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Winter mortality and supercooling point of the spruce beetle (Coleoptera: Curculionidae) not affected by host tree vigor in Nova Scotia, Canada

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ABSTRACT

At epidemic levels, the spruce beetle, *Dendroctonus rufipennis* **Kirby (Coleoptera**: Curculionidae), is able to spread from moribund to healthy trees by pheromone-mediated mass attacks, allowing insects to access additional, higher-quality food resources. Because host characteristics influence insect performance, we investigated the effect of host vigor on adult spruce beetle overwintering biology in Nova Scotia. Spruce beetles did not produce more cryoprotectants and did not exhibit lower supercooling points or greater winter survival on more vigorous trees. However, they exhibited high cold hardiness, as shown by low supercooling points and mortality rates (around 15%), for all levels of host vigor. Supercooling points reached temperatures as low as -44 °C in January, even though air temperature at the site did not fall below -23 °C. Also, as expected, the insect's lipid content decreased during the fall period but, surprisingly, was inversely related to tree vigor.

RÉSUMÉ

À des niveaux épidémiques, le dendroctone de l'épinette, *Dendroctonus rufipennis* **Kirby (Coleoptera** : Curculionidae), acquiert la capacité de se propager des arbres moribonds aux arbres sains en procédant à des attaques de masse. Cela lui procure une source de nourriture supplémentaire et de meilleure qualité. Puisque les caractéristiques de l'hôte ont une influence sur les performances des insectes, nous avons évalué l'effet de la vigueur de l'hôte sur la biologie hivernale des adultes du dendroctone de l'épinette en Nouvelle-Écosse. Ceux-ci n'ont pas produit plus de cryoprotectants et n'ont pas présenté des points de surfusion plus bas, ni un plus haut taux de survie sur les arbres plus vigoureux. Tout hôte confondu, ils ont présenté des points de surfusion et des taux de mortalité relativement bas (15%), démontrant ainsi une grande résistance au froid. Les points de surfusion sont descendus jusqu'à -44 °C en janvier alors que la température la plus froide enregistrée sur le site était de -23 °C. Comme prévu, le contenu en lipides chez les adultes a diminué durant l'automne, mais de façon, surprenante, il était inversement relié à la vigueur de l'arbre hôte.

INTRODUCTION

The spruce beetle, *Dendroctonus rufipennis* Kirby (Coleoptera: Curculionidae), is a destructive pest of mature spruce trees (Coulson and Witter 1984). This insect can be found throughout the geographic range of its hosts in North America (Bright 1976) from the western United States and Alaska to northeastern United States and

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northeastern Canada (Coulson and Witter 1984; Holsten et al. 1999). Spruce beetles overwinter mainly as larvae or teneral adults, depending on the length of the life cycle generated by the climatic conditions prevailing in the habitat (Werner and Holsten 1985a,b; Bentz et al. 2010).

Dendroctonus sp. are generally considered to be aggressive species as they prefer healthy and vigorous host trees (Coulson and Witter 1984; Hard 1985, 1989; Wallin and Raffa 2004) when populations reach epidemic levels. However, at endemic levels, these insects typically infest only stressed and moribund trees whose defenses are weakened.

Mortality from cold exposure is considered a key temperature-related factor in bark beetle population dynamics (Bentz and Mullins 1999; Bentz et al. 2010). Cold hardiness is the ability of an insect to survive at low temperatures and it has been well documented for a number of insect species in North America (Leather et al. 1993). With diapause, cold hardiness is considered as essential for winter survival in most insects (Denlinger 1991).

Strategies to survive extreme cold temperatures result from changes in behavioral and physiological processes in insects (Lombardero et al. 2000). Cold acclimation is the seasonal increase in cold hardiness from summer to winter (Leather et al. 1993). Generally, feeding ceases and an increase in levels of antifreeze agents like polyols and proteins occurs in response to a decrease in temperatures during autumn (Leather et al. 1993). Although more intermediate cold tolerance strategies exist to enable insects to survive low temperatures (Bale 1987, 1993; Sinclair et al. 2003), these have generally been divided into two categories: freeze-tolerant and freeze-intolerant (Salt 1961; Khani and Moharramipour 2010). The spruce beetle is considered as a freeze-intolerant insect (Miller and Werner 1987), which means it cannot survive the formation of ice in its body tissues and fluids (Leather et al. 1993). Freeze-intolerant insects predominate in cool and cold climates and freeze-tolerant species are more abundant in regions where winter frosts are extreme (Somme 1982; Leather et al. 1993). However, Turnock and Fields (2005) presented a more sophisticated situation for different habitats in temperate cold continental (e.g., Nova Scotia) and dry climates, where the incidence of freeze-tolerance among species increases with the severity of overwintering habitat. Bark beetles like the spruce beetle and the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae), accumulate cryoprotectants like glycerol as temperatures decline in fall (Miller and Werner 1987) and then acquire cold hardiness by lowering their supercooling point (SCP). The SCP can be measured by the temperature at which spontaneous freezing occurs and is used as an indicator of the lowest lethal temperature that an insect can survive (Leather et al. 1993). The SCP is the best documented parameter for describing levels of cold hardiness (Turnock and Fields 2005). At the SCP temperature, latent heat is emitted during the transition of water into ice (Leather et al. 1993).

Host tree vigor is used in many ways and describes a variety of physiological states (Barbosa and Wagner 1988), but indices are generally based on the expression of relative stem growth (Christiansen et al. 1987). According to Waring et al. (1980), healthy and vigorous trees are able to allocate part of their carbon to the stemwood, a lower priority level than roots or shoots (Waring et al. 1980). Access to vigorous trees provides the insect with an additional, higher-quality food source that is more nutritious (Waring and Pitman 1985; Sundberg et al. 1993), numerically more abundant (Raffa and Berryman 1983), and less likely to shelter interspecific competitors (Reid and Robb 1999; Wallin and Raffa 2001; Raffa et al. 2005). All of these benefits could possibly lead to a greater accumulation of reserve compounds such as lipids and sugars, which are closely related to the overwintering performance of the insect (Lombardero et al. 2000). Lipids are usually the main energy reserve for overwintering insects (Izadi et al. 2011). In freeze-tolerant and intolerant insects, lipids and glycogen are food reserves accumulated during summer and transformed into cryoprotectants for winter (Lencioni 2004). Cold hardiness may also be affected by several factors, including geographic location, environmental conditions, developmental stage, sex and age (Somme 1982). How host tree vigor affects overwintering survival and cold hardiness is not known for the spruce beetle.

Knowing that the performance of an insect under different climatic conditions is particularly useful for forecasting the risk of pest outbreaks and evaluating possible changes in its geographic distribution (Bale and Hayward 2010; Bentz et al. 2010), we tested the hypothesis that the overwintering performance of the spruce beetle would increase with increasing tree vigor. While deeply studied in cold climates, such as the one encountered in interior Alaska (Miller and Werner 1987) and in the Intermountain West (Bentz and Mullins 1999), no data are available for more temperate climates such as the one prevailing in eastern Canada. To assess the effect of host vigor, we evaluated overwintering mortality and cold hardiness of adult spruce beetles collected from trees exhibiting differing degrees of vigor evaluated by measuring annual tree growth rings.

MATERIALS AND METHODS

Study area and beetle sampling

Thirty-one red spruce trees, *Picea rubens* Sarg. (Pinaceae), were selected in a mature forest affected by a spruce beetle infestation near Cape d'Or, Nova Scotia, Canada (45°18'42" N, 64°45'20" W). These trees exhibited different degrees of vigor as judged by their outward appearance and all were infested by beetles. Foliage depletion and discoloration were important factors for this assessment. In fall 2008, four increment cores were taken at breast height (1.3 m) (Mulock and Christiansen 1986) at 90° from each cardinal direction from each tree with special attention being paid to finding the true center of the tree. The sapwood border was marked on all cores based on wood moisture content (Mulock and Christiansen 1986). Annual increments were measured using WinDENDRO software (Regent Instruments Inc. 2009). Tree vigor was assessed by calculating the percent ratio of 2008 annual ring basal area to sapwood basal area (Waring and Pitman 1980). Vigor indices ranged from 0 (dead) to 9.5% (healthiest). Air temperature and phloem temperature beneath the bark of one healthy and one dead tree were measured at 2 h intervals from 27 September 2008 through 24 April 2009 with a stainless steel probe connected to a temperature station (wireless leaf and soil moisture/temperature station, Davis Instruments, Hayward, CA). Probes were attached at a height of 3.5 m on the north side of the tree bole.

Adult spruce beetles were collected from each tree in September, October, November, December 2008, and in January, April 2009 by removing bark samples using a punch (diameter: 10.2 cm) modified based on Furniss (1962). Each time, enough bark samples were removed to obtain at least four beetles from each tree for metabolite analyses. Insect identification was based on Bright (1976). Beetle density was then estimated as counts of beetles per area of bark surface sampled. Mortality was expressed as the percentage of dead beetles over the total number of beetles sampled on a tree each month.

Metabolite analyses: measurement of lipid, trehalose and glycerol contents

Two adult beetles (observation units) were taken from each of the 31 trees on each of the six sampling dates for metabolite analysis. Fresh mass, dry mass, and water content were determined by weighing insects before and after freeze-drying. Lipid content was assessed using the phosphovanillin spectrophotometric method described by Kinn et al. (1994), which consists in dissolving the insects in sulfuric acid and adding a phosphoric acid and vanillin-based color reagent before comparing absorption at 530 nm with cholesterol standards using a spectrophotometer.

Trehalose and glycerol were identified in preliminary tests as the main sugar and polyol found in spruce beetle, respectively. Their levels were quantified using the method of Cho et al. (2007), with slight modifications as follows. Each insect was ground in 200 µL of methanol and centrifuged at 2000 g for 5 min, after which the supernatant was collected. The precipitate was recentrifuged twice and the supernatants were combined. The methanol was evaporated over 1.5 h using a Speed Vac Concentrator (Savant, Farmingdale, NY). The pellet that remained after evaporation was re-suspended in deionized water and filtered through a 0.45 µm syringe filter (Sarstedt, Montréal, QC). Trehalose and glycerol were measured using an HPLC (ProStar 420, Varian Canada, Mississauga, ON) equipped with a column (Sugar-Pak, Waters Corporation, Mississauga, ON) heated to 70°C. A 30 µL aliquot of the insect extract was eluted with deionized water for 20 min at a rate of 1 mL/min. Trehalose and glycerol were detected and quantified by a refractive index detector (ProStar 355, Varian Canada, Mississauga, ON).

Measurement of supercooling point

Two other adult beetles (observation units) were taken from each of the 31 trees on each of the six sampling dates for supercooling point measurements. Beetles were attached with tape to a support frame so that their abdomens were in contact with thermocouples (chromel/constantan Type E, Omega Engineering Inc., Laval, QC). This frame was placed in a Styrofoam box to slow the cooling rate of the samples to about 1 °C/min when placed in a freezer reaching -50 °C. A cooling rate of 1 °C min^{-1} remains the experimental standard (Sinclair et al. 2003). Temperature changes were recorded (±0.01 °C) with a data logger (CR10, Campbell Scientific Inc., Edmonton, AB) (Han and Bauce 1993). The supercooling point was considered as the lowest temperature reached before the release of latent heat associated with crystallization (Leather et al. 1993). Thirty beetles were kept after they reached their supercooling points and placed at ambient temperature on fresh bark to verify that they were dead after freezing.

Statistical analysis

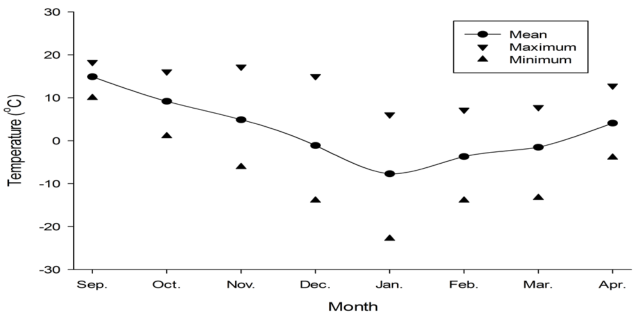
Linear regressions were used to study the association of tree vigor index and insect-related biological performance. Beetle density and mortality were analyzed using a generalized linear mixed model (PROC GLIMMIX, SAS Institute Inc. 2008). Beetle physiological characteristics were analyzed using a mixed model procedure (PROC MIXED, SAS Institute Inc. 2008). Beetle density and sampling time were considered in the mortality and physiological characteristics analysis. Beetle density was included as a covariate to ensure that observed variation was not due to intraspecific competition. Sampling time was evaluated as a subsample.

RESULTS

Study area

Air temperature and temperature beneath the bark of both healthy and dead trees were similar. A small buffer effect was observed beneath the bark of both tree types, but it only delayed by a few hours the time needed to reach the same temperature as the air. January was the coldest month, with minimum air temperature nearly reaching -23 °C (Figure 1). Temperatures recorded on site were representative of the normal trend reported for this region by Environment Canada (2010), in that no unusually cold or warm periods were observed in comparison with previous years.

Figure 1. Air temperature measured 3.5 m above ground at Cape d'Or, Nova Scotia, Canada, in 2008 and 2009.



Beetle density

Beetle density on trees significantly increased with tree vigor ($F_{1,29}$ = 13.42, P < 0.001). Densities varied from 253 to 2035 spruce beetles per m^2, from the least attacked tree to the most attacked one. For a constant area of bark surface sampled, there were 16% more beetles on the most vigorous trees compared with dead trees.

Overwintering mortality

Tree vigor had no significant effect on either adult ($F_{1,29}$ = 0.16, P = 0.69) or larval mortality ($F_{1,29}$ = 0.07, P = 0.79). Adult mortality fluctuated around 15% between sampling dates ($F_{5,149}$ = 2.69, P = 0.02). Larval mortality fluctuated around 10% ($F_{5,91}$ = 13.62, P < 0.0001), with a peak of 39% in January.

Metabolites and supercooling point

Tree vigor had no significant effect on beetle physiological characteristics except for lipid content (Table 1). Mean lipid content decreased from 17.1 to 15.2% of dry mass when tree vigor increased. Yet, the effect of the sampling date was significant for all physiological characteristics (Table 1).

Table 1. Results of analyses comparing physiological characteristics of adult spruce beetle with tree vigor for six sampling dates.

Variables/factors	DF	*F*-value	*P*
Fresh mass (366)			
Tree vigor	1	0.11	0.7394
Sampling date	5	12.73	<0.0001
Dry mass (366)			
Tree vigor	1	0.44	0.5060
Sampling date	5	26.42	<0.0001
Water (366)			
Tree vigor	1	1.74	0.1883
Sampling date	5	62.64	<0.0001
Lipid (366)			
Tree vigor	1	7.42	0.0068
Sampling date	5	21.09	<0.0001
Trehalose (370)			
Tree vigor	1	2.10	0.1481
Sampling date	5	30.85	<0.0001
Glycerol (363)			
Tree vigor	1	2.33	0.1280
Sampling date	5	366.16	<0.0001
Supercooling point (363)			
Tree vigor	1	1.90	0.1695
Sampling date	5	148.71	<0.0001

NOTE: Numbers in parentheses represent sample size.

Seasonal fluctuations in glycerol content were strongly complemented by shifts in supercooling points (Figure 2E,F). Mean glycerol content varied between 0 and 3.3% of dry mass across sampling dates. It was barely detectable throughout late summer to early autumn, and rose in late autumn (November) to a high concentration in mid-winter (January) before decreasing back down to zero in the spring (April).

During 2008 and 2009, lower lethal temperature of *Dendroctonus rufipennis* from Nova Scotia declined in October to reach a minimal threshold in January.

Supercooling means were around -20 °C in autumn. Their value decreased during the period when the insects were producing glycerol to reach a mean of minus 38 °C in January; some individuals even achieved a supercooling point of -44 °C. Both glycerol amounts and supercooling points got back to their autumn level in April.

None of the 30 beetles that were kept for observation after they reached their supercooling points survived, confirming that the spruce beetle is a freeze-susceptible insect.

Water and lipid reserves in the beetle decreased in tandem through autumn and winter before rising again in the spring (Figure 2C,D). Trehalose had a completely different pattern: increasing constantly until December (0.67% of dry mass), dropping abruptly to 0.08% of dry mass in January, and rising back almost to its previous level in April (Figure 2E). All of this variation resulted in an increase in beetle dry mass until November followed by a gradual decline (Figure 2B), while fresh mass fluctuated around 15 mg with a peak at 17.6 mg in November (Figure 2A).

DISCUSSION

Our study clearly demonstrated that differences in host vigor index ranging from 0 to 9.5% (Waring and Pitman 1980) evaluated with relative annual tree ring growth have no measured effect on some of the biological parameters related to the overwintering biology of the spruce beetle. It should be noted that all trees selected in this study were chosen because they had sufficient numbers of beetles for the experiment. Therefore, they possibly were not representative of the entire range of vigor that is exhibited by red spruce, and perhaps the range covered was too narrow to detect the effects of tree vigor on beetles. Moreover, the maximum vigor expressed by red spruce in terms of the Waring and Pitman index is not known. Studies performed on conifers (Waring and Pitman 1980; Waring et al. 1980; Kytö et al. 1998), including some spruce species (Mulock and Christiansen 1986; Münster-Swendsen 1987; Kytö et al. 1996), have not reported vigor estimates exceeding 15%. Thus, we are confident that the trees we selected were representative of low- and mid-range vigor of trees attacked by the spruce beetle. It would have been useful to also select unattacked, healthy trees to have a better picture of what the values we found mean. Further research should be conducted to identify the full range of vigor for trees that are attacked by the spruce beetle. For example, Waring and Pitman (1980) found that the mountain pine beetle only attacks individual lodgepole pines, *Pinus contorta* Dougl. ex Loud. (Pinaceae), with vigor estimates of 15% or less, and is able to kill trees exhibiting vigor of 8% or less. Such information would allow us to characterize trees that are more susceptible to insect attack and could be a useful tool for managers. A more appropriate vigor index could be the specific volume increment (SVI), as discussed by Bevilacqua (2002). The SVI could be more appropriate as it is less correlated with tree age.

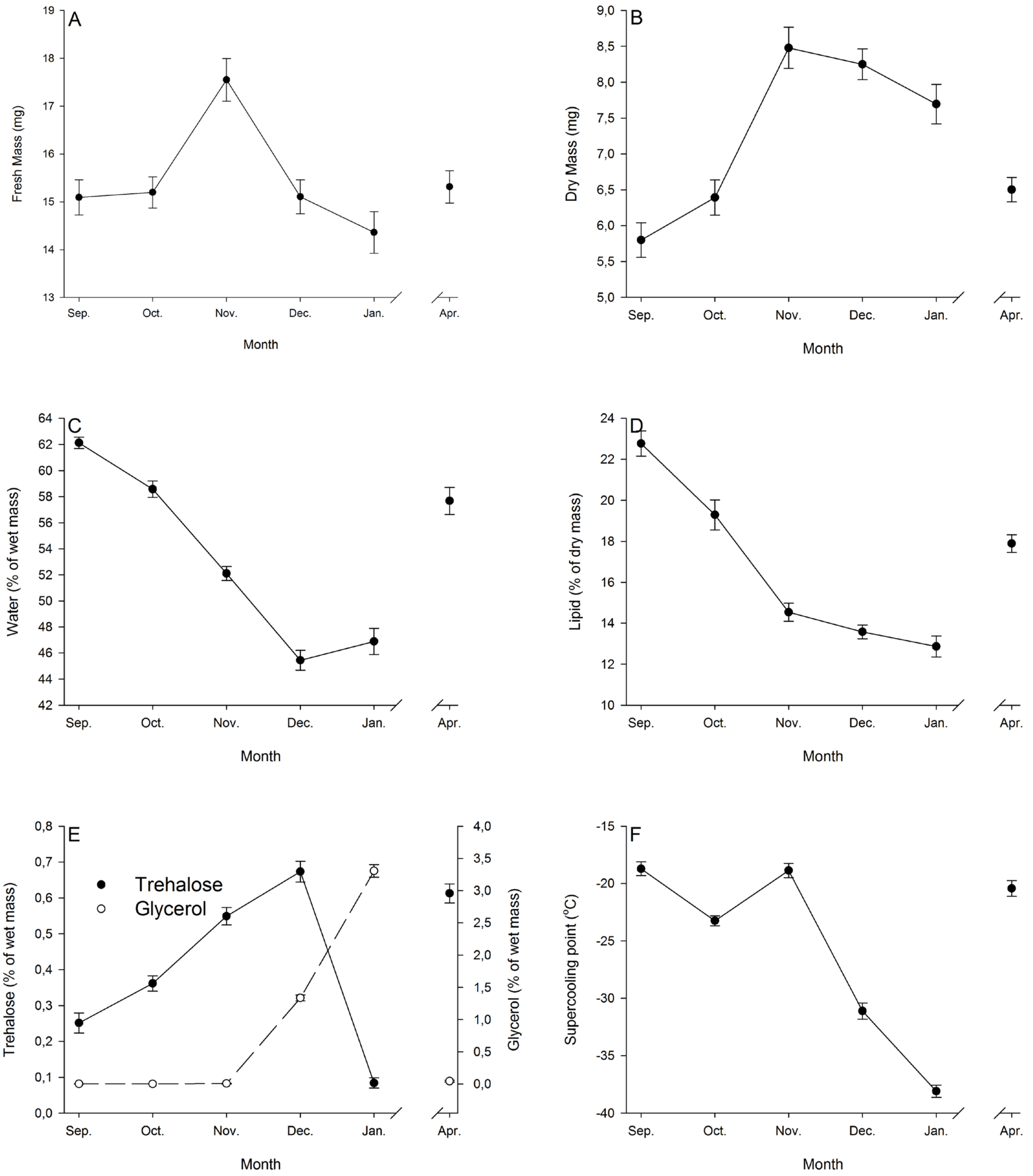
Our results concerning higher beetle densities on more vigorous but attacked trees are in agreement with the results obtained by Reid and Robb (1999). Their results suggest more nutrients in the phloem of vigorous trees. Also, from our results, the increase in beetle density with tree vigor is in keeping with the mass attack principle, implying that more beetles are required to overcome the defenses of a vigorous tree than those of a weak one (Waring and Pitman 1980; Mulock and Christiansen 1986; Christiansen et al. 1987). From a population dynamics point of view, these vigorous trees provide an additional food source to meet the needs of a growing population and reduce intraspecific competition (Raffa and Berryman 1983; Wallin and Raffa 2002, 2004).

For the spruce beetle, red spruce bark should not be considered as a protective layer against cold temperatures as indicated by the phloem temperature. Moreover, the temperature below the bark was similar whatever the vigor status and was similar to external temperature. Similar observations were made by Miller and Werner (1987) on white spruce trees in interior Alaska.

There were important changes in lipids and water content associated with the autumnal decline in *Dendroctonus rufipennis* supercooling point (Figure 2C,D). Similar trends were observed with *Dendroctonus rufipennis* in Alaska, *Dendroctonus ponderosae,* and *Ips pini* (Miller and Werner 1987; Bentz and Mullins 1999; Lombardero et al. 2000). In fall, during glycerol production, water is used, contributing to water loss in the insect, thus improving its cold tolerance (Lombardero et al. 2000).

Lipid content was inversely related to tree vigor. It is recognized that lipids stored in insects can be involved in the biosynthesis of cryoprotectants (Lombardero et al. 2000). This aspect was not validated here. Higher beetle density and consequent food competition on more vigorous trees could explain this reduction in the lipid content (Anderbrant et al. 1985). Even though we observed a slight decrease in body lipid content when tree vigor increased (Table 1), spruce beetle overwintering biology was not altered as no other characteristics of the insect, such as mortality and supercooling points, varied with tree vigor. However, the decrease in lipid content was not accompanied by a decrease in insect wet or dry mass. This suggests that other substances

Figure 2. Fresh mass (A), dry mass (B), water (C), lipid (D), trehalose and glycerol (E), and supercooling points (F) (mean ± SE) of overwintering adult spruce beetles. A mean value involves 31 replicates (trees), each of which contains two adult beetles.



could be produced by the spruce beetle when tree vigor increases and lipid content decreases. Such variation could be linked to the detoxification of terpenes, which are more abundant in vigorous trees (Wallin and Raffa 2002), but this speculation requires further investigation. With colonizing beetles, a reduction in the insect lipid reserves with tree vigor increase could be the result of the cost involved in the search for suitable host trees (Atkins 1969; Wallin and Raffa 2002). Reduced lipid reserves could lead to reduced fecundity or dispersal capability (Slansky and Haack 1986; Anderbrant 1988; Zhou et al. 1995).

During 2008–2009, our results showed that spruce beetles found in Nova Scotia exhibit a strong seasonal pattern and a high cold hardiness and can survive low temperatures, as demonstrated by their low supercooling points and mortality rates. For example, the mean supercooling point was as low as -38 °C in January, with some individuals reaching -44 °C, even though air temperatures measured in the study plot did not fall below -23 °C. This low SCP value for spruce beetle populations in eastern Canada can be easily compared with SCP measured on other bark beetles overwintering under the bark of their hosts like *Ips pini* and northern populations of *Ips grandicollis* (Lombardero et al. 2000). In comparison, Miller and Werner (1987) reported that spruce beetles in Alaska had a mean supercooling point of about -30 °C during the coldest part of winter, with some individuals reaching -37 °C. Differences between the supercooling points we measured and those of Miller and Werner (1987) could be due to variations in the cooling rates that were employed (Leather et al. 1993).

Low supercooling points likely explain, in part, why adult mortality rate was not affected by the temperature drop in January and why mortality remained quite low (15%) throughout winter. Even if Nova Scotia's climate is fairly warm compared with other regions infested by *Dendroctonus rufipennis*, high beetle mortality rates are possible in winter. As observed by Lombardero et al. (2000), even without water crystallization, a sustained chilling can induce *Ips grandicollis* and *Dendroctonus. frontalis* mortality. Also, beetle cold resistance needs a certain amount of time to develop, but can be lost very quickly if warm temperatures occur during winter (Bentz and Mullins 1999; Régnière and Bentz 2007). Yet, mortality rates in our study were comparatively low, given the values of 20 to 37% that were measured for spruce beetle in Colorado (McCambridge and Knight 1972) where air temperatures were similar to those measured in our study site. In a related species, the mountain pine beetle mortality rates of 90 to 95% have been reported following cold snaps in Alberta (A. Roe, University of Alberta, personal communication). Another way to test this mortality over the chilling point would have been to collect insects in the middle of winter, subject them to -20 °C, and pull samples of about 10-30 insects after different durations. This would have given us a lethal time to 50% mortality, and it would have been a more precise measure of cold hardiness.

Our results indicated higher levels of mortality for larvae than adults in January, which suggests lower cold tolerance in larvae. As Miller and Werner (1987) found that adults and larvae of the spruce beetle had similar supercooling points and glycerol contents, non-freeze mortality is apparently involved here. Thus, our results could indicate a lower accumulation of reserves in larvae due to curtailed feeding in autumn. Spruce beetles in Nova Scotia are thought to predominantly have a one-year life cycle. Therefore, larvae found in trees during winter are believed to come from late-emerging parents or from a second brood, which would not have had sufficient time to develop into adults or accumulate reserves before the onset of winter. As no measurements of metabolites and supercooling points were made on larvae, further research should be conducted with respect to spruce beetle larval cold hardiness in Nova Scotia.

The overwintering biology of the spruce beetle was not influenced by host vigor, at least within the range of the vigor index investigated in the current study. Glycerol content, supercooling points and overwintering mortality of the beetles remained the same regardless of host vigor. Thus, spruce beetles did not produce more cryoprotectants and did not exhibit lower supercooling points or greater winter survival on healthy and more vigorous trees. Although vigorous trees provide a source of high-quality food (Waring and Pitman 1985; Sundberg et al. 1993), these trees may not offer an added advantage to the spruce beetle with regard to winter survival and cold hardiness. Furthermore, our results showed that in Nova Scotia, spruce beetle exhibits high cold hardiness, as demonstrated by its low supercooling points and mortality rates. Winter temperatures probably exert negligible control on beetle populations in Nova Scotia, thereby contributing to persistent epidemic levels. Our study provided novel information regarding the overwintering biology of the spruce beetle in Nova Scotia, which will allow us to have a better understanding of the dynamics of this insect. This information should also be considered in the context of climate change to predict population fluctuations and their expansion into new areas.

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REFERENCES

Anderbrant, O. 1988. Survival of parent and brood adult bark beetles, *Ips typographus*, in relation to size, lipid content and re-emergence or emergence day. Physiological Entomology **13**: 121-129.

Anderbrant, O., Schlyter, F., and Birgersson, G. 1985. Intraspecific competition affecting parents and offspring in the bark beetle *Ips typographus*. Oikos **45**: 89-98.

Atkins, M.D. 1969. Lipid loss with flight in the Douglas-fir beetle. The Canadian Entomologist **101**: 164-165.

Bale, J.S. 1987. Insect cold hardiness: freezing and supercooling – an ecophysiological perspective. Journal of Insect Physiology **33**: 899-908.

Bale, J.S. 1993. Classes of insect cold hardiness. Functional Ecology **7**: 751-753.

Bale, J.S., and Hayward, S.A.L. 2010. Insect overwintering in a changing climate. Journal of Experimental Biology **213**: 980-994.

Barbosa, P., and Wagner, M.R. 1988. Introduction to forest and shade tree insects. Academic Press, New York, 639 pp.

Bentz, B.J., and 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., Régnière, J., Fettig, C.J., Hansen, E.M., Hayes, J.L., Hicke, J.A., Kelsey, R.G., Negrón, J.F., and Seybold, S.J. 2010. Climate change and bark beetles of the western United States and Canada: Direct and indirect effects. BioScience **60**: 602-613.

Bevilacqua, E. 2002. Using specific volume increment (SVI) for quantifying growth responses in trees - theoretical and practical considerations. *In*: Proceedings of the Third Annual Forest Inventory and Analysis Symposium. *Edited by* R.E. McRoberts, G.A. Reams, P.C. Van Deusen, and J.W. Moser. USDA Forest Service, North Central Research Station: Gen. Tech. Rep. NC-230. St. Paul, MN. pp. 195-201.

Bright, D.E., Jr. 1976. The insects and arachnids of Canada. Part 2: The bark beetles of Canada and Alaska (Coleoptera: Scolytidae). Agriculture Canada, Biosystematics Research Institute, Research Branch. Ottawa, Canada.

Cho, J.R., Lee, M., Kim, H.S., Kim, Y., and Boo, K.S. 2007. Cold hardiness in the black rice bug, *Scotinophara lurida*. Physiological Entomology **32**: 167-174.

Christiansen, E., Waring, R.H., and Berryman, A.A. 1987. Resistance of conifers to bark beetle attack: searching for general relationships. Forest Ecology and Management **22**: 89-106.

Coulson, R.N., and Witter, J.A. 1984. Forest entomology: Ecology and management. John Wiley & Sons, New York.

Denlinger, D.L. 1991. Relationship between cold hardiness and diapause. *In* Insects at low temperature. Chapman & Hall. pp. 174-198.

Environment Canada. 2010. Canadian climate normals or averages 1971-2000. Available from http://climate.weatheroffice.gc.ca/climate_normals/index_e.html [accessed 1 January 2010].

Furniss, M. 1962. A circular punch for cutting samples of bark infested with beetles. The Canadian Entomologist **94**: 959-963.

Han, E.-N., and Bauce, É. 1993. Physiological changes and cold hardiness of spruce budworm larvae, *Choristoneura fumiferana* (Clem.), during pre-diapause and diapause development under laboratory conditions. The Canadian Entomologist **125**: 1043-1053.

Hard, J.S. 1985. Spruce beetles attack slowly growing spruce. Forest Science **31**: 839-850.

Hard, J.S. 1989. Sequence of trees attacked by spruce beetles in a mature even-aged spruce stand in south-central Alaska. Northwest Science **63**: 5-12.

Holsten, E.H., Their, R.W., Munson, A.S., and Gibson, K.E. 1999. The spruce beetle. Forest Insect and Disease Leaflet 127. USDA Forest Service, Washington, DC.

Izadi, H., Samih, M.A., Behroozy, E., Hadavi, F., and Mahdian, K. 2011. Energy allocation changes during diapause in overwintering larvae of pistachio twig borer, *Kermania pistaciella* Amsel (Lepidoptera: Tineidae) in Rafsanjan. ARPN: Journal Agricultural and Biological Science **6**: 12-17.

Khani, A., and Moharramipour, S. 2010. Cold hardiness and supercooling capacity in the overwintering larvae of the codling moth, *Cydia pomonella*. Journal of Insect Science **10**: 1-12.

Kinn, D.N., Perry, T.J., Guinn, F.H., Strom, B.L., and Woodring, J. 1994. Energy reserves of individual southern pine beetles (Coleoptera: Scolytidae) as determined by a modified phosphovanillin spectrophotometric method. Journal of Entomological Science **29**: 152-163.

Kytö, M., Niemelä, P., and Annila, E. 1996. Vitality and bark beetle resistance of fertilized Norway spruce. Forest Ecology and Management **84**: 149-157.

Kytö, M., Niemelä, P., and Annila, E. 1998. Effects of vitality fertilization on the resin flow and vigour of Scots pine in Finland. Forest Ecology and Management **102**: 121-130.

Leather, S.R., Walters, K.F.A., and Bale, J.S. 1993. The ecology of insect overwintering. Cambridge University Press, Cambridge, UK.

Lencioni, V. 2004. Survival strategies of freshwater insects in cold environments. Journal Limnnology. **63** (Supplement 1): 45-55.

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

McCambridge, W.F., and Knight, F.B. 1972. Factors affecting spruce beetles during a small outbreak. Ecology **53**: 830-839.

Miller, L.K., and 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.

Mulock, P., and Christiansen, E. 1986. The threshold of successful attack by *Ips typographus* on *Picea abies*: a field experiment. Forest Ecology and Management **14**: 125-132.

Münster-Swendsen, M. 1987. Index of vigour in Norway spruce (*Picea abies* Karst.). Journal of Applied Ecology **24**: 551-561.

Raffa, K.F., and Berryman, A.A. 1983. The role of host plant resistance in the colonization behavior and ecology of bark beetles (Coleoptera: Scolytidae). Ecological Monographs **53**: 27-49.

Raffa, K.F., Aukema, B.H., Erbilgin, N., Klepzig, K.D., and Wallin, K.F. 2005. Interactions among conifer terpenoids and bark beetles across multiple levels of scale: an attempt to understand links between population patterns and physiological processes. Recent Advances in Phytochemistry **39**: 79-118.

Regent Instruments Inc. 2009. WinDENDRO, version 2009a. Regent Instruments Inc., Québec, Canada.

Régnière, J., and Bentz, B. 2007. Modeling cold tolerance in the mountain pine beetle, *Dendroctonus ponderosae*. Journal of Insect Physiology **53**: 559-572.

Reid, M.L., and Robb, T. 1999. Death of vigorous trees benefits bark beetles. Oecologia **120**: 555-562.

SAS Institute Inc. 2008. SAS/STAT User's Guide, version 9.2. SAS Institute Inc., Cary, NC.

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

Sinclair, B.J., Vernon, P., Klok, C.J., and Chown, S.L. 2003. Insects at low temperatures: An ecological perspective. Trends in Ecology and Evolution **18**: 257-262.

Slansky, F., and Haack, R.A. 1986. Age-specific flight behavior in relation to body weight and lipid content of *Ips calligraphus* reared in slash pine bolts with thick or thin inner bark (phloem). Entomologia Experimentalis et Applicata **40**: 197-207.

Somme, L. 1982. Supercooling and winter survival in terrestrial arthropods. Comparative Biochemistry and Physiology - Part A: Molecular and Integrative Physiology **73**: 519-543.

Sundberg, B., Ericsson, A., Little, C.H.A., Näsholm, T., and Gref, R. 1993. The relationship between crown size and ring width in *Pinus sylvestris* L. stems: dependence on indole-3-acetic acid, carbohydrates and nitrogen in the cambial region. Tree Physiology **12**: 347-362.

Turnock, W.J., and Fields, P.G. 2005. Winter climates and cold hardiness in terrestrial insects. European Journal of Entomology **102**: 561-576.

Wallin, K.F., and Raffa, K.F. 2001. Effects of folivory on subcortical plant defenses: can defense theories predict interguild processes? Ecology **82**: 1387-1400.

Wallin, K.F., and Raffa, K.F. 2002. Density-mediated responses of bark beetles to host allelochemicals: a link between individual behaviour and population dynamics. Ecological Entomology **27**: 484-492.

Wallin K.F., and Raffa, K.F. 2004. Feedback between individual host selection behavior and population dynamics in an eruptive herbivore. Ecological Monographs **74**: 101-116.

Waring, R.H., and Pitman, G.B. 1980. A simple model of host resistance to bark beetles. Oregon State University, School of Forestry, Forest Research Laboratory, Corvallis, OR, Research Note 65.

Waring, R.H., and Pitman, G.B. 1985. Modifying lodgepole pine stands to change susceptibility to mountain pine beetle attack. Ecology **66**: 889-897.

Waring, R.H., Thies, W.G., and Muscato, D. 1980. Stem growth per unit of leaf area: a measure of tree vigor. Forest Science **26**: 112-117.

Werner, R.A., and Holsten, E.H. 1985a. Effect of phloem temperature on development of spruce beetles in Alaska. *In* The role of the host in the population dynamics of forest insects: Proceedings of the IUFRO conference, 4-7 September 1983, Banff, Canada. *Edited by* L. Safranyik. Canadian Forestry Service, Pacific Forestry Centre, Victoria, BC, Canada. pp. 155-163.

Werner, R.A., and Holsten, E.H. 1985b. Factors influencing generation times of spruce beetles in Alaska. Canadian Journal of Forest Research **15**: 438-443.

Zhou, X., Honek, A., Powell, W., and Carter, N. 1995. Variations in body length, weight, fat content and survival in *Coccinella septempunctata* at different hibernation sites. Entomologia Experimentalis et Applicata **75**: 99-107.