## LOW TEMPERATURE IN THE HEMLOCK WOOLLY ADELGID SYSTEM

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## ABSTRACT

Hemlock woolly adelgid (HWA) vary in their susceptibility to temperatures considerably below freezing. Many individuals may die by -20°C (-4°F), but more cold-tolerant individuals will allow populations to persist. Managers wanting to gauge temperature impacts on future adelgid pressure to hemlock forests may benefit from using available daily records of minimum low temperatures. More robust data on adelgid temperature response will enhance development of models/tools to assist in their forecasts. Exotic predators being released to suppress adelgid populations (*Sasajiscymnus tsugae*, *Scymnus sinuanodulus*, *Scymnus ning-shanensis*, and *Laricobius nigrinus*) may be slightly less cold-tolerant than the adelgid, but the cold-tolerance does not appear to be affected by feeding. These predators are able to feed at near 0°C temperatures. In areas with relatively cold climates, the low winter temperature and the four predators tested could work together to regulate HWA populations.

## **KEYWORDS**

cold tolerance, predator, microclimate, range

## INTRODUCTION

Cold weather—or rather, low winter temperature—has a dynamic role in impacting populations of hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, and defining the limits of their geographic expansion. The consequences of low temperature extends beyond mortality within HWA populations to influences on predators that might serve to reduce HWA numbers. A variety of predators are being released in the hemlock ecosystem in attempts to stabilize HWA populations at levels that will reduce their impacts to hemlock forests. We need not only a better understanding of predator survival at low temperatures but also of the behaviors they might have to protect themselves as temperatures decline and whether bouts of feeding during warming periods affect their cold tolerance. Temperature within an ecosystem environment can be measured as ambient (i.e., the temperature without influences of solar radiation) or it can be measured as the microenvironment that a target organism might experience. Depending on where an insect is located at any given time in its lifecycle, its microhabitat might be on an hemlock needle, in the cracks of bark, or in the soil, among other places. Previous research (Costa et al. 2004) during the winter season in Massachusetts and Connecticut has compared the microclimatic extremes on hemlock foliage with ambient temperature records obtained in adjacent weather stations. Daily maximum winter temperatures on foliage were increased several degrees Celsius over ambient, with increases corresponding to recorded periods of sunlight. However, the difference between daily minimum temperature on hemlock foliage and ambient records fluctuated much less. The reason for this disparity between how maximum and minimum microenvironment temperatures reflect ambient conditions is that the lowest temperatures in the hemlock forest generally occurred at night when there was no sunlight to warm needles.

The similarity between ambient recordings of daily minimum temperatures and that found in the HWA microenvironment suggests the usefulness of ambient temperature records for assessing impacts of low temperature on HWA survival. Conveniently, this relationship makes the vast resources of meteorological data that is annually collected of great potential value in predicting areas where HWA pressure may be currently mitigated or expected to escalate in the coming year. There is a limited amount of information available on HWA cold tolerance to give a general indication of the range of HWA susceptibility to cold (Parker et al. 1999; Skinner et al. 2003). Data that exams mortality at narrower temperature increments is critical for more precise determinations of HWA response to cold.

The results presented in this paper provide updated data on HWA cold tolerance, the low temperature and feeding response of several HWA predators, and initial observations on predator low-temperature behavior.

#### SUPER-COOLING POINT

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For freeze-intolerant insects (as opposed to those that can tolerate freezing without dying), the temperature that freezing occurs can be considered the lowest possible temperature before mortality is expected to occur. The primary measurement used for determining the lethal temperature is the observation of super-cooling points (SCP) for individual insects. The basis of super-cooling point determination is the fact that, as water crystallizes or freezes, it releases a small amount of heat, which can be detected with a suitably sensitive temperature probe.

The term 'super cooling' stems form the observation that water can freeze at temperatures below 0°C (32°F) and thus allow organisms to survive normal freezing conditions. To assess the freezing point, insects are subject to rapidly decreasing temperatures (1-2°C/minute) while their temperature is monitored at very short intervals (every second in our research). Later, the temperature records are examined and the lowest temperature achieved before the brief release of heat occurs is the super-cooling point for that individual.

#### SIGNIFICANCE OF HWA SUPER-COOLING POINT VARIATION

The super-cooling point varies among individuals of a particular species and can also be influenced overall in a population by various factors such as time of year, preconditioning, and geographic sources of the insect, among other factors. Figure 1 depicts the SCP for HWA collected in the southern and northern extents of their range, and each individual point in the scatter plot represents an SCP determination. In general, many of the insects tested had a SCP around -18°C (0°F), and those from northern regions tended to tolerate slightly lower temperatures.

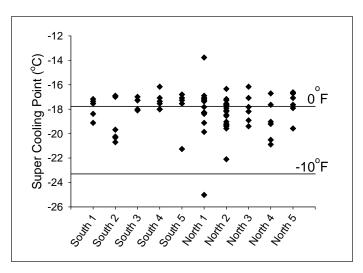


Figure 1. Super-cooling point of hemlock woolly adelgid collected during winter and early spring in the southern region (one site in Georgia, three in North Carolina, and one in Virginia) and northern region (one site in Pennsylvania, three in Connecticut, and two in Massachusetts). Each triangle represents a single determination. Horizontal lines provide reference between temperature scales.

Knowing the proportion of insects that will die at a particular temperature can have considerable value when coupled with temperature records for a given region. Importantly, Figure 1 shows that there are several insects that survive at very low temperatures. These few individuals can have profound affects on HWA populations in an area after what would appear to be a substantial winter cold event, especially with the high fecundity of HWA. However, for this kind of information to be of increased practical value for predicting temperature influence on upcoming HWA pressure in a forest, a more complete characterization of the frequency distribution of SCP values would be a benefit. Trotter is developing a comprehensive Hemlock Woolly Adelgid Simulation Model based on our research and a multitude of other available data. A subcomponent of this model involving HWA overwintering mortality could be adapted as a Forest Health HWA Impact Tool.

The practical impact that temperature can have on HWA populations and the synchrony between the SCP data in Figure 1 and what occurs in a forested setting can be deduced from results obtained under natural settings. Data collected in Massachusetts and Connecticut on the mortality of HWA over time in relation to microclimate low temperature shows that there was limited mortality during early January of those sistens of HWA that had survived summer aestivation. However, two low-temperature events occurred in mid-January in which microclimate temperatures fell to below -20°C. Subsequent to this, HWA mortality dramatically increased at both locations with more dying in Massachusetts, where temperatures were lower. The observation that 99.5% of the individuals died at Mount Tom, Massachusetts,

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and that damaging populations still persist there today highlights the significance of the few individuals with appreciable lower SCP values.

#### PREDATOR LOW-TEMPERATURE FEEDING AND COLD TOLERANCE

A data set is developing on four predators being released for management of HWA (*Sasajis-cymnus tsugae* Sasaji and McClure, *Scymnus sinuanodulus* Yo and Yao, *Scymnus ningshanensis* Yo and Yao, and *Laricobius nigrinus* Fender) in relation to their ability to feed at temperatures ranging form 0-10°C. The adult beetles were placed individually in tubes, brought to 0°C in a low temperature bath, and then provided 15-25 HWA eggs, after which the temperature was raised to the target test temperature for 20 hours. Subsequent egg counts revealed that the beetles were able to feed down to 2.5°C (Figure 2; similar results were obtained with *L. nigrinus* but are not presented). This suggest that beetles active during the winter months are able to feed as temperature several degrees (Costa et al. 2004), it is likely that higher levels of feeding are possible or that beetles may feed even when ambient temperature conditions suggest otherwise.

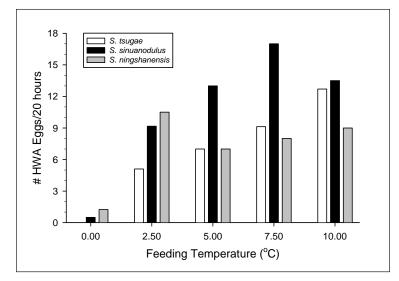


Figure 2. The feeding activity of *Sasajiscymnus tsugae*, *Scymnus sinuanodulus*, and *Scymnus ningshanensis* on hemlock woolly adelgid eggs at the temperatures indicated. Similar results were obtained with *Laricobius nigrinus*, although response at 2.5°C was not examined.

For insects feeding during the cold season, a question arises as to whether the consumption of food increases their susceptibility to cold. Initial observations made by determining the SCP of insects from the feeding trial above suggest that there is little difference in cold tolerance among fed and starved beetles (Figure 3; similar results were obtained with *L. nigrinus* but are not represented. The lack of influence on SCP favors the survival of insects feeding in an environment where temperatures can change rapidly and refuge from low temperatures is not readily available. These results coincide nicely with previous unpublished data for low temperature survival of *S. tsugae*. Note that the scatter of the SCP response (Figure 3) tends to be slightly above -18°C, whereas those for HWA (Figure 1) tended to be more around this point. Regardless, the differences are relatively minor, which is promising news for the geographic (environmental) range compatibility of HWA and the predators being released for its management.

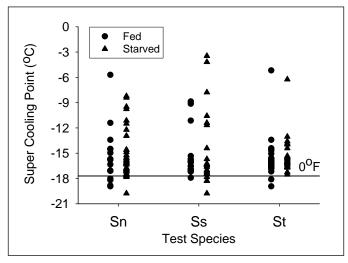


Figure 3. Super-cooling point of three hemlock woolly adelgid predators (*St* - *Sasajiscymnus tsugae, Ss* - *Scymnus sinuanodulus,* and *Sn* - *Scymnus ningshanensis*) that were either fed (●) or starved (▲) at low temperatures indicated in Figure 2. Each symbol represents a single determination. Horizontal lines provide reference between temperature scales. Similar results were obtained with Laricobius nigrinus.

#### BEHAVIORAL RESPONSE TO LOW TEMPERATURE

A critical question concerns the behavioral responses predators may have to protect themselves from freezing as temperatures fluctuate in the winter. For instance, do they remain on the foliage or do they seek refuge in bark or in the soil? Technology previously used to monitor the flight behavior of fruit flies under micro-gravity conditions (Miller et al. 2002) was adapted to monitor beetle behavioral responses to changing temperatures. The basic setup involves closed insect runways (10 lanes) etched into Plexiglas where the passage of beetles down the channel is detected at two points as they pass through infrared beams. To simulate a nonfreezing refuge (e.g., soil), the temperature at the bottom of the channels can be regulated (5°C for this study) while the rest of the unit is cooled (i.e., via immersion in a low temperature bath). Temperatures were monitored at the top and bottom of the behavior chamber, which currently is placed vertically in the bath. This technology became available during early 2008 and we are just beginning its use as a research tool. The question being addressed is: Where do beetles go as the temperature drops?

Initial observations with *L. nigrinus* found considerable activity in the upper part of the chamber when temperature in the entire observation chamber was maintained at 15°C. But as temperatures began to decline beetle activity diminished. The temperature in the upper chamber dropped to near zero, while temperatures in the bottom chamber did not go under 5°C. As this occurred, the activity records indicated that the beetles moved downward toward the area of temperature refuge (i.e., where it was warmer). Our preliminary observations with *S. tsugae* were less convincing because of their reluctance to move; however, the activity profiles suggested that *S. tsugae* remained in the top of the chamber as temperatures declined. Conceivably, these preliminary results might suggest that, in the natural environment *L. nigrinus* either

seeks refuge near warmth or by moving downward in response to declining temperatures, whereas *S. tsugae* may remain where they are located (upper chamber), presumably where they had been feeding on HWA.

#### HWA SYSTEM CONSIDERATIONS

The use of existing ambient low temperature records has excellent potential as a valuable resource for gauging impacts on HWA overwintering mortality. To increase the robustness of this approach for incorporation into a Forest Health HWA Impact Tool there is a need for expanded data on HWA SCP that captures the overall frequency distribution of SCP responses. The somewhat similar SCP profiles of HWA and its predators is a good sign and signals hope for wide compatibility within their geographic range. Additionally, there is indication that these predators may impact HWA populations when ambient and/or microenvironment temperatures rise modestly above freezing. These data suggest that in relatively cold climates the low winter temperature and the four predators tested could work together to regulate HWA populations.

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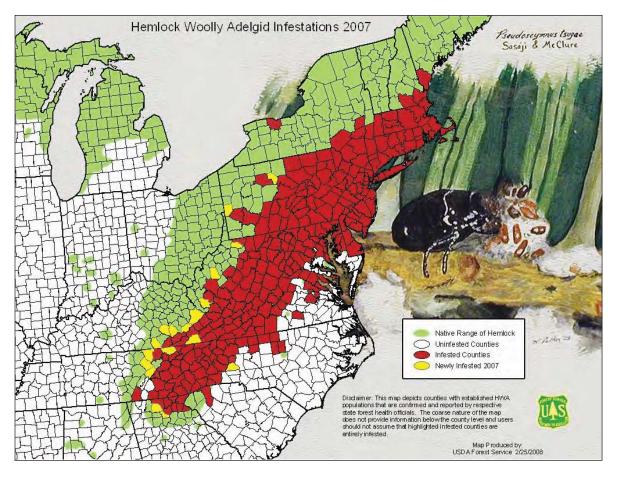
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