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Cold tolerance of mountain pine beetle among novel eastern pines: A potential for trade-offs in an invaded range?

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ABSTRACT

Novel hosts may have unforeseen impacts on herbivore life history traits. The mountain pine beetle (*Dendroctonus ponderosae* Hopkins) is a tree-killing bark beetle native to western North America but constrained by cold temperatures in the northern limits of its distribution. In recent years, this insect has spread north and east of its historical range, and continued expansion, or accidental introduction, could result in the mountain pine beetle becoming invasive in eastern North America. The limiting effect of cold temperatures among novel host pines is unknown, yet crucial for understanding the risk posed to northeastern North American forests. We report the susceptibility of mountain pine beetle to cold temperatures while overwintering in six different pine species. Brood developed in two western pine hosts (*Pinus contorta* Dougl. var. *latifolia* Engelm. and *P. ponderosa* Dougl. ex. Laws. var. *scopulorum* Engelm.) as well as four eastern pines (*P. banksiana* Lamb., *P. resinosa* Ait., *P. strobus* L. and *P. sylvestris* L.) novel to this insect. The cold tolerance and cold tolerance strategy of the most common overwintering stage varied by host and year. Models describing lower lethal temperatures more accurately predicted observed field mortality of overwintering larvae than models based on temperatures at which larvae froze. Rapid development to less cold tolerant pupal and adult stages by brood in novel hosts prior to winter may constitute a trade-off between increased host suitability and winter mortality. We demonstrate that overwintering survival of mountain pine beetles in novel hosts depends on a match between the climate and ecophysiological effects of pine species. These results have implications for risk assessment models and management planning for eastern forests as mountain pine beetle continues to expand its range.

1. Introduction

Climate change is having significant impacts on many insect populations, resulting in the shifting of historical ranges and modifications to activity and development (e.g., Bentz et al., 2010; Weed et al., 2013; Bebber et al., 2013). Among phytophagous forest insects, the effects of climate change can be particularly important due to the ecological and economic significance of many of these species (Gandhi and Herms, 2010). Mitigating the effects of climate change will require effective management of these insects in new ranges and among novel hosts (Raffa et al., 2015; Tobin et al., 2014). However, novel hosts may influence the life history traits of these insects in unexpected ways (Awmack and Leather, 2002), complicating assessments of forest vulnerability (Fuentealba et al., 2013).

The mountain pine beetle (*Dendroctonus ponderosa* Hopkins), a tree-killing bark beetle native to western North America, is expanding its range due to climatic warming (Carroll et al., 2004; Raffa et al., 2015; Safranyik et al., 2010). Recent outbreaks have killed multiple pine species (*Pinus* spp.) across millions of hectares (Meddens et al., 2012) and contributed to a depletion of the forest carbon stock similar to that of all forest fires in the same region combined (Hicke et al., 2013). Winter temperatures have historically limited the northern range of this insect to southern British Columbia, Canada (Safranyik et al., 1975). In recent years, mountain pine beetle has been spreading north and east due to increasingly suitable climate (Carroll et al., 2004; Safranyik et al., 2010). Spread into western Alberta, Canada (de la Giroday et al., 2012) has coincided with expansion through the lodgepole-jack pine hybrid zone and recently into jack pine (*P. banksiana* Lamb.) of

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the boreal forest, a pine species and ecosystem historically naïve to this insect (Cullingham et al., 2011; Erbilgin et al., 2014). Continued eastward spread through the boreal forest, or anthropogenic movement of infested wood, could introduce this insect to other pine species that have no coevolutionary history with it (Raffa et al., 2015). Some assessments suggest that with continued climate change, most North American pine forests will have winters that are moderately to highly suitable for mountain pine beetle survival (Bentz et al., 2010).

Under historical climatic conditions and among western hosts, mountain pine beetle generally exhibits one generation per year. Broods initiated in late summer typically advance through four instars prior to winter, although developmental rate may differ among hosts (Cerezke, 1995). Development at low temperatures is more rapid for early instars than late instars (Bentz et al., 1991; Régnière et al., 2012), which enables early instars to "catch up" with siblings oviposited earlier, synchronizing much of the population as late instars prior to winter (Powell et al., 2000). This strategy supports mass attack behavior by adult beetles on the following summer that can overwhelm the defenses of trees when insects are at outbreak levels.

Overwintering success is critical for sustained mountain pine beetle outbreaks (Cole, 1981; Langor, 1989; Safranyik and Linton, 1991) and can be affected by the cold tolerance of overwintering life stages and the severity and duration of cold exposure. Eggs and early instars are less cold tolerant than late instars (Reid and Gates, 1970; Safranyik and Linton, 1998), and pupae and teneral adults rarely survive the winter (Amman, 1973; Reid, 1963). Supercooling points (i.e., the temperatures at which insect bodily fluids begin to freeze) for late instars vary, but average between −26.5 (Bentz and Mullins, 1999) and −36.7 °C (Cooke, 2009) in midwinter. Much research on mountain pine beetle cold tolerance and modeling climatic suitability has focused on supercooling point (Bentz and Mullins, 1999; Cooke, 2009; Somme, 1964; Strongman, 1982) although freezing is not coincidental with mortality in all insects (Sinclair, 1999). For mountain pine beetle, early instars (Somme, 1964) and late instars in lodgepole pine (*P. contorta* Dougl. var. *latifolia* Engelm.) (Bentz and Mullins, 1999) are reported to die upon freezing, but survive exposure to low, nonfreezing temperatures (Bentz and Mullins, 1999). Larvae can adjust cryoprotectant concentrations (Somme, 1964) and cold tolerance (Yuill, 1941) in response to winter temperature fluctuations. However, pine hosts may also affect cold tolerance of larvae as evidenced from laboratory experiments (Wygant, 1940; Yuill, 1941), and field observations (Langor and Spence, 1991) among hosts in western North America. Indeed, increasing evidence from several other insect-host systems suggests that host may play a role in cold tolerance and winter survival (Feng et al., 2016; Gash and Bale, 1985; Morey et al., 2016; Trudeau et al., 2010). Thus host species may be an important factor to consider when assessing cold tolerance among novel hosts.

The stage distribution and cold tolerance of mountain pine beetle overwintering in novel hosts from eastern North America have not yet been assessed, and knowledge of how hosts may mediate life-history traits may improve our understanding of climatic suitability (Bentz et al., 2010; Régnière and Bentz, 2007). Here, we report the results of experiments designed to assess host effects on mountain pine beetle overwintering success. We measured supercooling points, lower lethal temperatures and development rates of overwintering brood in six pine species. Two species, lodgepole and ponderosa pine (*P. ponderosa* P. & C. Lawson) were historical hosts, and four pine species, jack, red (*P. resinosa* Ait.), eastern white (*P. strobus* L.) and Scots pine (*P. sylvestris* L.) had no historical association with mountain pine beetle. We hypothesized that the insects would be freeze-intolerant and that cold tolerance would differ among host species.

2. Methods

2.1. Material preparation

These experiments required us to work with cut logs because (i) many of the *Pinus* spp. of interest do not grow within the current range of mountain pine beetle; (ii) adult beetles will only colonize large diameter trees; and (iii) we cannot introduce mountain pine beetle into the environs of eastern North America. A total of eight trees of each species were harvested, four in 2013 and four in 2014. Each year uninfested trees were cut on two occasions, with two trees of each species being harvested each time, one week apart in early August, spanning peak flight time. Lodgepole pine were harvested in the eastern Big Horn mountains of Wyoming west of Big Horn, WY the first year (latitude, longitude: 44.60337, −107.21505 and 44.62710, −107.16303) and west of Buffalo, WY the second year (44.31865, −106.94633 and 44.22341, −106.93212). Ponderosa pine were harvested in the Black Hills near Nemo, SD the first year (44.12955, −103.48513) and near Silver City, SD the second year (44.12587, −103.56700). Jack, red, eastern white and Scots pines were harvested at the Cloquet Forest Research Center, MN (46.701735, −92.521798). All pines were 23–30 cm diameter at breast height (DBH, approximately 1.3 m above ground) and had a full crown with no signs of bark beetle attack and no visible mistletoe infection. The main stem of each tree was cut into 1 m sections, immediately waxed to slow desiccation, and placed into zippered tarapuan body bags (BP medical supplies, Brooklyn, NY, USA) for transport to the Black Hills, where they were further cut into 40 cm bolts. The ends of the bolts were waxed, and material was stored indoors.

Beetles used to infest bolts were caught in 12-funnel Lindgren funnel traps baited with pheromone and kairomone lures (trans-verbenol, exo-brevicomin, and myrcene) (Contech Enterprises Inc, Delta, BC) in an ongoing outbreak in ponderosa pine forests in the Black Hills of South Dakota, U.S.A. near Silver City. Beetles were immediately sexed by stridulation (Rosenberger et al., 2016) and stored in Petri dishes on moist KimWipes (Kimberly-Clark, Irving, TX) at approximately 5 °C for ≤3 days before use. Within 36–48 h of being harvested, six holes in 2013 and seven in 2014, each 5 cm from one cut end, were drilled on each bolt to the phloem with a 0.63 cm-diameter drill bit. A female beetle was placed in a microcentrifuge tube that was gently inserted into the hole to allow the beetle to enter the phloem. Bolts were stored upright with beetles near the base. Beetles were checked after 12 h for boring dust, evidence of initial host acceptance. Dead or inactive beetles were replaced. Males were added to three of the holes within 24 h of female introduction and replaced after 12 h if they did not enter the gallery. Bolts were covered in aluminum screening to keep other insects from infesting them. The bolts were hung for 5 days in the Black Hills National Forest as part of a separate experiment on colonization dynamics on novel hosts (Rosenberger et al., 2017) before moving them to the USDA Forest Service Mystic Ranger Station outside of Rapid City, SD to overwinter. In mid-October 2013, temperature probe data-loggers (HOBO, Onset Computer Corporation, Bourne, Mass.) were inserted under the bark on the north and south sides of two bolts of each species. In 2014, probes were inserted immediately after being moved to the overwintering location. Air temperatures were also recorded 30 cm above the ground.

2.2. Beetle extraction and storage

A subsample of the total infested bolts representing two bolts of each of the four trees of each species were moved from Rapid City, SD to St. Paul, MN in late December 2013 and early January 2015 in their aluminum screening in construction-grade plastic bags inside zippered tarpaulin bags. The bolts remained in bags and were stored in a secured but unheated building (temp. <0 °C). Individual insects were carefully extracted by removing the bark with a chisel as needed over the course of the experiments in January and early February. All life stages except eggs (i.e., early instar, late instar, pupae, teneral adult, and parent) of the mountain pine beetle were collected and tallied to determine proportion of brood at each stage of development prior to winter. Larvae with head capsules less than approximately 0.75 mm wide were likely first or second instars, while larvae with head capsules greater than approximately 0.75 mm were likely third and fourth instars (Rosenberger, 2016). Fourth instars used in cold tolerance tests were further differentiated with 98% accuracy from third instars by visual estimation of head capsule width and body size (Rosenberger, 2016). Individuals to be used for cold tolerance tests were placed immediately into individual 1.5 ml microcentrifuge tubes, and all brood were stored at −5 °C. All tests were completed within four days of individuals being removed from a bolt.

Discolored (i.e., darkened) larvae were considered dead and not used in cold tolerance tests (Amman, 1973; Wygant, 1940). Discolored larvae were prevalent in 2015, likely due to an early November 2014 cold snap and subsequent warming that would have allowed for decomposition to begin.

2.3. Cold tolerance tests

Tests to determine supercooling points and lower lethal temperatures of fourth instar mountain pine beetles (i.e., the most commonly found stage) from each pine species were conducted in January and early February 2014 and in January 2015. Insects were extracted from eight trees of each species across the two years of the experiment. Microcentrifuge tubes containing insects were removed from storage at −5 °C and placed in contact with a copper-constantan thermocouple (Stephens et al., 2015). Temperatures starting from a room temperature of 21 °C were recorded once per second via an analog data acquisition unit (USB-TC, Measurement Computing Corporation, Norton, MA) and TracerDAQ Pro software (Measurement Computing Corporation, Norton, MA). In 2014, individual microcentrifuge tubes with the insects and thermocouples were cooled at ~1 °C per minute (Carrillo et al., 2004). In 2015, a refrigerated bath circulator (A40, Thermo Fisher Scientific, Newington, NH) with SIL-180 silicon oil was used to cool the insects at ~1 °C per minute. The microcentrifuge tubes containing the thermocouple and beetle were placed in glass test tubes and lowered into the coolant bath.

2.4. Supercooling point tests

To determine the effect of host on larval supercooling points, we used a randomized complete block design in which larvae from each pine species were tested in the same run (i.e., block). Supercooling points were determined by recording the lowest temperature before the exotherm as the latent heat of fusion was released. Larvae were removed after approximately 2–3 min, once the exotherm returned to the supercooling point. Larval mass was measured following treatment.

While most overwintering mountain pine beetles were larvae, in 2014, some pupae and teneral adults were also available for testing. The supercooling points of pupae and male and female teneral adults from each of the pine species were measured. Individuals from the different pine species were pooled for statistical analysis.

2.5. Lower lethal temperature tests

We used a randomized complete block design for lower lethal temperature tests, similar to above. Fourth instar larvae extracted from bolts into microcentrifuge tube and stored at −5 °C were placed in contact with a thermocouple, as above, and cooled from 21 °C to a randomly chosen temperature between −20 and −42 °C, inclusive, in one degree increments in 2014 and between −21 and −38 °C in 2015. In 2015, half the batches were cooled to a randomly assigned temperature and half were removed directly after the exotherm when the temperature of the insect had returned to the supercooling point. This approach allowed us to obtain a large number of insects from the same populations for which we had both supercooling point measurements and lower lethal temperature measurements. After the target temperature was achieved, larvae were removed from the cooling unit, weighed, and warmed to room temperature while remaining in microcentrifuge tubes. For every two lower lethal temperature treatment batches, control larvae from each pine species were set aside and remained at room temperature to estimate mortality not due to cold exposure. Supercooling point measurements obtained from lower lethal temperature experiments were combined with the supercooling point data for analysis. We tested 40 blocks (one larvae from each pine species in each block) in 2014 and 63 blocks in 2015.

2.6. Mortality evaluation

We modified a method from Wygant (1940) to assess survival after cold exposure: larvae were held in the microcentrifuge tubes that were stuffed with 2.5 × 2.5 cm pieces of KimWipe to simulate a pupal cell. The KimWipe was dampened with 2 mL of deionized water, and a small hole was made in the cap for ventilation. Tubes were stored on their sides to allow larvae to move and kept in a dark box at room temperature. Individuals that bored into the moist Kim Wipe or molted to the next developmental stage were considered alive. Survival was assessed after 1, 2, 4, and 7 days after cold exposure and then twice a week until the insect reached adulthood or died. Thus in our experiments, we assessed effects that were not immediately lethal, yet still result in mortality. Approximately 10% of control and 15% of chilled larvae did not move when warmed to room temperatures, indicating that some healthy-appearing larvae were dead or mortally injured during extraction from bolts (Wygant, 1940), which could affect lower lethal temperature curves. Thus, insects that never resumed movement in lower lethal temperature tests were removed from further analysis allowing us to account for prior unobservable mortality. Because beetles can rapidly de-acclimate at room temperature (Cooke, 2009) (Supplementary Material), assessment of mortal injury before cold exposure was not possible.

2.7. Statistical analysis

All data were analyzed using R v. 3.1.0 (R Development Core Team, Vienna, Austria).

2.7.1. Development stage

To test whether proportions of brood at each stage differed between pine host species we used a generalized linear logistic model for binomial data ("lme4" package) in R. The binomial response variable was the presence of a specific stage "1" (early instar, late instar, pupae or adult), or that of another stage "0". Values for each tree species were separated using a Tukey HSD post-hoc test with the "multcomp" package in R (Hothorn et al., 2008).

2.7.2. Survival of early winter cold snap

To determine the effect of host on larval mortality or developmental stage on mortality in bolts exposed to sub-zero temperatures in November 2014, proportions of discolored (dead) vs. creamy (likely alive) larvae were analyzed by using a generalized linear mixed effects model with a binomial distribution in the lme4 package (Bates et al., 2015). A term for host species was included as a fixed effect. To account for multiple individuals assessed from the same bolt and multiple bolts from the same tree, terms for bolt and tree were included as random effects. Chi square tests were used to test for differences in survival between larval stages and between species.

2.7.3. Overwintering supercooling points between stages

Mixed effects ANOVA models (“nlme” package in R) (Pinheiro et al., 2013) were used to evaluate the effect of natal host on the supercooling points of larvae and to compare supercooling points of pupae and adults. Model assumptions of homoscedasticity and normality of errors were assessed via inspections of residual plots. For all models, terms for tree and bolt within tree were included as random effects. *F*-tests were used to obtain a global estimate of the treatment effect, and means were separated between species using a Tukey HSD post-hoc test with the “multcomp” package in R.

2.7.4. Comparison of lower lethal temperature data between species and between years

Lower lethal temperature data were analyzed by using logistic regression. Survival curves were modeled by using logistic regression (binomial distribution and logit link function) and compared between years to determine if data could be pooled. Within year, modeled survival curves were compared between species to determine whether mortality rates at 0 °C (intercept) and/or the change in mortality with each degree change in temperature (slope) differed between species. For this analysis a positive event (i.e., survival) was the dependent variable and temperature, data group (i.e., year one or two for comparison of years, and species for comparison between species curves) and their interaction were included as independent variables. Wald tests, using a marginal fit, were used to compare these models.

2.7.5. Comparison of lower lethal temperature and supercooling point

Likelihood of larvae freezing as temperature declined was modeled using logistic regression to obtain a modeled cumulative supercooling point curve. At a population level, the cumulative supercooling point curve described the entire proportion of individuals that were expected to have started freezing if exposed to a specified temperature, x, with some individuals beginning to freeze at a temperature $\geq x$ ($-45\,°C \leq x \leq 0\,°C$, in our application). Modeled cumulative supercooling point curves modeled lower lethal temperature survival curves were compared using logistic regression as above. Comparisons of these curves allow us to determine whether the extent of freezing and mortality are equivalent at a specified temperature for this insect (i.e., evidence of freeze-intolerance), or whether mortality occurs before or after freezing, which would indicate chill intolerance or freeze-tolerance, respectively (Cira et al., 2016; Stephens et al., 2015). To determine overall similarity between freezing and mortality for each year, the dependent variable was a positive event (i.e., survival for lower lethal temperature data and freezing for the cumulative supercooling point curve). Independent variables were temperature, data group (lower lethal temperature or supercooling point), and an interaction between the two variables. The same analysis was then conducted to compare freezing and mortality at the natal pine species level by constructing separate models for each species in each year. Estimated LT_{50} and LT_{90} values and associated variances were obtained from the lower lethal temperature curves with the “MASS” package (Venables and Ripley, 2002) in R by using the dose.p function.

2.7.6. Field validation

To determine whether our models were able to predict survival at a specified temperature, we used the predict.glm function in R to obtain predicted mortality for fourth instar larvae at the minimum temperature (i.e., the average of minimum temperatures recorded from underbark probes on the north and south side of two bolts of each species) recorded in each pine species prior to debarking in January 2014. Only 2014 models were used as the early cold snap in November 2014 likely killed beetles prior to winter acclimation (Somme, 1964) and our models are constructed for winter acclimated insects. Observed survival was obtained by tallying the proportion of dark (dead) versus creamy (alive) larvae from eight bolts from four trees of each pine species in January and February 2014. We considered models in which observed survival fell within 95% confidence limits to be superior.

3. Results

3.1. Development and early winter field mortality

In both years of these experiments, we observed significant effects of host pine species on the proportion of brood at each stage of development by January (Table 1). In the first year we found predominantly pupae and teneral adults in the novel hosts and larvae in the historical hosts (Table 1). We observed little natural mortality (8.2%) as indicated by dark larvae (Wygant, 1940) among brood overall the first year, despite under-bark temperatures reaching around −21 °C in early to mid-December (Fig. A.1). In the second year, most individuals were larvae (Table 1) and higher mortality (25%) was observed. An early winter cold snap occurred the second year when daily low air temperature rapidly dropped from 2 °C on 09-Nov-2014 to −22 °C on 13-Nov-2014. Daily low average underbark temperatures (averaged for the north and south faces of the cut bolts) reached −13 °C (Fig. A.1). Host had a modest effect on survival of early instar larvae the second year, with a greater proportion of larvae surviving in ponderosa, jack and red pines than in lodgepole, eastern white or Scots pines (Fig. 1, $\chi^2 = 10.04$, $df = 5$, $P = 0.07$). There was no clear effect of pine species on survival of late instar larvae (Fig. 1, $\chi^2 = 5.83$, $df = 5$, $P = 0.32$). Larval stage did affect survival ($\chi^2 = 145.6$, $df = 1$, $P < 0.001$), with early instar larvae in all but jack pine exhibiting lower survival than late instars (Fig. 1).

3.2. Life-stage and host effects on supercooling points

In the first year, supercooling points differed among mountain pine beetle life stages tested (i.e., fourth instar, pupa, and teneral adult) ($F_{3,295} = 149.0$; $P < 0.0001$). Fourth instar larvae had significantly lower supercooling points than pupae or male or female teneral adults (Fig. 2) or all teneral adults combined (mean ± SE: −16.6 °C ± 1.3). The mean supercooling points of pupae and male teneral adults were not significantly different but were both significantly warmer than those of female teneral adults. The second year, the mean fourth instar supercooling point (−30.7 °C ± 0.22) was similar to the first year ($F_{1,46} = 0.021$; $P = 0.89$). The minimum fourth instar supercooling point observed over both years in our experiments was −38.4 °C and the maximum was −19 °C. Natal pine species affected supercooling points of larvae in the first ($F_{5,18} = 9.07$; $P = 0.0002$), but not the second ($F_{5,18} = 1.14$; $P = 0.38$) year (Table 2). Fourth instars from lodgepole and Scots pine had the lowest and highest mean supercooling points respectively, each year although the average supercooling point from lodgepole

Table 1
Stage distribution of mountain pine beetles in the winters of 2013–14 and 2014–15 from evolutionarily novel and historical host species reared in eight logs of each pine species each year in Black Hills, SD. Stage distribution was determined in January of each year. Data were analyzed for differences between hosts using a generalized linear model. Proportions with the same letter within a column and year are not significantly different from each other.

				Proportion			
Year	Host species	Host Type	*n*	Early Instar	Late Instar	Pupae	Adults
2013–14	Ponderosa	Historical	1085	0.08b	0.45ab	0.19a	0.28d
	Lodgepole	Historical	694	0.09b	0.51a	0.15ab	0.24d
	Jack	Novel	856	0.06b	0.37c	0.14b	0.43bc
	Red	Novel	985	0.01c	0.22e	0.13b	0.64a
	Eastern white	Novel	1399	0.07b	0.41bc	0.15ab	0.38c
	Scots	Novel	686	0.15a	0.28d	0.12b	0.44b
			ξ^2_5	139.6	227.3	21.7	387.6
			P	<0.0001	<0.0001	<0.001	<0.0001
2014–15	Ponderosa	Historical	1393	0.04c	0.94a	0.004b	0.002c
	Lodgepole	Historical	747	0.17a	0.82c	0.000ab	0.003bc
	Jack	Novel	701	0.08b	0.90b	0.001ab	0.016ab
	Red	Novel	878	0.09b	0.86bc	0.019a	0.035a
	Eastern white	Novel	1213	0.03c	0.96a	0.001b	0.002c
	Scots	Novel	534	0.11b	0.89b	0.004ab	0.000abc
			ξ^2_5	149.9	156.4	38.3	78.0
			P	<0.0001	<0.0001	<0.0001	<0.0001

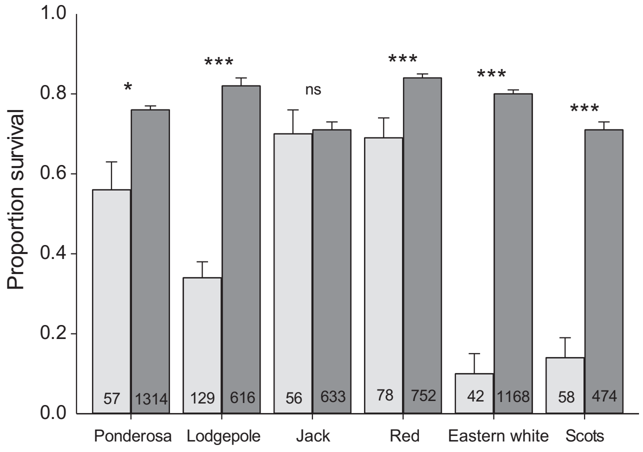

Fig. 1. Proportion of early (light bars) and late (dark bars) instars in overwintering pine bolts infested in August 2014, that appeared to survive through January 2015 after experiencing a cold snap in early November 2014. Discolored larvae were classified as dead, and cream colored larvae were classified as alive. Numbers on bars indicate the total number of early or late instar larvae for that pine. Late instars were more likely to survive than early instars ($\chi^2 = 145.6$, $df = 1$, $P < 0.001$). Stars indicate a significant difference between survival of small and large larvae (ns: not significant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

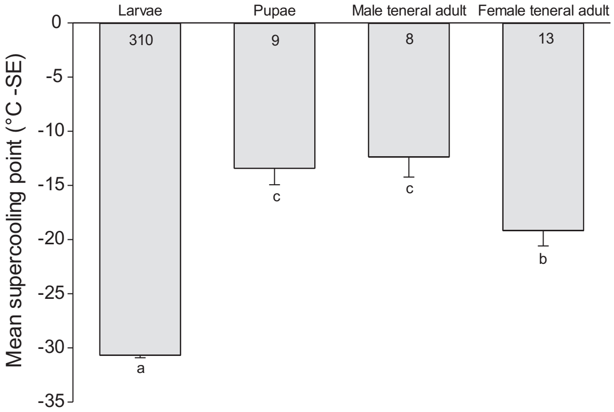

Fig. 2. Mean (-SE) supercooling points of fourth instar (−30.7 °C ± 0.23SE), pupae (−13.4 °C ± 1.5SE), adult male (−12.4 °C ± 1.9SE) and adult female (−19.2 °C ± 1.4SE) mountain pine beetle in 2014. Insects were extracted from all six pine species. Numbers on bars represent sample sizes. Bars with the same letter are not significantly different from each other.

was not significantly different from jack and eastern white, and Scots was not different from ponderosa and red (Table 2). An interaction between pine species and year precluded us from combining larval supercooling point data from both years. Live mass of larvae did not affect supercooling point the first ($F_{1,267} = 0.003$; $P = 0.96$) or second year ($F_{1,223} = 0.665$; $P = 0.42$).

3.3. Lower lethal temperatures

Lower lethal temperature curves described survival as a function of exposure to sub-zero temperatures. Comparisons of these curves among the six pine species indicated that projected mortality at 0 °C (i.e., the intercept) was not affected by host in the first ($\chi^2 = 4.34$, $df = 5$, $P = 0.50$) or second year ($\chi^2 = 8.0$, $df = 5$, $P = 0.16$). The change in survivorship with each degree of cooling (i.e., the slope) was also not affected by host in either year (Year 1: $\chi^2 = 4.22$, $df = 5$, $P = 0.52$; Year 2: slope: $\chi^2 = 6.26$, $df = 5$, $P = 0.28$). However, host may have affected the temperature at which mortality occurred as we observed a 4 °C spread in LT_{50}s between the coolest and warmest values the first year (comparison of red pine to Scots pine: $t = 1.97$, $df = 62$, $P = 0.053$) and 5.5 °C the second year (comparison of red pine to jack pine: $t = 2.18$, $df = 63$, $P = 0.033$) (Table 2). Survival among controls was 84.4% overall, but no effect of species on control mortality was observed ($\chi^2 = 2.7$, $df = 5$, $P = 0.75$).

3.4. Comparison of modeled supercooling point and lower lethal temperature

We found that fourth instars demonstrated similar freezing and mortality relationships with temperatures in some pines, but not in others (Fig. 3). In 2014, the proportion of larvae that froze or died at 0 °C (i.e., intercepts) was similar for the individuals from ponderosa, jack, red and Scots pines, as was the rate of change in the proportion of individuals that died or began to freeze as temperatures declined below 0 °C. In contrast, freezing and mortality appeared to be unrelated among larvae from lodgepole and eastern

Table 2
Mean supercooling point and temperatures required for 50 % and 90 % mortality of fourth instar mountain pine beetles from different pine species. Mean supercooling points with the same letter are not significantly different form each other.

	2014					2015				
	SCP (°C ± SE)	*n*	LT50 (°C ± SE)	LT90 (°C ± SE)	*n*	SCP (°C ± SE)	*n*	LT50 (°C ± SE)	LT90 (°C ± SE)	*n*
Ponderosa	−30.3 ± 0.46bc	56	−32.1 ± 1.5	−38.2 ± 2.7	30	−30.0 ± 0.68	44	−28.3 ± 1.3	−36.5 ± 2.8	50
Lodgepole	−33.2 ± 0.43a	49	−30.6 ± 1.8	−37.6 ± 3.8	30	−31.6 ± 0.48	39	−29.2 ± 1.0	−34.4 ± 1.4	53
Jack	−31.2 ± 0.51ab	53	−31.1 ± 1.0	−34.6 ± 1.6	35	−30.5 ± 0.51	46	−25.9 ± 2.4	−37.2 ± 3.5	51
Red	−28.8 ± 0.63c	51	−33.3 ± 1.5	−38.6 ± 2.6	29	−31.0 ± 0.48	45	−31.4 ± 0.9	−36.8 ± 1.7	55
Eastern white	−33.1 ± 0.47ab	45	−31.6 ± 1.6	−39.3 ± 3.1	34	−31.6 ± 0.48	42	−27.7 ± 1.5	−36.4 ± 2.4	56
Scots	−28.2 ± 0.47c	57	−29.4 ± 1.4	−35.5 ± 2.4	34	−29.6 ± 0.61	38	−27.5 ± 1.8	−39.6 ± 4.2	58

white pines because mortality commenced before freezing began (Fig. 3). Factors other than freezing contributed to mortality at low temperatures among these hosts. In 2015, freezing was only associated with mortality in ponderosa and red pines, while other factors appeared to be involved in mortality among larvae from lodgepole, jack, eastern white and Scots pines in which a high proportion of mortality occurred in the population before freezing (Fig. 3, Table A.1). For larvae from ponderosa, jack, red, and Scots pines (i.e., hosts from which the rates of change in the proportion of individuals that began to freeze and that began to die were the same), the mean supercooling points and LT_{50}s were not different except in red pine ($t = 2.88$, $df = 39$, $P = 0.0064$), which had a warmer mean supercooling point than LT_{50} in 2014. Larvae from red pine demonstrate some degree of freeze-tolerance.

3.5. Survival of supercooling

In addition to observing mortality before and at the point of freezing, we also observed that 13.5% of fourth instars survived after an exotherm was detected. Natal host did not significantly affect the proportion of individuals that survived the onset of freezing ($\chi^2 = 8.79$, $df = 5$, $P = 0.12$), although four times as many larvae reared in red pine survived ice formation, compared with larvae from lodgepole or eastern white pine (Fig. 4).

3.6. Field validation of models

We used under-bark temperatures and the supercooling point and lower lethal temperature models from 2014 (Fig. 3) to determine expected mortality of late instar larvae in overwintering bolts given a certain exposure temperature. Cumulative supercooling point models consistently overestimated the proportion of live late instar larvae found in bolts (Table 3). However lower lethal temperature models provided generally more reliable estimates of mortality, and all observed values fell within 95% confidence intervals around the predicted value (Table 3).

4. Discussion

Our work empirically demonstrates that the ecophysiological effects of host species on mountain pine beetle can affect cold tolerance and pre-winter development rate, two life history traits that may mediate the population dynamics of this insect (Bentz and Powell, 2014; Régnière and Bentz, 2007). These findings have critical implications for the suitability of novel hosts for this insect, and thus the potential for management of outbreaks among novel hosts in eastern forests.

4.1. Effects of life stage and sex on cold tolerance

Previous work has suggested that different life stages of the mountain pine beetle may have different tolerances to cold temperatures (Cooke, 2009; Lester and Irwin, 2012; Safranyik and Linton, 1998; Strongman, 1982; Wygant, 1940). This study shows, for the first time in a common garden environment, that fourth instars are significantly more cold tolerant than pupae or teneral adults (Fig. 2). Indeed, supercooling points of fourth instars were 12–18 °C lower than later life stages. Our results also suggest a sex-related difference in cold tolerance among overwintering adults (Fig. 2). This bimodality has previously received little attention (Renault et al., 2002; Salin et al., 2000), yet may have important implications for sex ratios and colonization dynamics of mountain pine beetle throughout its range as males may be less likely to survive winter, possibly contributing to previously observed female bias in this species (James et al., 2016). The mean supercooling point of teneral adults in our study (−16.6 °C) was substantially warmer than reported for diapausing adults (−28.7 °C) that were overwintering after depositing brood (Lester and Irwin, 2012). The reason for this difference is unclear but may be related to exposure to different temperature regimes (Bentz and Mullins, 1999) or to the significant shifts in adult physiology that occur during and post host colonization (Pitt et al., 2014).

We observed higher mortality in early than in late instars (Fig. 1), similar to others (Reid and Gates, 1970; Safranyik and Linton, 1998), despite reports of little difference in supercooling points among instars (Bentz and Mullins, 1999; Somme, 1964). Similar supercooling points, yet higher mortality in early instars (Fig. 1) may suggest potential chill intolerance in these early stages. While we investigated the relationship between cold and mortality (cold tolerance strategy) of fourth instars, further work is needed to determine the cold tolerance strategy of other life stages.

4.2. Host mediated trade-offs

The combination of rapid pre-winter development, and differences in cold tolerance among life stages, may constitute a trade-off in novel hosts. More rapid development is often positively correlated with other performance traits (Amman, 1982; Bentz, 1999; Cerezke, 1995; Langor, 1989) and is likely an indicator of greater nutrition (Goodsman et al., 2012) and a higher quality host (Amman, 1982; Safranyik and Linton, 1983). However, a trade-off may occur as the likelihood of cold induced mortality increases in advanced life stages. Overwintering in pupal and adult stages is uncommon in the beetle's historical range (Reid, 1963). This may be due to host specific selection pressure for appropriate development rates in lodgepole and ponderosa pines (Bentz et al., 2014). Indeed, we observed a low proportion of brood in these late stages in historical hosts the first year (Table 1). In novel hosts this interaction between development rates and host species may be disrupted. Brood in red pine in particular developed at faster rates both years (Table 1), resulting in brood in these hosts generally becoming less cold tolerant as a population once winter arrived (due to some insects reaching less cold tolerant stages). Faster development prior to winter and variable rates of develop-

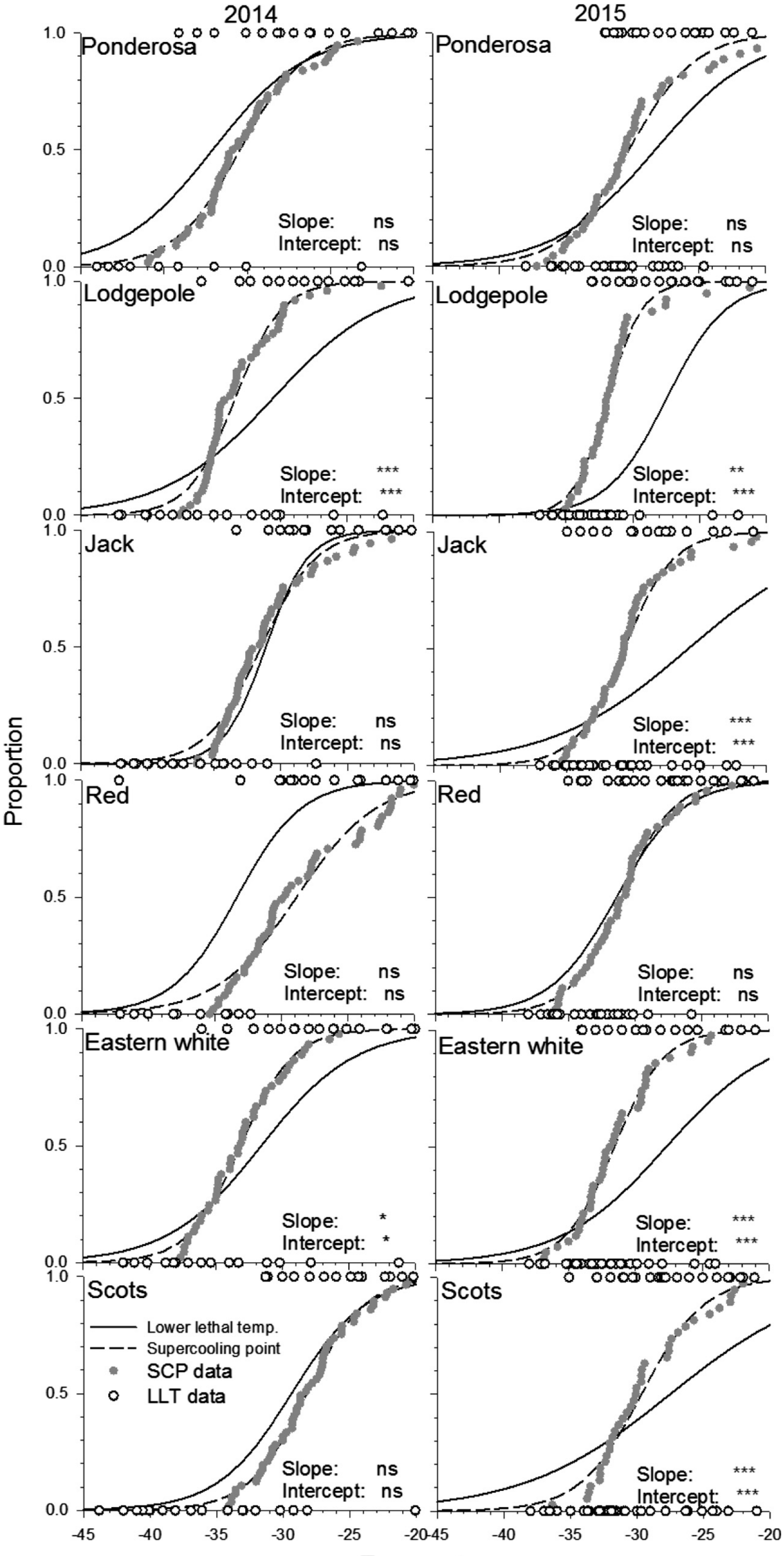

Fig. 3. Proportion of fourth instar mountain pine beetles from different hosts that had survived (i.e., lower lethal temperature) or had not yet started to freeze (i.e., reached the supercooling point) when exposed to various temperatures. Filled circles indicate individual supercooling points. Dashed lines describe the modelled proportions of individuals that had not given an exotherm when cooled to the specified temperature. Open circles and solid lines describe observed and modelled survival of individuals cooled to the specified temperature respectively. Models were based on logistic regressions. In each panel, tests compare slopes and intercepts of the two models: ns, not significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

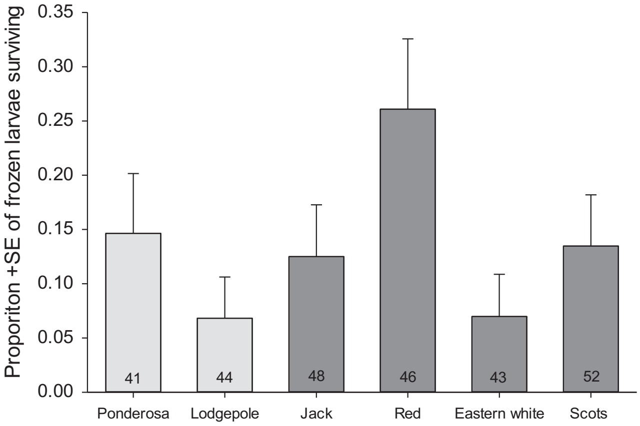

Fig. 4. Proportion of fourth instar mountain pine beetles within each pine species that survived freezing in lower lethal temperature experiments. Survival was determined by evidence of feeding or molting. Numbers on bars are the total number of larvae tested. Light bars are historical hosts and dark bars are novel, potential hosts. The proportion of larvae that survived freezing was not affected by host ($\chi^2 = 8.79$, $df = 5$, $P = 0.12$).

ment among pine species within a stand may result in earlier and desynchronized emergence the following year. Establishment of brood earlier in the summer could result in an even larger proportion of the population developing to pupal or adult stages prior to winter. Thus, differences in rates of development could have cascading impacts on the population dynamics of the mountain pine beetle among novel hosts and in eastern forests.

4.3. Host impact on cold tolerance

Contrary to our expectations, mortality was not strictly associated with freezing in all hosts, with many larvae in hosts such as lodgepole and eastern white pine, for example, dying before freezing (Fig. 3), thus demonstrating chill-intolerance. Mortality prior to freezing in an otherwise freeze-intolerant insect has been observed by others (Renault et al., 2002), but the possible role of host in mediating survival of chilling is intriguing.

Our results showing little fourth instar survival of the onset of ice formation in lodgepole pine are consistent with other studies (Bentz and Mullins, 1999; Somme, 1964). In contrast, survival of the onset of freezing was more prevalent in red pines, where over 25% of the larvae survived (Fig. 4). We did not quantify the extent of ice formation or assess survival after holding individuals in a semi-frozen state for an extended period (e.g., >24 h), two metrics often used to determine freeze-tolerance (Wygant, 1940). Thus, we are reluctant to suggest that mountain pine beetle larvae can be fully freeze-tolerant, but posit that these insects could be considered partially freeze-tolerant. This finding is ecologically significant because it indicates that the supercooling points may be a less reliable measure of overwintering capacity than lower lethal temperatures for overwintering fourth instar larvae of mountain pine beetle.

In addition to how larvae respond to freezing, we found that the effects of cold temperatures on larval mortality differed among pine species (Table 2). Previous studies of lower lethal temperatures conducted in common garden environments (Wygant, 1940; Yuill, 1941), as well as field observations of mountain pine beetle in mixed stands (Langor and Spence, 1991), have suggested that larvae experience different lower lethal temperatures among different western hosts. Host effects on cold tolerance of insects are becoming more widely documented in recent years (e.g., Liu et al., 2007; Trudeau et al., 2010; Feng et al., 2016; Morey et al., 2016). In fourth instars, we observed a spread in LT_{50}s of 4 °C the first year and 5.5 °C the second year (Table 2), similar to previously reported spreads of 3 °C (Yuill, 1941) and 5 °C among historical hosts (Wygant, 1940). Similarly, differences in supercooling points among hosts varied by as much as 5 °C (Table 2). Thus a greater proportion of larvae may be killed in Scots, jack and eastern white than in red pine in a mixed stand exposed to the same temperatures.

4.4. Parameters for climatic suitability models

By utilizing under-bark temperatures in the different pine hosts, we were able to compare observed and predicted mortality (Table 3). In this field validation of cumulative supercooling point and lower lethal temperature models (Fig. 3), supercooling point models were consistently conservative in their estimate of mortality, while our lower lethal temperature models more accurately predicted observed mortality in most cases (Table 3). Host-mediated differences in cold tolerance may reduce the accuracy of models, parameterized on a single host, to accurately project winter mortality. Our results for supercooling points for lodgepole pine (−33.2 °C ± 0.43SE in Year 1, and −31.6 °C ± 0.48SE in Year 2) are consistent with the global value (−32.3 °C ± 0.06SE) for full cold tolerance used in the model developed by Régnière and Bentz (2007), parameterized from a sample of larvae from primarily lodgepole pine (Bentz and Mullins, 1999). However, mean supercooling point values from larvae from other pines in our study deviated from that value by as much as 4 °C (Table 2). Deviation from the global value further increased by 6 °C when LT_{50}s were used. However while host may exact varying effects, we observed LT_{90}s among all hosts to be warmer than a threshold of −40 °C (Safranyik et al., 1975), an absolute lower limit for survival (Carroll et al., 2004; Safranyik et al., 2010, 1975).

Table 3
Predicted and observed survival of late instar mountain pine beetles overwintering in the Black Hills, SD in December 2013 in eight bolts of each of six pine species. Predicted survival is based on the minimum observed temperature (averaged for the north and south sides of each log) and January-February 2014 laboratory measures and subsequent models of the supercooling point (SCP) and lower lethal temperature (LLT). The supercooling model assumes that each individual dies as it starts to freeze.

				2014 SCP model			2014 LLT model		
Species	*n*	Observed Temp °C	Observed Survival	Lower 95% CI	Predicted Survival	Upper 95% CI	Lower 95% CI	Predicted Survival	Upper 95% CI
Ponderosa	489	−20.3	91.2%	99.2%	99.5%	99.7%	77.9%	98.6%	99.9%
Lodgepole	356	−21.7	92.7%	99.7%	99.8%	99.9%	54.0%	88.4%	98.0%
Jack	316	−21.1	97.2%	98.7%	99.2%	99.5%	83.8%	99.8%	100.0%
Red	213	−21.2	80.8%	91.1%	92.8%	94.2%	80.8%	99.4%	100.0%
Eastern white	569	−21.8	95.3%	99.5%	99.7%	99.8%	68.5%	94.3%	99.2%
Scots	194	−21.4	90.2%	94.8%	95.9%	96.7%	65.4%	94.7%	99.4%
All samples	2137	−21.4	92.3%	98.1%	98.2%	98.3%	91.7%	96.8%	98.8%

4.5. Significance to eastern forests

Our results have implications for mountain pine beetle winter survival should it reach forests of eastern North America. In the beetle's native range, natural selection acts to match development rate and cold tolerance to the climate of the region (Bentz et al., 2014), facilitating outbreak potential (Sambaraju et al., 2012; Stahl et al., 2006; Weed et al., 2015). In areas of eastern North America with winter temperatures similar to western forests (e.g., central Great Lakes region and New England), more rapid development in certain novel hosts may result in high mortality among pupae and adults. In areas with winters typically colder than the native range of the insect (e.g., Northwestern Great Lakes region and areas north of New England), brood developing in red pine may be particularly successful due to generally greater cold tolerance and reduced likelihood of reaching less cold tolerant life stages prior to winter (Table 1, Fig. 2). However, the risk of sudden drops in temperature could still limit success. Further research is needed to more fully integrate climate, development, and cold tolerance throughout the range most at risk for invasion.

4.6. Conclusions

This research was inspired by a simple, pragmatic question: could mountain pine beetle survive winters in northeastern North America? The question cannot be answered without considering the effects of novel hosts. Indeed, the effects of novel host plants on an insect herbivore's capacity to successfully overwinter are likely important to consider in assessments of climatic suitability for other insects that are experiencing range expansions, or introduced to new regions (Morey et al., 2016). Our study revealed host mediated trade-offs between development and cold tolerance. If future, extreme-low temperatures are similar to historical norms, winter mortality of mountain pine beetle may be an important driver of population dynamics among novel hosts due to rapid development to less cold tolerant stages, and serve to limit range expansion and outbreak potential (Bentz et al., 2010). But, if low-winter temperatures rise, that rate of range expansion may accelerate due to faster development on novel hosts than historical hosts.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foreco.2017.05.031.

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