# Cold Temperatures. Will They Limit Range Expansion?

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## Abstract

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, primarily infests trees along the eastern seaboard in USDA plant hardiness zones where the minimum low temperature does not exceed –28.8°C. Laboratory studies were done to determine the low lethal temperature of HWA collected in January, February, and March from locations within different USDA plant hardiness zones. The Mt. Tom, Massachusetts HWA population, which exists in the coldest location, does not survive under laboratory conditions (at –35 to –40°C). It was evident that HWA cold hardiness declined as the winter progressed. HWA that has an ability to tolerate short periods of exposure to low temperatures also were able to tolerate longer periods. This suggests that time of exposure is not a major factor in survival. HWA cold hardiness differs depending on the geographical location of the population and the time of year. Most sensitive were populations collected from a site with a low minimum temperature not exceeding –20.5°C. In March only about 5% of the HWA survived an exposure of –20°C. Only HWA collected from Mt. Tom were able to survive an exposure of –30°C. The northerly spread of this pest may be impeded or even prevented by the cold winter temperatures in more northerly locations.

## Keywords:

Cold hardiness, minimum low temperatures, plant hardiness zones.

## Introduction

The overwintering adult hemlock woolly adelgid, *Adelges tsugae* Annand (HWA), is the source for the coming year's population and little is known about the cold tolerance of these wingless forms in North America. Range expansion of HWA may be limited by cold temperatures such as found in Vermont, where this pest is not known to occur currently (H.B.Teilon unpublished data). Winter survival and cold hardiness are the primary factors governing subsequent population levels of some temperate species (Bale 1991). In addition, cold winter temperatures may significantly reduce established populations in an infested area.

In Japan, approximately 25% of the HWA die over the winter at elevations of 1,650 m or lower where winter temperatures often reach – 35°C (McClure 1996). We report here on laboratory studies to determine the lowest temperature at which HWA collected from infested areas in Massachusetts and Connecticut in January, February, and March are able to survive. The studies were done from 1996 to1999 and originally reported in Parker et al. (1998, 1999) and Skinner et al. (2002, in review).

## Materials and Methods

In each area where samples were taken (Table 1), approximately five 30 m tall dominant eastern hemlock, *Tsuga canadensis* (L.), were randomly chosen. The sample trees showed a similar level of slight decline because of adelgid feeding but all had healthy branches with new growth and an abundance of HWA. We hypothesized that HWA on these branches would be well nourished and healthy and the most tolerant to cold temperatures. On sample days five 2 m long branches were pruned from the mid- and upper canopy of each tree using either a hydraulic bucket truck or a tree climber. From each branch we cut apical twigs (10 cm long) with one or more branchlets containing healthy green needles and adelgids. These were placed individually in glass test tubes (2.5 cm diameter by 25 cm long) containing 20 g of clean, dry sterilized sand. The base of each twig was pressed into the sand. Tubes were stored at 0°C until tested.

**Table 1. Geographical Information for HWA Sample Sites**

| Site | Longitude | Latitude | USDA Plant Hardiness Zone | Low Temperature Range |
|---|---|---|---|---|
| Mt. Tom, MA | 42°12'N | 72°37'W | 5a | -26.5 to –28.8°C |
| Meriden, CT | 41°32'N | 72°44'W | 6a | -20.6 to –23.3°C |
| Guilford, CT | 41°15'N | 72°44'W | 6b | -17.8 to –20.5°C |

HWA cold hardiness was assessed at -15, -20, -25, and -30°C (in 1996); at -20, -25, -30, -35 and -40°C (in 1997); and -20, -25 and –30°C (in 1999). Temperature selection for this study was done based on two factors: low annual temperature in USDA plant hardiness zones where HWA presently exists in New England, and low annual temperatures in USDA plant hardiness zone located one cold hardiness zone colder than the most northerly cold hardiness zone, where HWA is now. HWA held at 0°C were used as controls. All testing was done in an alcohol bath (Model 2103, Forma Scientific, Marietta, Ohio). HWA were exposed to these low temperatures for time periods ranging from 1to 24 hours. The alcohol bath was programmed to decrease from 0°C to the target temperature at the rate of 4°C per hour. Because the optimal cooling rate of HWA is not known, we selected a rate that is considered slow (Baust and Rojas 1985) and approximated conditions that occur naturally in some locations in New England.

Following low temperature exposure, tubes were removed from the alcohol bath, and 4 ml of sterile distilled water was added to the sand in each to maintain twig condition and prevent desiccation of

HWA. Tubes were capped with parafilm and held at room temperature (approximately 20°C). Mortality assessments were delayed 7 days to permit the insects to adapt to the warmer conditions (recommended by M.S. McClure personal communication) and to wait for typical mortality symptoms to develop (Baust and Rojas 1985).

To assess mortality, a twig was removed from the tube and viewed under 40x magnification. A randomly selected area of the twig, which appeared infested with live HWA, was examined, and the first 10 individuals were evaluated. These data were converted to the percentage of live HWA for each treated and control tube for analysis purposes. The following three criteria were used to determine if HWA were alive: overall appearance, a positive haemolymph pressure response to probing, and leg or body movements. The objective of this study was to determine the effect of our treatments on the survival of presumably live adelgids. It therefore differed from methods used to assess overwintering mortality in field populations, when counts are made of all individuals – live or dead on a twig.

Several sequential steps were involved with the data analyses. For specific explanations refer to Parker et al. (1998, 1999).

## Results

***1996 Experiments with HWA Collected From Mt. Tom, Massachusetts***. Evaluation of the field population of HWA, which had been placed at 20°C following sampling, revealed that 70%, 66%, and 45% of the adelgids assessed were alive in the January, February, and March samples, respectively. This provided evidence of the level of naturally occurring mortality at the time of sampling. A gradual decline in the actual percentage of live HWA was evident among the treated samples as time of exposure increased. A noticeable decline in cold hardiness throughout the winter was found among samples exposed to -20°C for 24 hours. The difference in live HWA between treated and control samples were more than five times greater in March than in January. A similar but smaller effect was obtained after exposure for shorter time periods and at -25°C.

A significant effect of temperature on HWA coldhardiness was detected. The colder the treatment and the longer the exposure to low temperature, the greater the difference in the percentage of live HWA between the treated and the control samples. The lowest percentage of live HWA was 5% found in the -30°C treatment held for 24 hours. Complete mortality was not obtained in any of the cold temperatures and exposure times used.

***1997 Experiments with HWA Collected From Mt. Tom, Massachusetts.*** The assessment of field-collected HWA prior to experimentation revealed 77%, 75%, and 67% live individuals in January, February, and March, respectively. The minimum temperature recorded at Mt. Tom during this period was -21°C. Changes in HWA cold hardiness from January to February were minimal. Our temperature exposures indicated that by mid-March most HWA had lost their ability to tolerate even relatively mild cold conditions. Without any statistical manipulations, at -25°C, 14 to 22% of the January-collected HWA; 8 to12% of the February-collected HWA; and 0 to 4% of the March-

collected HWA were alive. Ten percent of the March-collected HWA survived exposure to -20°C. The next coldest plant hardiness zone north of the Mt. Tom infestation has a low temperature range of -28.9 to -31.6°C. Slightly further north minimum temperatures are -31.7 to -34.4°C. Therefore, only a small percentage of HWA from the currently infested locations in the Mt. Tom area are likely to survive the low temperatures that occur in the next coldest plant hardiness zones. Our data also suggests that individual adelgids with an ability to survive subzero temperatures for short periods of time also can tolerate longer exposures to cold. The 1997 experiments demonstrated that HWA does not survive, under laboratory conditions, at -35 and -40°C. A short 2-hour exposure to these low temperatures is sufficient to kill them.

***1998 Experiments with HWA Collected From Mt. Tom, Massachusetts, Meriden, Connecticut and Guilford, Connecticut.*** HWA from these locations were living in different plant hardiness zones with different mean low temperature ranges (Table 1).

When we combined mortality data from all treatments and exposure times it became evident that mortality of HWA collected from Meriden and Guilford was greater than HWA mortality of those collected at Mt. Tom. As temperature was decreased, mortality of HWA increased. This was true for collections made in January, February, and March.

In all months adelgids collected from Meriden and Guilford did not survive exposure to -30 or -35°C. Less than 3% of the adelgids sampled in January and February from Mt. Tom, our northern site, survived exposure to -30°C and none survived exposure to -35°C in two of the three sample months. This suggests that -30°C was a critical temperature below which there was little or no survival among adelgids for all sample locations throughout the winter.

## Discussion

Our laboratory results demonstrate that HWA cold hardiness differs depending on the geographical location of the population and the time of year. In January the three populations we sampled and tested had a similar level of cold hardiness. They were fairly tolerant to -15 and -20 °C however, tolerance to -25, -30, and -35°C was minimal or lacking. In February, HWA collected at Mt. Tom showed a far greater tolerance to cold than those collected in Guilford. Mortality among those collected in Guilford at this time was relatively high after exposure to -15°C. By March a loss in cold tolerance was detectable in all three sites where HWA were sampled. Survival was slightly higher at -15 °C in the Mt. Tom adelgids than in the Guilford or Meriden populations. HWA may naturally lose an ability to tolerate cold temperatures as ambient temperatures rise in late winter and they begin to feed more extensively.

The mechanisms that influence cold hardiness in insects are complex and not fully understood for HWA. Our studies confirm that cold temperatures are likely to significantly reduce field populations, particularly in the northern range of this pest, and may limit their range expansion in the future.

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