within two 20-cm-diameter PVC pipes that had previously being inserted into the soil with a minimum of disturbance to the soil and litter. In January and March 1999, pipes were removed with soil and litter intact and sliced into 2.5 cm and 1 cm sections respectively. Beetles within each of these depths were separated, identified, and scored as dead or alive.

Results

Freeze Tolerance. All four species of bark beetles were freeze-susceptible. No individuals of any species survived freezing. A few adults were able to move their antennae after freezing but otherwise never recovered normal movements. *I. pini* adults within a litter box exposed to New Hampshire air temperatures sustained complete mortality (100% of 60 individuals). Some immatures of *I. pini* and *I. grandicollis* survived temporarily after brief freezing but were apparently injured because they were unable to resume development. There was no survival of larvae or pupae in logs exposed to −17°C for 7 d; 1 mo after treatment, logs contained a single fresh gallery that was excavated by one adult female and contained no eggs. In contrast, the control logs contained 13 new galleries with eggs and larvae.

When exposure to low temperatures was brief (0.2 h), the probability of survival for *I. grandicollis* and *D. frontalis* was accurately predicted by the mean and standard deviation of supercooling points measured on a subset of the same animals (Table 1). When exposure to low temperatures was increased to 48-72 h, the supercooling point still accurately predicted survival of *I. grandicollis*, but *I. pini* and *D. frontalis* had significantly lower survival than expected based on the probability of freezing (Table 1). Apparently, supercooling point is an accurate measure of maximum cold tolerance, but sustained chilling can sometimes produce mortality without causing water crystallization.

Table 1. Survival of beetle adults exposed to temperatures near the average supercooling point

Species	Origin	No. of adults	Exposure temperature, °C	Exposure duration, h	Observed survival, %	Expected[a] survival, %	Chi-square	*P*
I. grandicollis	WI	30	−8	0.2	70	78	1.06	0.30
I. grandicollis	WI	68	−9	72	65	66	0.05	0.83
I. grandicollis	LA	37	−10	0.2	100	95	1.87	0.17
I. pini	WI	50	−12.3	48	72	91	23.04	0.0001
I. pini	WI	60	−11.3	72	25	62	34.49	0.0001
D. frontalis	LA	17	−12.5	0.2	41	57	1.69	0.19
D. frontalis	LA	100	−7	48	25	85	276.1	0.0001

Chi-square statistic tests the null hypothesis that survival equals the probability of not freezing.

[a] Expected survival calculated from a normal distribution with mean and standard deviation of supercooling point measured on a matched set of control animals using the normal protocol (cooling rate of 0.20°C/min).

Contrary to our expectations, adults were more cold tolerant than larvae and pupae in all species (Table 2). Larvae tended to be more cold tolerant than pupae (Table 2). At least in the case of *I. pini*, there was no conspicuous difference in average supercooling point between first instars and third instars (mean ± SE = −8.82 ± 0.55 versus −7.17 ± 0.75, respectively; F = 3.12; df = 1, 18; P = 0.09). Eggs of *I. pini* had relatively high cold tolerance, with values similar to adults (mean ± SE = −15.63 ± 1.72).

Seasonal Patterns in Cold Tolerance. *D. frontalis* and southern populations of *I. grandicollis* showed no seasonal changes in the cold tolerance of any life stage (Table 2 shows winter months). Values remained similar during April, May, and September for *I. grandicollis* (supercooling points ± SE = −13.82 ± 1.08, −10.55 ± 0.56, and −11.88 ± 0.30, respectively); and were slightly lower for *D. frontalis* in June (−14.07 ± 0.31). There were no systematic trends across seasons for either population. In contrast, there were strong seasonal patterns in the supercooling point of *I. pini* and northern populations of *I. grandicollis* (Figs.1–2; date effect: F = 2.81; df = 9, 222; P = 0.004 for *I. pini* and F = 5.73; df = 5, 135; $P < 0.001$ for *I. grandicollis;* the effect of date was significant even excluding the anomalously low supercooling point observed in August 1998 for *I. pini*). During 1997–1998, lower lethal temperature of *I. pini* from Wisconsin declined in November and then increased back to near summer levels by midwinter (Fig. 1). During the following winter (1998–1999), supercooling points followed a similar trend, except that the supercooling points increased later in the winter and less dramatically than in 1997–1998. During 1997–1998, *I. grandicollis* and *I. perroti*, like *I. pini*, had lower supercooling points in early winter than in midwinter (mean ± SE = −20.32 ± 0.47 versus −14.18 ± 1.83 in November versus January for *I. grandicollis* and −16.43 ± 1.18 versus −13.43 ± 1.12 for *I. perroti*). *I. grandicollis* showed a somewhat different pattern during 1998–1999 in that the lowest supercooling points were in the middle of the winter (Fig. 2).

Acclimation Experiments. *Short-Term Acclimation.* Throughout the winter, in all treatments, all four species responded rapidly to warming by beginning to move and walk within 5 min of being moved to room temperature. However, there was no evidence for short-term changes in cold tolerance of any species. The supercooling point of *D. frontalis* adults acclimated at −5°C for 48 h before measurement did not differ from that of animals remaining at 22°C before measurement (mean ± SE = −11.42 ± 0.72 versus −10.47 ± 0.56, F = 1.06; df = 1, 46; P = 0.31). Similarly, there was no effect of warming *I. pini* adults for 1–3 h before measurement of supercooling point: mean ± SE = −14.60 ± 1.16 versus −15.12 ± 1.76 versus −12.96 ± 1.76 for no warming, 1 and 3 h warming, respectively (F = 0.42; df = 2, 23; P = 0.66). Nor were there differences between *I. pini* adults warmed for 72 h at 22°C versus those that were measured immediately after being retrieved in January from their overwintering microcosms (mean ± SE of warmed versus control = −8.11 ± 0.9 versus −9.00 ± 0.69 for field microcosms and −7.3 ± 0.31 versus −8.47 ± 0.94 for laboratory microcosms at 0°C). Furthermore, there were no differences between the supercooling point of *I. pini* adults measured immediately after removal from the field at the normal cooling rate of 0.20°C/min versus beetles cooled very slowly (cooling rate of 0.003°C/min) versus beetles cooled for 48 h at

Table 2. Supercooling temperatures during winter months for *Ips* spp. and *D. frontalis*

		Supercooling point ± SE (°C)					
Species	Stage	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Ips spp. (WI)	Larvae		−12.99 ± 1.11	−8.35 ± 0.99	−8.95 ± 0.88	−6.1 ± 0.84	
	Pupae		−12.33 ± 0.94	−8.20 ± 0.66	−8.44 ± 0.24	−5.05 ± 0.14	
D. frontalis	Larvae	−12.95 ± 0.23	−12.40 ± 0.30				−8.98 ± 0.40
	Pupae	−9.54 ± 0.61	−11.24 ± 0.53				−7.43 ± 0.16
	Adult	−12.23 ± 0.38	−13.41 ± 0.27	−12.11 ± 0.41	−12.81 ± 0.7		−10.83 ± 0.44
I. grandicollis (LA)	Adult	−11.72 ± 0.87	−12.46 ± 0.71		−11.72 ± 0.49		−12.24 ± 0.79

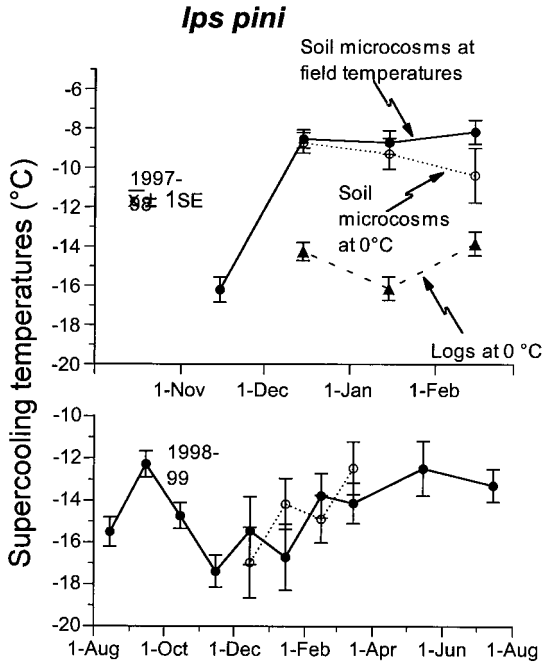

Fig. 1. Seasonal patterns in the supercooling point of *I. pini* adults during winters of 1997–1998 and 1998–1999. Samples include beetles that were acclimated in soil microcosms at field temperatures, in soil microcosms at a constant 0°C, and within logs at 0°C.

0.003°C/min and then warmed for 48 h at 22°C and then measured with normal cooling rate: mean ± SE = −9.00 ± 0.73 versus −7.70 ± 1.69 versus −9.51 ± 1.19, respectively (F = 0.38; df = 2, 39; P = 0.68). Finally, mortality was not affected by cooling rates; there were no differences between the survival of *I. pini* adults cooled as slowly as 0.003°C/min until −12.3°C (=mean supercooling point + 1 SD) compared with beetles placed directly at −12.3° (5 of 18 versus 3 of 17)

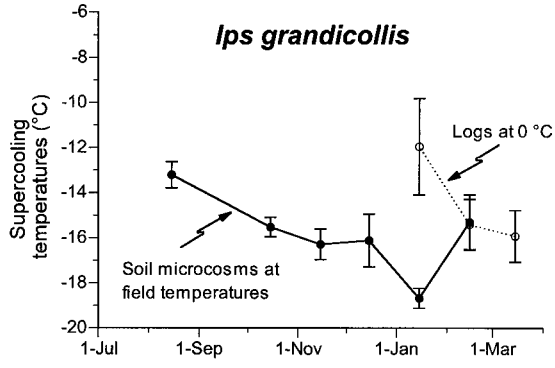

Fig. 2. Seasonal patterns in the supercooling point of northern populations of *I. grandicollis* adults during winter of 1998–1999. Samples include beetles that were acclimated under field conditions, and in soil microcosms at a constant 0°C.

(G = 0.52, df = 1, P = 0.47). For both treatments, mortality was indistinguishable from that expected based on the mean and standard deviation of supercooling points measured with the normal cooling rate of 0.20°C/min (χ^2 = 0.25; df = 1; P = 0.60).

Long-Term Acclimation. There were no changes in the supercooling point of *D. frontalis* adults from the field measured in October compared with those adults that were acclimated for 4 mo at 0°C before measurement: mean ± SE = −12.23 ± 0.40 versus −12.85 ± 0.42, F = 1.12; df = 1, 42; P = 0.29). Apparently, *D. frontalis* can survive and slowly develop even at 0°C, because there were individuals of all life stages alive after 4 mo at 0°C. Similarly, southern populations of *I. grandicollis* showed no obvious acclimation after 6 wk at 0°C: supercooling point (mean ± SE) of beetles measured in March from natural populations versus a subset of those beetles measured in May after 6 wk at 0°C versus another set of beetles collected from nature in May = −12.24 ± 0.76 versus −9.70 ± 1.23 versus −10.09 ± 0.84 (F = 2.48; df = 2, 43; P = 0.09).

During 1997–1998, there were no differences in the supercooling points of *I. pini* adults in litter microcosms in the field compared with those in litter microcosms in the incubator at 0°C (F = 2.31; df = 1, 197; P = 0.13 for main effect of environment; F = 0.71; df = 2, 197; P = 0.49 for date × environment interaction; Fig. 1). There were differences in 1997–1998, but not in 1998–1999, between beetles overwintering in the microcosms in the field and beetles overwintering in logs at 0°C (1997–1998: F = 69.82; df = 1, 191; P < 0.0001 for main effect of environment and F = 0.35; df = 2, 191; P = 0.70 for date × environment interaction; 1998–1999: F = 0.36; df = 1, 154; P = 0.55 for effect of environment; F = 0.66; df = 3, 154; P = 0.57 for date × environment interaction; Fig. 1).

Southern and northern populations of *I. grandicollis* differed in their winter behavior when compared in common environments. Under greenhouse temperatures, the southern population colonized fresh logs within 1 wk during mid-December 1998, whereas northern populations of *I. grandicollis* and *I. pini* needed 1 mo before any individuals initiated galleries and began to oviposit. Larvae of southern populations survived for 7 wk in logs at 0°C but appeared to completely cease development. At the same temperature, larvae from northern populations were able to develop slowly (the proportional abundance of larva:pupa: adults changed from 28:41:31 in November to 0:0:100 in March). Southern populations did not survive field temperatures in New Hampshire (minimum air temperature = −27°C) and died in the logs without going to the soil; under the same conditions, northern populations entered the litter beneath the logs and largely survived (Fig. 3; chi-square comparing survival of adults in field versus greenhouse for Louisiana populations = 258.24, df = 1, P < 0.0001; same chi-square for Wisconsin populations = 1.28, df = 1, P = 0.26). There were no significant differences in the supercooling point of beetles in the field versus the greenhouse versus an incubator at 0°C, although supercooling temperatures tended to be lower in field

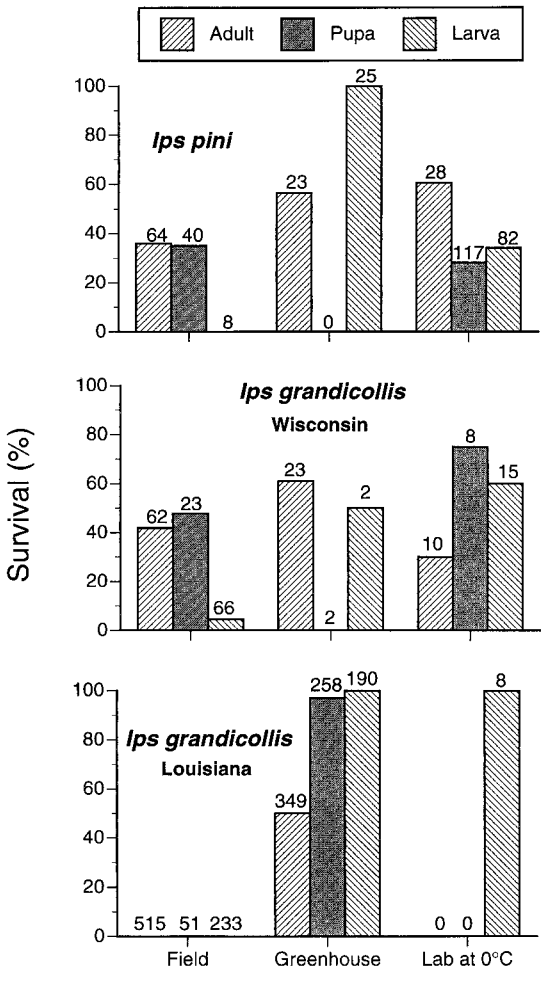

Fig. 3. Survival of *I. pini* from Wisconsin and *I. grandicollis* from Wisconsin and Louisiana after 7 wk of exposure to winter temperatures in New Hampshire, greenhouse temperatures of ≈25°C, or a constant temperature of 0°C. Numbers indicate sample size.

individuals for both species (for *I. pini*, mean ± SE = −17.39 ± 1.42 versus −14.56 ± 2.14 versus −15.79 ± 1.03, $F = 0.71$; df = 2, 50; $P = 0.49$; $n = 16, 7, 30$, respectively; for *I. grandicollis* from Wisconsin, mean ± SE = −16.80 ± 1.07 versus −15.94 ± 1.20 versus −13.25 ± 1.34, $F = 2.20$; df = 2, 43; $P = 0.12$; $n = 21, 15, 12$, respectively). The supercooling point of *I. grandicollis* from Louisiana in the greenhouse was −13.36 ± 0.54 ($n = 43$).

Water and Lipid Content. The water content of *I. pini* overwintering in the field declined from 61% in late summer to a low of 47% in December before gradually increasing during the remainder of the winter (Fig. 4; $F = 28.72$; df = 6, 163; $P < 0.0001$). The water content of northern populations of *I. grandicollis* in the field remained relatively stable from November through February, (Fig. 4; $F = 3.47$; df = 3, 44; $P = 0.02$ for date effect). The lipid content of *I. pini*

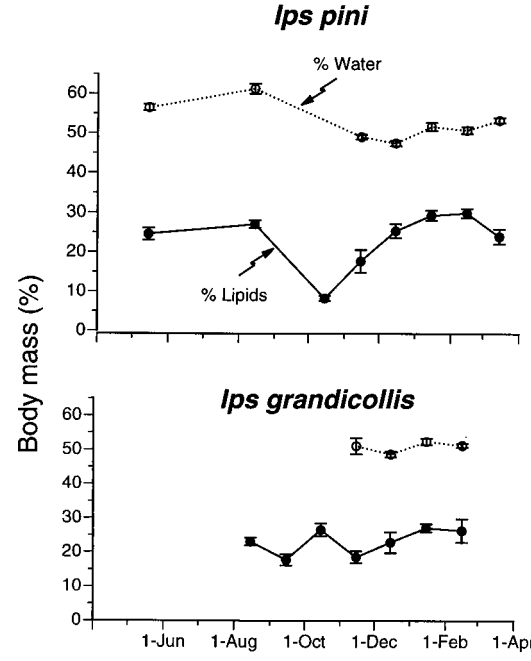

Fig. 4. Seasonal patterns of lipid content and water content of *I. pini* and *I. grandicollis* adults that were acclimated to field temperatures during 1998–1999.

changed markedly through the winter (Fig. 4; $F = 33.71$; df = 6, 162; $P < 0.0001$). A sharp decline from 27.0 ± 0.94% in August to 8.2 ± 0.62% in October (mean ± SE) coincided with a lowering of the supercooling point from −12.3 ± 0.22°C to −15.3 ± 0.80°C (Fig. 1). Among individual beetles measured on the same date in October, animals with lowest lipid content also had the lowest supercooling points (Fig. 5). The lipid content of *I. pini* gradually returned to 25–30% by February. *I. pini* that overwintered at 0°C in the laboratory showed a very similar seasonal pattern (returning from a low of 9% in October to 20–25% in February and March). In contrast, *I. grandicollis* did not show any strong seasonal changes in lipid content

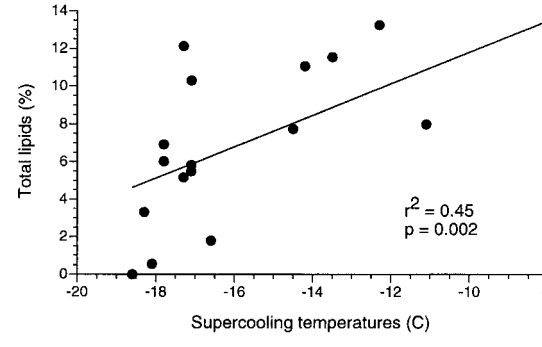

Fig. 5. Relationship between supercooling points and lipids content of *I. pini* adults measured in October 1998.

Table 3. Overwintering microhabitats of *I. pini* and *I. grandicollis* adults in January and March 1999

Depth below litter surface, cm[a]	*Ips pini*			*Ips grandicollis*		
	No.	% alive	% in stratum	No.	% alive	% in stratum
			January 1999			
<2–3 (litter)	47	55	22	465	85	81
2.5–5	163	82	77	108	88	19
5–7.5	2	50	1	0	0	0
7.5–12	0	0	0	0	0	0
			March 1999			
<1	1	0	1	3	100	1
1–2	6	50	8	128	89	32
2–3	46	98	64	247	99	61
3–4	19	100	26	24	100	6
4–6	0	0	0	0	0	0

[a] Litter was ≈3 cm of pine needles.

either in the field (Fig. 4) or at 0°C in the laboratory, although there were some detectable differences among dates ($F = 3.53$; df = 6, 161; $P = 0.003$). Adults of both *I. pini* and *I. grandicollis* that overwintered in the field had significantly higher lipid content in the late winter, especially in February, than those at 0°C in the laboratory: mean ± SE for field versus laboratory = 29.8 ± 1.84 versus 15.6 ± 2.49 for *I. pini* ($F = 21.70$; df = 1, 124; $P < 0.0001$) and 26.0 ± 2.54 versus 13.2 ± 2.54 for *I. grandicollis* ($F = 10.35$; df = 1, 45; $P = 0.002$).

Overwintering Habitats. When allowed to choose their own overwintering microhabitats, most *I. pini* adults moved to within 1 cm of the bottom of the litter layer, which was ≈2–3 cm deep and comprised chiefly of pine needles (Table 3). In the January sample, 78% of 210 *I. pini* were beneath the litter layer, compared with only 19% of 573 *I. grandicollis* ($\chi^2 = 234.54$, df = 1, $P < .0001$). The majority of *I. pini* had burrowed about one body length into the sandy soil below the litter layer, whereas *I. grandicollis* almost never burrowed into the soil beneath the litter. *I. pini* were also in deeper microhabitats than *I. grandicollis* in the March samples (Table 3; $\chi^2 = 39.09$; df = 2, $P < 0.001$ for comparison of <2 cm versus 2–3 cm versus >3 cm).

Ips that had chosen their own overwintering microhabitats in Wisconsin had similar, but slightly warmer, supercooling points than individuals from the same population that experienced field and laboratory acclimation regimes in New Hampshire (compare Fig. 1 to Fig. 6). The lipid content and water content of these beetles also matched that of field and laboratory acclimation regimes: mean ± SE for January and March = 26.5 ± 1.52 and 22.6 ± 1.34% lipids for *I. pini;* 28.2 ± 1.02 and 23.4 ± 1.50% lipids for *I. grandicollis;* 52.0 ± 0.63 and 53.9 ± 0.55% water for *I. pini;* and 50.8 ± 0.47 and 52.0 ± 0.70% water for *I. grandicollis.* There were no significant differences in supercooling point, lipid content, or water content between *Ips* that were overwintering at different depths, although there was a tendency for beetles that were deeper to have lower supercooling points (Fig. 6). Overwintering survival in natural habitats was ≈80% for *I. pini* and ≈90% for *I. grandicollis.* Survival was significantly lower in *I. pini* that overwintered in the litter (55% survival at <2–3 cm) compared with those that overwintered in the soil beneath the litter (82% survival at >3 cm; $\chi^2 = 14.54$, df = 1, $P < 0.01$; Table 3). Survival of *I. grandicollis* did not vary with microhabitat. By comparison, overwintering survival in field microcosms in New Hampshire was ≈70% for both *I. pini* and *I. grandicollis* during 1998–99 and ≈ 37% for *I. pini* during 1997–1998 (Fig. 7).

Discussion

Acclimation and Winter Behavior. During 1997–1998, the supercooling point of Wisconsin populations of *I. grandicollis*, *I. pini*, and *I. perroti* reached their minimum during autumn, and then increased by midwinter to values as high as during summer. This pattern, which matches the autumn-dynamic strategy characterized by Merivee (1978), could be an adaptive response to climatic patterns in the Great Lakes

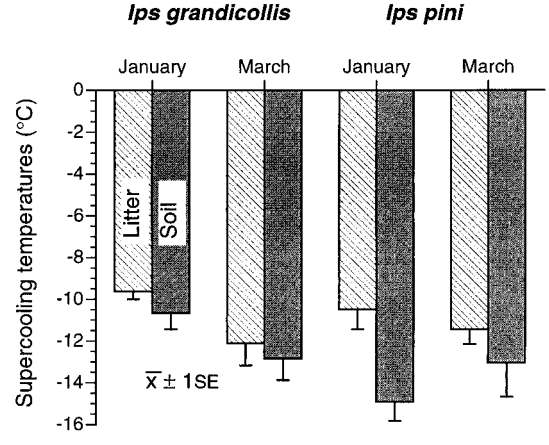

Fig. 6. Supercooling points of *I. pini* and *I. grandicollis* adults that selected their own overwintering sites within the litter or soil at Colfax, WI, during 1998–1999.

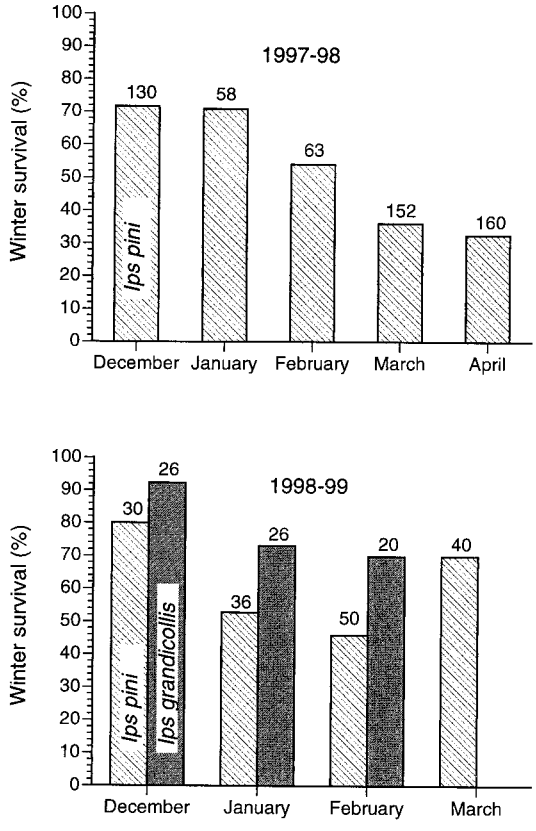

Fig. 7. Survival of *I. pini* and *I. grandicollis* adults in soil microcosms at field temperatures during 1997–1998 (upper) and 1998–1999 (lower).

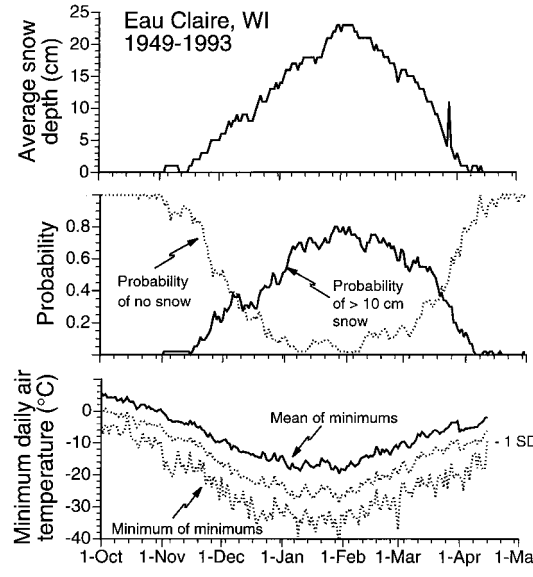

Fig. 8. Climatic patterns of snow cover and air temperatures in west-central Wisconsin.

states. For insects that overwinter in the forest litter, snow cover provides a strong buffer against low temperatures (Bale 1991). Consequently, the greatest risk of mortality from low temperatures comes from the combination of no snow and low temperatures. At our study sites in Wisconsin, there is a window of 4–5 wk during the autumn when the probability of no snow is >0.2 and air temperatures can drop below −20°C: 5 November to 15 December in Eau Claire, WI (Fig. 8). During most years, air temperatures drop below the lower lethal temperature for *Ips* adults sometime during November (mean ± SD of November minimum air temperature = −18.7 ± 4.5 for Eau Claire). Based on the same climatic criteria, there also appears to be a window of vulnerability to freezing during spring (7 March to 7 April), but we saw no evidence in either year of increased cold tolerance during spring. In addition to insulating beetles from winter temperature extremes, snow cover may be of additional ecological importance by allowing *I. pini* to raise their water content back toward summer levels and reduce the risk of dessication (Fig. 4).

The autumnal decline in supercooling point, and return to near summer levels during winter, was evident in *I. pini* adults placed within litter microcosms in the field and in the incubator at a constant temperature of 0°C. The return to warmer supercooling points was less rapid during the winter 1998–1999, but still evident by late winter. Furthermore, some beetles that chose their own overwintering sites in Wisconsin during 1998–1999 also had relatively warm supercooling points of −10°C when they were measured in January.

Temperature and photoperiod are the most common environmental cues that trigger acclimatization of insect cold tolerance (Sømme 1964, Baust and Miller 1972, Baust 1982, Horwath and Duman 1982, Rojas et al. 1983, Nordin et al. 1984, Baust and Rojas 1985, Pio and Baust 1988, Beck 1991). Extremely rapid cold-hardening has been observed in some insects (Lee et al. 1987). In our studies, all species, populations, and life stages, under all acclimation regimes, showed an immediate response to temperature by beginning to move and crawl within 5 min of exposure to room temperatures. However, an assortment of temperature manipulations provided no evidence of plasticity in cold tolerance. We were unable to induce changes in supercooling point with any acclimation regime, even a month at greenhouse temperatures. Consequently, it is difficult to explain seasonal changes in cold tolerance, or year-to-year variation in the seasonal pattern of change, based upon differences in temperature regime. The autumnal decline in supercooling points may be regulated by endogenous rhythms, photoperiod, or a combination.

Comparisons of *I. grandicollis* populations in reciprocal thermal environments provided further evidence that seasonal changes in cold tolerance are not a simple response to temperature regime. Even when exposed to winter conditions in New Hampshire, *I.*

grandicollis populations from Louisiana remained active and attempted to colonize new logs. They died in the process. In the same environment, Wisconsin populations survived by moving into the litter beneath the logs. Southern *I. grandicollis* at greenhouse temperatures also colonized new logs, but successfully, whereas the Wisconsin population remained largely inactive, just as they did under field temperatures. Thus, the dramatic differences between populations in their survival under New Hampshire winter temperatures was largely a result of choosing different microhabitats (litter versus pine phloem) with different temperature regimes. We hypothesize that the Wisconsin populations in both environments were in a state of light diapause. Birch (1974) provided evidence of diapause in *I. pini* by showing that their respiration rate and response to pheromones decreased during the winter. Somewhat surprisingly, there were no differences in supercooling point associated with the behavioral differences between populations. The literature contains other examples of a physiological independence between diapause and cold tolerance (Lees 1955, Salt 1961, Sømme 1964, Ring 1972), although there are also numerous examples of a physiological correlation between behavioral inactivity and cold tolerance (Asahina 1969; Mansingh 1971, 1974; Pullin 1996). In contrast to the case for bark beetle adults, our studies showed no evidence of diapause or reduced activity in the larvae or pupae of any species or population. In fact, larvae of both species of *Ips* from Wisconsin continued feeding and development at 0°C. It remains an open question whether endogenous rhythms, photoperiod, or some other cues control the onset and termination of behavioral inactivity in northern populations of *Ips.*

Energy Reserves and Cold Tolerance. There were striking changes in lipid stores associated with the autumnal decline in *I. pini* supercooling points (Figs. 1, 4, and 5). Lipid stores in insects can be associated with high survival, high dispersal ability, and competitive advantage (Hagen and Atkins 1975, Anderbrant et al. 1985, Anderbrant 1988). Lipids can also be directly involved in the biosynthesis of cryoprotectors. Glycerol, one of the most common cryoprotectors (Sømme 1964, 1982), is synthesized from lipids in *Epiblema scudderiana* (Clemens) during the autumn and converted back into lipids and glycogen in the spring (Rickards et al. 1987, Joanisse and Storey 1996). Similarly, seasonal changes in the cold tolerance of *I. pini* could occur via interconversion of lipids and glycerol. If so, fat is hydrolyzed in autumn to yield glycerol, which lowers the supercooling point and acts as a cryoprotectant. Water is used in this step, so the process would tend to dehydrate beetles, thereby further contributing to cold tolerance. Dessication problems are frequently associated with overwintering strategies of insects (Zachariassen 1991). The interplay between cold tolerance and dessication could also be involved in the selection of overwintering microsites. For example, the litter microsites preferred by *I. grandicollis* must commonly reach colder temperatures than the soil microsites selected by many *I. pini* (Table 3), but it could be easier for *I. grandicollis* to maintain high levels of cryoprotectants if the frozen litter is less hyperosmotic to beetles than the sand beneath the litter. The conversion of glycerol back to lipids or glycogen could also be dictated by energetic demands. This physiological model predicts that a significant pool of glycerol accumulates within the hemolymph of *I. pini* during late autumn and then declines during mid winter.

Neither *I. grandicollis* (this study), *I. typographus* (L.) (Khansen et al. 1980) or *D. frontalis* (Hedden and Billings 1977) showed the same pattern as *I. pini* of correlated seasonal changes in lipids, water, and cold tolerance. Apparently, there are differences among bark beetles species in the cryoprotectants or metabolic pathways that produce and break down the cryoprotectants.

Patterns of Cold Tolerance Among Species and Populations. There were important differences among bark beetle species in their adaptations for overwintering. *D. frontalis* did not show any seasonal acclimatization to winter temperature. Its supercooling points were the same during winter months as during the rest of the year. Neither does this species have any behavioral traits that allow escape from winter temperatures. Instead, individuals of this species remain active and exposed to temperatures near air-temperature while feeding and reproducing within the phloem of infested trees. This suggests that the northern distribution limits of *D. frontalis* could be constrained by winter temperatures. Indeed, climatic analyses indicate that this species occurs as far north as it possibly could given its cold tolerance (Ungerer et al. 1999). However, *D. frontalis* is unusually well adapted for remaining active during the winter in that adults are able to fly at temperatures as low as 6.7°C, the lowest flight temperature known for any bark beetle (Moser and Thompson 1986).

There is a geographical pattern in the winter biology of *Ips* species. Southern populations of *I. grandicollis*, which are sympatric with *D. frontalis*, have the same overwintering behavior as *D. frontalis* in that they continue to reproduce and develop under the bark of their host plants throughout the winter and do not show any seasonal acclimatization of lower lethal temperature. However, there is regional differentiation in the overwintering biology of *I. grandicollis* that is apparently important in allowing their distribution to extend well north of *D. frontalis.* Pine bark provides insignificant insulation against low air temperatures (Bolstad et al. 1997; unpublished data). Therefore, Wisconsin populations migrate during winter into the forest litter, where they are insulated from extremes in air temperatures. They also acquire greater cold tolerance during the winter than populations in Louisiana and Alabama. Our population comparisons in common environments suggest that this geographic variation in winter biology is genetically controlled.

Investigations of insect cold tolerance have frequently attempted to classify insect species based on their overwintering strategies (Salt 1961, Merivee 1978, Young and Block 1980). However, our study adds

to the evidence that ecologically important aspects of winter biology can be flexible within species (Sømme 1965, Baust et al. 1979, Baust and Lee 1981, Bale 1993, Tanaka 1997; but see Dautel and Knuelle 1996). In some species, overwintering biology can vary from year to year within the same population (Chansler 1966, Kukal and Duman 1989, Bentz and Mullins 1999), even to the extent of switches between freeze tolerance and freeze intolerance (Duman 1984, Horwath and Duman 1984). Vernom et al. (1996) reported a mix of freeze-tolerant and freeze-intolerant individuals within the same population. Investigations of *I. grandicollis* populations along a cline from Louisiana to Wisconsin could help to clarify the ecological factors that shape the evolution of overwintering strategies.

The supercooling points of all four scolytid species measured here were always above −20°C, which is relatively warm compared with those of some scolytids that spend the winter under the bark [−31 to −39°C in *Pityogenes chalcographus* (L.), *Polygraphus polygraphus* (L.), and *Crypturgus cinereus* (Herbst)] but matches that of *I. sexdentatus* (Boern) and *Tomicus piniperda* (L.), which have minimum supercooling points of approximately −18°C and typically overwinter in the litter (Sømme 1982). Similarly, Chansler (1966) reported 100% mortality for *I. confusus* (LeConte) and *I. lecontei* Swaine from Arizona and New Mexico that were exposed to temperatures of −17 to −18°C. Species differences among immature stages are more dramatic. Temperatures of −10°C were usually lethal to immature stages of *I. pini* and *I. grandicollis*, so they are less cold tolerant than adults of the same species and far less cold tolerant than larvae of some other species that can sustain temperatures of −31 to −53°C (Miller 1982, Sømme 1982). In Wisconsin, large numbers of *I. pini* immatures can be found within the phloem of infested logs nearly every winter, and significant numbers of *I. grandicollis* in many winters. Our cold tolerance measurements and climatic analyses suggest that these animals must nearly always perish. Indeed, we have never found any immatures that survived the winter in Wisconsin. Other reports indicate that *I. pini* immatures also cannot overwinter successfully in Minnesota (Leach et al. 1934, Orr 1935) or Ontario (Thomas 1961). Apparently, overwinter mortality of immatures exerts strong effects in most years on *I. pini* populations in the Great Lakes region.

There appear to be at least two different strategies for bark beetles that inhabit cold environments. Some species have a high cold tolerance of both adults and larvae, which allows beetles of all life stages to survive winters within the habitat where they feed (phloem of host trees). Other species have limited cold tolerance, but compensate with a behavioral strategy in which adults migrate to the forest soil to overwinter. Species with the former strategy have greater flexibility in their life histories. For example, *D. rufipennis* (Kirby) has a facultative multi-year life cycle in high latitude forests that depends on larvae being able to survive the winter within their feeding habitat. Populations with the latter strategy may be quite sensitive to climatic variation that results in a phenological mismatch between the onset of winter and the movement of adults into the soil. For example, *I. pini* populations in Wisconsin could suffer very high mortality in a year with cool late-summer temperatures that prevent completion of the last generation before winter and, conversely, could have very high population growth during a year when fall temperatures allow an additional complete generation. In general, populations with the latter strategy may be more sensitive to environmental vagaries because population persistence depends on the survival of one life stage that is concentrated within one habitat. *I. acuminatus* Gyllenhall may represent an exception to this dichotomy of strategies in that adults are the only life stage that survive the winter in Norway, but they have a relatively low supercooling point (approximately −30°) and remain under the bark of their host trees throughout the winter (Gehrken 1985).

Improved understanding of cold tolerance and overwintering behavior may have applied value in predicting the population dynamics of forest pests (Werner 1978; Virtanen et al. 1996, 1998; Ungerer et al. 1999). Improved understanding of variation among species and populations may have value in assessing the risks associated with exotic pests (McClure 1989, Niemelä and Mattson 1996) and the potential benefits from biological control agents (Turnock et al. 1990). In North America, the spread of *Adelges tsugae* Annand (Homoptera: Adelgidae) seems to be constrained by winter temperatures (McClure 1989). In Australia, introduced populations of *I. grandicollis* can sustain high mortality during winter (Lawson 1993), partly because they remain within their host trees throughout the winter, rather than moving into the forest floor where they would be buffered by temperature extremes (suggesting that the introduction was from southern North America). Secondary introductions of those species from northern latitudes could increase damage. A better knowledge of overwintering biology, in combination with physiologically explicit climatic analyses, could aid in projecting the regions that are at risk from introduced pest species that have been recently discovered in North America such as *Tomicus piniperda* (L.) (Scolytidae; Carter et al. 1996) and *Anoplophora glabripennis* (Motschulsky) (Cerambycidae; Cavey et al. 1998).

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