Comparative cold tolerance and climate matching of coastal and inland *Laricobius nigrinus* (Coleoptera: Derodontidae), a biological control agent of hemlock woolly adelgids

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**Abstract**

*Laricobius nigrinus* (Coleoptera: Derodontidae) was first collected near the coastal city of Victoria, British Columbia, Canada for release as a biological control agent to suppress tree-killing densities of hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae), in the eastern United States. Beetles established in warm areas of the invaded range of *A. tsugae*, but had a low probability of establishment in cold areas. With the goal of locating beetles with greater cold-tolerance, we collected hundreds of adults in the northern Rocky Mountains. To support planned releases of these inland *L. nigrinus*, the cold tolerance of field-collected coastal (Seattle, WA) and inland (Coeur d’Alene and Moscow, ID) adults and climate match index scores (CLIMEX v.2) of these collection areas and parts of the eastern United States were compared. We found that individuals of inland *L. nigrinus* were more cold tolerant than those of coastal *L. nigrinus*, based on higher survival in a winter field cage study in Massachusetts and a lower supercooling point in a laboratory assay. Inland *L. nigrinus* also had higher survival than coastal *L. nigrinus* after an 18 h exposure to sub-zero temperatures in another laboratory assay. Areas of the eastern United States infested with *A. tsugae* and receiving *L. nigrinus* releases matched the climate of Seattle and Coeur d’Alene reasonably well, but Coeur d’Alene had higher index values over a considerably larger area than Seattle. Accordingly, inland *L. nigrinus* appears preferable for release in the colder portions of *A. tsugae*’s invaded range in the eastern United States.

**1. Introduction**

*Adelges tsugae* Annand (Hemiptera: Adelgidae), native to Asia and western North America, was accidentally introduced to eastern North America from Japan (Havill et al., 2006) and is recognized as the most important pest of eastern hemlock, *Tsuga canadensis* (L.) Carrière, and Carolina hemlock, *Tsuga caroliniana* Engelmann (Anonymous, 2005). *Laricobius nigrinus* Fender (Coleoptera: Derodontidae), a predator released against *A. tsugae* in the eastern United States, is native to the northwestern United States and western Canada (Fender, 1945). The original releases in the eastern United States were made with beetles collected from the Puget Trough area (e.g., Victoria, British Columbia and Seattle, Washington), which has a mild maritime climate. After their release, beetles from Victoria established in the mid- and southern Appalachian Mountains (Lamb et al., 2006; Mausel et al., 2010), where they proved to be well synchronized and demonstrated an impact on *A. tsugae* populations (Mausel et al., 2008). This “coastal” *L. nigrinus*, however, had a low probability of establishment in colder areas, either in more northerly locations or at high elevations, likely due to winter low temperatures (Mausel et al., 2010) that exceeded the cold tolerance limits of the beetle (Humble and Mavin, 2005).

If available, release of a *L. nigrinus* “biotype” with greater cold tolerance could improve both establishment probability and impact on *A. tsugae* because *L. nigrinus* is most active during the cold seasons (Zilahi-Balogh et al., 2003a,b) and release areas in the eastern United States are colder than the Puget Trough (Humble and Mavin, 2005). *L. nigrinus* has been recorded from the Rocky Mountains (e.g., Coeur d’Alene and Moscow, Idaho in this study) of the northwestern United States and Canada (Fender, 1945; Lawrence, 1989; Bright, 1991), an area with a continental climate. In this “inland” area, winters are colder and snowier than in the coastal areas from which the original *L. nigrinus* beetles were collected for release. Additionally, inland summers are warmer and precipitation occurs year-round, while on the coast summers are cooler and drier (Humble and Mavin, 2005). The similarity of seasonal temperature and precipitation patterns between collection and release
sites (i.e., climate matching) is fundamental to natural enemy introductions, and mismatched natural enemies can fail to establish or spread throughout a pest’s invaded range (Hopper et al., 1993).

Another principle of classical biological control is to collect large numbers of a natural enemy over a wide variety of environments in its native range to capture population variation in traits that may influence effectiveness (Hopper et al., 1993). Coastal and inland L. nigrinus are disjunct, being isolated by a lack of suitable host plants for adelgids in the arid Columbia Plateau area (Burns and Honkala, 1990), which divides the Cascade Mountains from the Rocky Mountains. Small but consistent differences in COI gene sequences between coastal and inland L. nigrinus (0.5% sequence divergence) suggest that these beetles represent distinct populations but that they are the same species (Havill, Personal Communication). Despite a lack of extensive data on the actual boundaries of L. nigrinus populations in a genetic context, restricted gene flow between coastal and inland sources implies a potential for long-term genetic differentiation in important life-history features such as host-range and cold tolerance. Laricobius spp. are considered adelgid specialists (Lawrence and Hlava, 1979) and host-range testing of inland L. nigrinus suggests low risk to non-target organisms (Mausel et al., unpublished data) as determined previously for coastal L. nigrinus (Zilahi-Balogh et al., 2002).

2.1. Insect populations

The “coastal” L. nigrinus adults used in the following experiments were collected in early November 2007 and 2009 from A. tsugae-infested western hemlock, Tsuga heterophylla (Raf.) Sarg., at the Washington Park Arboretum, Seattle, King Co., WA (47.6 N, 122.3 W; elevation 24 m) using canvas beat sheets (Bioquip, Rancho Dominguez, CA). “Inland” adults were collected during the same periods either from A. tsugae-infested western hemlock in Coeur d’Alene, Kootenai Co., ID (47.7 N, 116.7 W; elevation 658 m) or from western white pine, Pinus monticola Douglas ex D. Don, infested with Pinesus similis (Gillette) (Hemiptera: Adelgidae) at the University of Idaho Arboretum, Moscow, Latah Co., ID (46.7 N, 117.0 W; elevation 805 m). Beetles were shipped overnight on excelsior in escape-proof containers to the University of Massachusetts, Amherst, and were placed in environmental chambers at 5 °C day and 2 °C night, 75% relative humidity (RH), seasonally adjusted photoperiod, where they were fed locally collected A. tsugae ad libitum before use in experiments.

Adult beetles from both Coeur d’Alene and Moscow, ID (collected from different adelgid species) were confirmed morphologically to be L. nigrinus by both Zilahi-Balogh (Canadian Food Inspection Agency) and Vandenberg (US Department of Agriculture, Agriculture Research Service). Furthermore, DNA sequences from the mitochondrial COI gene from Coeur d’Alene and Moscow beetles were indistinguishable from each other but were distinguishable from coastal beetles (Havill, Personal Communication). The adelgid on western white pine was identified as P. similis by comparing its COI sequence with other specimens determined by morphology (Havill, Personal Communication).

2.2. Experiments

2.2.1. Survival in field cages

To test for differences in survival of coastal (Seattle) and inland (Coeur d’Alene and Moscow) L. nigrinus adults under harsh winter conditions, we conducted a field cage study from 20 December 2007 to 17 January 2008 in Leverett, MA (42.4 N, 72.5 W) using eastern hemlock at a site with 183 m elevation and an eastern aspect with 20% slope. Ten A. tsugae-infested eastern hemlock trees, separated by ≈20 m along a transect, were selected for use, with treatments arranged in a randomized complete block design (n = 10). Cages were bags made of sewn polyester fabric (0.25 mm mesh size), 0.6 m wide, and 0.9 m long (National Filter Media Corp., Wallingford, CT). Three branches (>2 m height) with high adelgid densities (i.e., nearly every needle base having an adelgid) were tagged per tree, and each caged branch was randomly assigned to a beetle treatment (Seattle, Coeur d’Alene, or Moscow). Ten adult beetles were placed inside each cage. The cage was pulled over the branch, and tightly closed with a cable tie around the bag and stem. One temperature logger (iButton, Maxim Integrated Products Inc., Sunnyvale, CA) was deployed per tree in a cage and data were recorded at 1-h intervals. After 1 month, branches with cages were cut and returned to the laboratory and kept at −3 °C until inspection (within 3 h). Cages were opened inside a Bug Dorm (Bioquip, Rancho Dominguez, CA) in a cool room (15 °C) to recover live and dead L. nigrinus. Despite repeated searching, four Seattle (4% of total), three Coeur d’Alene (3%), and two Moscow (2%) adults were not recovered and these individuals were excluded in the calculation of percent survival. Two-way ANOVA was used to determine whether a significant portion of the variation in mean percent survival was attributable to the L. nigrinus source, followed by a Tukey’s HSD test to separate significantly different means (p = 0.05) using SPSS 16.0 (SPSS Inc., 2007). Proportion data were arcsine square root transformed before analysis to account for unequal variance. Averages are presented as means ± standard error (SE).

2.2.2. Supercooling points

Supercooling points of coastal (Seattle) and inland (Coeur d’Alene and Moscow) L. nigrinus adults were tested on 14–16 January 2008 (n = 21). Beetles were placed on excelsior in plastic containers without food or water and kept at 0 °C for 48 h before the test. The supercooling point testing equipment and methods used were the same as described in (Bentz and Mullins, 1999), with the exception of a new circulator bath (Lauda K-2/R, Lauda-Brinkmann, Delran, NJ), data acquisition module (DaqTemp, Iotech
Inc., Cleveland, OH), and software (Daqview v.7.13.14, iotech Inc., Cleveland, OH). Four adults were tested at a time and each adult was placed between two thermocouples (accurate to 0.1 °C) in a drop of zinc oxide thermal grease. Adults were cooled at a rate of 0.4 °C/min from 24 to 5 °C, and 0.4 °C/min from −10 °C until the adult frozed. As ice formed in tissues, a temperature spike occurred due to the release of latent heat of fusion. The supercooling point was recorded as the mean temperature of the thermocouples on the dorsal and ventral side of each adult, one second before the spike. One-way ANOVA was used to determine whether a significant portion of the variation in mean supercooling point was attributable to the L. nigrinus source area, followed by a Tukey’s HSD test to separate significantly different means, as described previously.

2.2.3. Sub-zero temperature exposure

Survival of coastal (Seattle) and inland (Coeur d’Alene) L. nigrinus adults after a long exposure to temperatures 1.5 °C above the mean supercooling point of coastal beetles were compared on 15–16 April 2010 (n = 75). Individual beetles were placed into ventilated plastic containers and held at 5 °C, 75% RH, and 12:12 photoperiod on excelsior (without food or water) for 24 h before the test. In an environmental chamber (Percival Scientific, Inc. Model LT36VL, Perry, IA) the mean temperature of the beetles during the experiment was 0.6 ± 0.03 °C for 2 h, −8.0 ± 0.03 °C for 2 h, and then −15.4 ± 0.02 °C for 18 h. The mean (±SE) temperatures inside the chamber was calculated from data logger (iButton) readings at 2-min intervals. At the end of the test, beetles were warmed gradually over 2 h to 21.0 ± 0.02 °C and placed on A. tsuga-infested eastern hemlock with moistened filter paper in plastic containers for 4 days. At the end of 4 days, coastal and inland beetle survival was recorded. Dead beetles were unresponsive and were monitored periodically over a day to account for death feigning. Live beetles exhibited normal walking and feeding behavior. The binomial comparative trial (Zar, 1999) data were analyzed using the conservative Yates continuity correction in SPSS 16.0 (p = 0.05).

2.2.4. Climate matching

The basic ‘match climates’ algorithm was used in CLIMEX v.2 (Sutherst et al., 2004; Sutherst, 2005) to create two maps comparing the climate match index of the Seattle (47.8 N, 122.3 W) and Coeur d’Alene (47.8 N, 116.8 W) collection areas to parts of eastern North America. The climate match index is similar to a correlation coefficient and values vary from zero (no match) to 1.0 (an exact match). Generally, an index >0.8 is a close match, 0.6 < x < 0.8 is reasonable, and <0.6 is poor (Sutherst et al., 2004). The climate match index we used was a composite of minimum temperature, maximum temperature, total rainfall, and seasonal rainfall pattern estimated from the 0.5° grid of meteorological data (mean monthly norms, 1961–1990) included in CLIMEX v.2 (New et al., 1999). Two other maps were produced comparing mean monthly minimum temperature (from October to April) of the collection areas to eastern North America, as establishment was positively related to minimum winter temperature in earlier studies (Mausel et al., 2010). In addition, we compared the climate and minimum temperature match indices between the collection areas and recent coastal and inland L. nigrinus release areas in five northeastern States with paired t-tests (n = 5). These recent L. nigrinus release areas were near Caywood, NY on the Finger Lakes National Forest (42.8 N, 76.8 W); Slate Run, PA on the Tiadaghton State Forest (41.3 N, 77.3 W); Wendell, MA in the Wendell State Forest (42.8 N, 72.3 W); Brattleboro, VT on Cersosimo Lumber Co. land (42.8 N, 72.8 W); and Kittery, ME on non-industrial private forestland (43.3 N, 70.8 W).

3. Results

3.1. Survival in field cages

Field survival of coastal and inland L. nigrinus adults in cages differed significantly (F = 34.7; df = 2, 18; p < 0.0001). Mean survival of coastal adults (Seattle = 49% ± 0.07) was lower than that of inland adults (Coeur d’Alene = 90% ± 0.02 and Moscow = 90% ± 0.05), despite the fact that one cage from the Seattle treatment became buried in snow during the experiment and had 90% survival (Fig. 1). Average temperature recorded in the cages during the 1 month period was −1.1 ± 0.04 °C, average minimum temperature was −18.1 ± 0.2 °C during a bout of arctic air on 2–3 January 2008, and average maximum temperature was 12.1 ± 0.6 °C. Sub-zero temperatures above the coastal L. nigrinus mean supercooling point (i.e., 0 to −16.9 °C, see experiment below).

Fig. 1. Survival of coastal (Seattle, WA) and inland (Coeur d’Alene and Moscow, ID) L. nigrinus adults over a 1 month long field cage exposure study on eastern hemlock branches in Leverett, MA (20 December 2007–17 January 2008).

Fig. 2. Supercooling points of adult L. nigrinus from coastal (Seattle, WA) and inland (Coeur d’Alene and Moscow, ID) collection areas.
occurred for 374.4 ± 3.2 h and below the supercooling point (i.e., <−16.9 °C) for 13.5 ± 1.9 h.

3.2. Supercooling points

Supercooling points of coastal and inland *L. nigrinus* adults differed significantly (*F* = 4.7; df = 2, 60; *p* = 0.01) (Fig. 2). The mean supercooling point of coastal adults (Seattle = −16.9 ± 0.3 °C) was 2.3 °C higher than that of inland adults from Coeur d’Alene (−19.2 ± 0.7 °C). However, the supercooling point of inland adults from Moscow (−18.6 ± 0.6 °C) was not significantly different from those of Seattle or Coeur d’Alene.

3.3. Sub-zero temperature exposure

Survival of coastal and inland *L. nigrinus* after an 18 h exposure to −15.4 °C differed significantly (χ² = 9.6, df = 1, *p* = 0.002). Only 36% of coastal (Seattle) adults survived while 63% of inland (Coeur d’Alene) adults survived.

Fig. 3. Climate match index maps produced by CLIMEX v.2 to predict areas of reasonable-to-good climatic similarity (0.6–1.0 indices) in the eastern United States for *L. nigrinus* collected from source areas in (A) Seattle, WA and (B) Coeur d’Alene, ID. The index is a composite of mean monthly minimum temperature, maximum temperature, total rainfall, and seasonal rainfall pattern (1961–1990), with 1 being an exact match.
3.4. Climate matching

The areas of the eastern United States currently infested with *A. tsugae* matched the climates of both Seattle and Coeur d’Alene reasonably well (i.e., >0.6) (Fig. 3). However, Coeur d’Alene had higher climate index values over a considerably larger area than Seattle. The eastern areas that best match Seattle (i.e., 0.68–0.76) were in the mid- to southern Appalachian Mountains, coastal mid-Atlantic States, coastal southern New England, some areas of the midwestern States, and southeastern Ontario. Coeur d’Alene’s best matches (i.e., 0.68–0.80) include the entire Appalachian Mountain chain, mid- to southern New England, coastal New England, midwestern States, and some areas of southern Ontario, Quebec, and the Atlantic Provinces.

The eastern United States areas that matched the mean monthly minimum temperature of Seattle reasonably well (i.e., >0.6) were the southeastern States and mid-Atlantic coast (Fig. 4). The eastern areas that matched the minimum temperature of Coeur d’Alene reasonably well were the entire Appalachian Mountain chain, mid-Atlantic States, southern and coastal New England, the midwestern States, southeast Ontario, and southern Nova Scotia.

At five *L. nigrinus* release areas in the northeastern United States, significantly better climate matching resulted from the

![Fig. 4. Minimum temperature match index maps produced by CLIMEX v.2 to predict areas of similarity in the eastern United States for *L. nigrinus* collected from source areas in (A) Seattle, WA and (B) Coeur d’Alene, ID. The index is based on mean monthly minimum temperature only (1961–1990), with 1 being an exact match.](image-url)
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cooling point, as suggested by our sub-zero temperature exposure

due to the nearly 400 h of sub-zero temperatures above the super-

difference in Moscow beetles) is probably explained by mortality

pared to the small differences in supercooling points (or no

Climate and minimum temperature match indices of coastal (Seattle, WA) and inland (Coeur d’Alene, ID) *L. nigrinus* collection areas with experimental release areas in five northeastern United States using CLIMEX (v.2). 

<table>
<thead>
<tr>
<th>Release area</th>
<th>Climate match index</th>
<th>Minimum temperature match index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seattle, WA</td>
<td>Coeur d’Alene, ID</td>
</tr>
<tr>
<td>Caywood, NY</td>
<td>0.671</td>
<td>0.770</td>
</tr>
<tr>
<td>Slate Run, PA</td>
<td>0.696</td>
<td>0.760</td>
</tr>
<tr>
<td>Wendell, MA</td>
<td>0.691</td>
<td>0.740</td>
</tr>
<tr>
<td>Brattleboro, VT</td>
<td>0.661</td>
<td>0.726</td>
</tr>
<tr>
<td>Kitley, ME</td>
<td>0.711</td>
<td>0.753</td>
</tr>
<tr>
<td>Average ± SE</td>
<td>0.686 ± 0.009</td>
<td>0.750 ± 0.008</td>
</tr>
</tbody>
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* a Mean monthly minimum temperature, maximum temperature, rainfall total, and rainfall pattern (1961–1990).

* b Mean monthly minimum temperature from October to April (1961–1990).

Coeur d’Alene collection area versus Seattle in terms of the climate match index ($t_{4} = 6.5; p = 0.003$) and minimum temperature match index ($t_{4} = 57.8; p < 0.0001$) (Table 1). The mean climate match index improved 6% points and the minimum temperature index improved 39% points when comparing Seattle to Coeur d’Alene collecting areas.

4. Discussion

Because *L. nigrinus* is active and is released for potential biological control of *A. tsugae* during fall through early spring, winter survival is critical for population establishment. Results from our studies suggest that inland *L. nigrinus* adults (e.g., northern Idaho) are more cold tolerant and more likely to survive the winter conditions found in cold areas of the eastern United States than are those of the coastal population (e.g., Seattle, WA). Inland beetles may therefore have a higher probability of establishment and impact. However, our field cage study could have artificially restricted some of the beetle’s normal behaviors. Surviving *L. nigrinus* adults were typically clustered where the cage was cinched to the branch stem suggesting they were trying to move off the branch towards the tree bole and perhaps the forest floor, which would likely have increased survival. Adult aggregations were also found in branch axes inside the cage. Such sheltering behavior is known in *Ips grandicollis* (Eichoff) (Coleoptera: Scolytidae) adults, which in Wisconsin migrated to the soil litter layer and survived $27{^\circ}$C (Lombardero et al., 2000). Mean supercooling point temperatures were lower for *L. nigrinus* adults from Coeur d’Alene compared to Seattle, but by only a couple of degrees and the importance of this difference might be reduced if beetles sought shelter in thermally buffered microsites.

Freeze avoidance is the most common adaptation in arthropods to avoid lethal freezing in cold environments and the supercooling point represents the lower lethal temperature (Block, 1990). Previous work has shown that coastal *L. nigrinus* adult survival declines with increasing time at temperatures just above the mean supercooling point (Humble and Mavin, 2005) and this aspect of cold tolerance must be understood to fully assess relative cold tolerance (Block, 1990; McDonald et al., 2000). For example, in a comparative cold tolerance study of two populations of *Delia radicum* (L.) (Diptera: Anthomyiidae), temperature changes and duration of sub-zero temperatures were more biologically important than the supercooling point (Turnock et al., 1990). Similarly, the large population differences in *L. nigrinus* survival in our cage study compared to the small differences in supercooling points (or no difference in Moscow beetles) is probably explained by mortality due to the nearly 400 h of sub-zero temperatures above the supercooling point, as suggested by our sub-zero temperature exposure experiment. Many coastal and some inland beetles that were alive immediately after the exposure experiment exhibited leg or antennal movement but in a manner that did not allow them to walk, articulate the head, or feed, and they soon died.

Climate matching of collection and release areas is considered a fundamental requirement in choosing agents for establishment and impact in classical biological control projects (Debach and Rosen, 1991). Climatic matching provided further insights into areas at which inland *L. nigrinus* might have a survival advantage over coastal beetles. In terms of climatic similarity of collection areas to intended release areas, we found that coastal beetles were best matched with warm areas as compared to colder areas, a conclusion borne out by empirical results of actual patterns of establishment (Mausel et al., 2010). Based on the high match indices between the inland collection area and colder areas of the *A. tsu-
ge-invaded range, we anticipate better establishment rates of inland *L. nigrinus* in these areas, where the coastal *L. nigrinus* has not yet demonstrated establishment. Furthermore, *A. tsugae* has not occupied its entire potential range in the northeastern States, mid-western States, and Canada and may become a pest in these cold areas due to continued genetic selection on the pest for increased winter cold tolerance (Butin et al., 2005) and climate change (Paradis et al., 2008).

A difference between *L. nigrinus* populations in cold tolerance in our common environment studies suggests a genetic basis for the trait, but maternal effects were not controlled. Crossing or half-sib analysis should be done to verify and investigate if the phenotypic variation we observed has a genetic basis (Hopper et al., 1993). Because *L. nigrinus* is collected yearly from the western United States for direct biological control release in the eastern United States and to supply mass-rearing laboratories, maternal effects on cold tolerance are potentially important for establishment and should not be ignored. To test the prediction of improved establishment and impact by inland *L. nigrinus* when released directly in the field, we have initiated paired coastal and inland beetle releases and control sites for long-term experimental assessment of establishment, population growth, and impact on *A. tsugae* and hemlock health. Previously, large coastal *L. nigrinus* release sizes were recommended to establish populations in the climatically unfavorable cold areas of the eastern United States (Mausel et al., 2010). It is possible that the biological control of *A. tsugae* with *L. nigrinus* could be more efficient by allocating coastal and inland beetles to the areas of greatest climate match.

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References


