*Agricultural and Forest Entomology* (2007), **9**, 215–219 DOI:10.1111/j.1461-9563.2007.00334.x

# Factors affecting settlement rate of the hemlock woolly adelgid, *Adelges tsugae*, on eastern hemlock, *Tsuga canadensis*

**Elizabeth Butin*†, Evan Preisser*‡ and Joseph Elkinton***

*Department of Plant, Soil, and Insect Sciences, University of Massachusetts at Amherst, Amherst, MA 01003, U.S.A., †Holyoke Community College, Holyoke, MA 01040, U.S.A. and ‡Department of Biological Sciences, University of Rhode Island, Kingston, RI 02881, U.S.A.*

**Abstract**

1 We assessed the importance of several factors potentially affecting the settlement rate of the invasive hemlock woolly adelgid *Adelges tsugae* (Hemiptera: Adelgidae) on uninfested foliage of the eastern hemlock, *Tsuga canadensis*. We conducted our experiments in Massachusetts (U.S.A.) with overwintering sistens adelgids, and applied standard densities of infested foliage to uninfested branches in a planned multiple-comparison design.

2 Settlement rates of progrediens crawlers produced by the overwintering sistens were highest when adelgid-infested foliage was loosely attached to uninfested foliage and both branches were then enclosed in a mesh sleeve.

3 Early-emerging crawlers settled at a higher rate than did late-emerging crawlers.

4 Increasing the density of infested branches did not affect settlement rates.

5 We also tested whether less severe winter conditions improved settlement, and found that overwintering infested foliage in a refrigerator decreased settlement rate relative to foliage overwintered outdoors.

6 Our results suggest a protocol for adelgid inoculations that could substantially increase the success rate of experimental manipulations and encourage additional research on the population dynamics of this pest.

**Keywords** *Adelges tsugae*, herbivory, inoculation, *Tsuga canadensis*, *Tsuga caroliniana*.

## Introduction

The hemlock woolly adelgid *Adelges tsugae* Annand (Hemiptera: Adelgidae) (HWA) is a major invasive pest of eastern hemlock [*Tsuga canadensis* (L.) Carriere] and Carolina hemlock (*Tsuga caroliniana* Engelm.) throughout the eastern portion of the U.S.A. First reported in Richmond, Virginia in the 1950s (Souto *et al*., 1996), HWA spread throughout the eastern portion of the U.S.A. in the late 1970s and had entered southern New England by 1985 (McClure, 1989b). It has been detected south of the Great Smoky Mountains National Park in Tennessee (Johnson *et al*., 2005), and as far north as Portsmouth, New Hampshire (Anonymous, 2001). Although induced plant defenses have been shown to affect arthropod abundance in other systems (Rodriguez-Saona & Thaler, 2005), there is no evidence that eastern hemlock is capable of suppressing adelgid reproduction. The impact of this forest pest is such that it is feared that continued spread of the adelgid will lead to extinction of the Carolina hemlock in its native range and the extirpation of eastern hemlock from much of the U.S.A. (Esham *et al*., 2005; Tighe *et al*., 2005).

The HWA undergoes two generations per year (the overwintering sisten and spring/summer progredien) (McClure, 1991). Adult fecundity varies widely between generations, with approximately 50 offspring per sisten and approximately 20 per progredien (McClure, 1989a). These mobile 'crawler' offspring can either crawl to nearby branches or disperse (via wind or phoretically) to other eastern or Carolina hemlock trees (McClure, 1989b, 1992). Although winged sexuparae are also produced alongside the crawlers, they lack a suitable host plant in the eastern U.S.A. and thus perish (McClure, 1989a). Once the crawler locates a suitable feeding site, it moults into the sessile adult stage and feeds on the ray parenchyma cells, causing needle loss and tree death in as little as four years (McClure, 1992; Young *et al*., 1995).

Understanding the dynamics of HWA infestation and its interaction with hemlock has been a major focus of both basic and applied research (Reardon *et al*., 2005). Such research often requires the use of HWA-infested branches to infest target branches with crawlers that settle on the branch and

Correspondence: Evan Preisser, Department of Biological Sciences, 100 Flagg Road., University of Rhode Island, Kingston. RI 02881, U.S.A. Tel.: +1 401 874 2120; fax: 401 874 4256; e-mail: preisser@uri.edu

develop into adults. Unfortunately, the published experimental protocols for this procedure are often vague and fail to mention the large percentage of infestation attempts in which HWA settlement is very low or does not occur at all. As a result, different research programmes use different inoculation methods, and the research community as a whole relies on unpublished anecdotal information concerning the success rates of differing protocols.

The present study evaluated a variety of factors potentially affecting adelgid settlement. Our aim was to identify factors affecting the success of HWA settlement, and to offer a protocol for researchers attempting to experimentally manipulate HWA populations.

## Materials and methods

On 15 January 2005, we collected 40, 20-cm branches of HWA-infested foliage from eastern hemlocks in Forest Park (East Longmeadow, Massachusetts, U.S.A.; 42°09′N, 72°57′W). We chose this site because it offered abundant infested foliage and because we have had success using foliage from this area to inoculate branches with HWA (Butin *et al*., 2005). We kept these branches hydrated using florist's foam, and placed them in a refrigerator set at 0 °C until 16 March 2005. The temperature in the refrigerator was raised to 5 °C on 17 March 2005, to 10 °C on 1 April 2005, and to 15 °C on 14 April 2005. These branches were then examined using a dissecting microscope; 37 of 40 had live HWA and, of these, 13 branches appeared healthy enough to use in inoculations (see below).

On 22 April 2005 we collected approximately 120 20-cm branches of HWA-infested foliage from the same Forest Park location. One hundred and twelve of these branches were individually placed in a water-filled aquapic (Diamond Line Containers, Akron, Ohio), exposing approximately 15 cm of HWA-infested foliage per branch.

On 20 April 2005, we chose 16 mature (approximately 0.5 m in diameter at breast height) hemlocks in Cadwell Forest (Pelham, Massachusetts; 42°22′N, 72°25′W), an experimental forest managed by the University of Massachusetts at Amherst (Amherst, Massachusetts). Although this forest is south of the most northerly populations of HWA recorded in MA, numerous surveys have found no HWA in the forest or on any of the 16 trees chosen for this experiment (J. Elkinton, personal communication). We selected seven healthy, partially shaded, branches on each of the 16 trees, and applied one of seven treatments to each of the selected branches on 22 April 2005 (because of a shortage of aquapics, treatments D, E, F, and G were applied 3 days later on 25 April 2005). This procedure was replicated for each of the 16 trees.

*MA*. One HWA-infested branch applied to the top of the uninfested foliage at the terminal end of the branch, held on loosely by two loops of gardening wire, with the 50 cm of uninfested foliage closest to the terminal end of the branch enclosed in a 60-cm long by 30-cm wide mesh sleeve (1 $mm^2$ mesh size; Kleantest Products, Milwaukee, Wisconsin).

*MA-under.* As with MA, but HWA-infested branch applied to underside.

*MA high density.* As with MA, but with three HWA-infested branches.

*Wrapped.* As with MA, but HWA-infested branch held tightly against uninfested foliage with flagging tape.

*No bag.* As with MA, but no mesh sleeve.

*Field.* As with MA, but foliage collected from a single marked tree in Forest Park on 25 April 2005.

### Refrigerator

As with 'Field', but foliage collected from the same single marked tree on 1 January 2005 and overwintered in the refrigerator.

Two weeks after starting the above experiment, we ran a parallel experiment examining whether HWA-infested branches cut during the first phases of HWA crawler emergence were better inoculants than branches cut later in the period of crawler emergence. Once crawler emergence was detected in Forest Park, we applied HWA-infested foliage using the protocol outlined in treatment (MA), above, to five mature hemlock trees located near the 16 trees used in the first experiment. We applied freshly collected foliage from Forest Park to uninfested hemlock branches (one branch per tree per date) on 2, 9, 16, 23 and 30 May 2005. The replicates from 2, 9 and 16 May were considered 'Early Emergence', whereas the replicates from 23 and 30 May were considered 'Late Emergence.' We stopped the experiment after 30 May 2005 because the number of emerging crawlers diminished considerably during the first week of June.

### Data collection

After applying the treatments, we waited until the progrediens crawlers had emerged, settled on the branch, and the adults had formed the woolly masses typical of mature insects. On 15 August 2005, we collected the inoculated branches from each of the trees and brought them back to the laboratory. Inoculated branches that were damaged (0–3 replicates per treatment) were excluded from further analysis. We measured the total length of all branchlets emerging within 50 cm from the terminal end of the target branch. To assess the amount of uninfested foliage within the mesh sleeve of each branch available for colonization by HWA crawlers, we followed a similar procedure for the 50 cm of branch closest to the terminal end for the 'No Bag' treatment. After measuring the total length of all branchlets per treated branch, we then counted the number of settled HWA progrediens on the measured foliage. Finally, we divided the number of settled HWA per replicate by the total length of all foliage within the mesh sleeve (or a similar branch area for the 'Refrigerator' treatment) to determine HWA density per cm branch.

### Statistical analysis

We used univariate analysis of variance (ANOVA) to test for among-treatment differences in treatments A through G (i.e. all of the treatments that were begun on 22–25 April 2005 and collected on 15 August 2005). We also included the site variable 'tree' to test for differences in the response of individual hemlocks to the treatment. If the ANOVA revealed significant among-treatment differences, we tested for differences in HWA settlement between all pairs of means using Tukey's test, which controls for the increased possibility of type I errors due to multiple paired comparisons. In a separate analysis, we also tested for differences in HWA settlement between the 'Early Emergence' (infested branches applied on 2, 9 and 16 May 2005) and 'late emergence' (infested branches applied on 23 or 30 May 2005) treatments using univariate ANOVA. The dependent variable in all of the analyses was the number of settled HWA per cm branch, and all analyses were performed using JMP-IN, version 5.1 (SAS, 2004). We aimed to consider: (i) is there an effect of applying HWA-infested branches to the top vs. the bottom of the branch; (ii) is there an effect of bagging branches inoculated with HWA-infested branches in mesh sleeves; (iii) is there an effect of attaching HWA-infested branches tightly vs. loosely around the branch; (iv) is there an effect of applying one vs. three HWA-infested branches to each branch; (v) is there an effect of applying HWA-infested branches overwintered in a refrigerator vs. branches overwintered outdoors and (vi) is there an effect of applying HWA-infested branches collected early vs. late during crawler emergence?

## Results

Treatments A through G differed significantly in the number of settled HWA per cm branch (ANOVA: $F_{6,85} = 6.43$, $P < 0.001$). There were no significant differences between trees at ($F_{15,85} = 1.70$, $P = 0.065$).

HWA settlement was not affected by applying HWA-infested branches on the bottom of the branch vs. the top of the branch ('MA' vs. 'MA-under'; Fig. 1).

HWA settlement was 56% lower when branches inoculated with HWA-infested branches were not enclosed in mesh bags ('MA' vs. 'No Bag'; Fig. 1).

HWA settlement was 56% lower when HWA-infested branches were wrapped tightly vs. loosely around the branch ('MA' vs. 'Wrapped'; Fig. 1).

HWA settlement was not significantly affected by the number of branches applied to uninfested branches ('MA' vs. 'MA High Density'; Fig. 1).

HWA settlement was 66% lower when HWA-infested branches were kept in the refrigerator from January until deployment in the spring ('Refrigerator' vs. 'Field'; Fig. 1).

HWA settlement was 96% lower in the 'Late Emergence' treatment than in the 'Early Emergence' treatment ($F_{1,20} = 12.1$, $P = 0.002$; Fig. 2). Neither treatment, however, had settlement rates nearly as high as branches in the 'MA' treatment (0.73 ±0.102 HWA per cm branch) that were collected from the same location on 22 April 2005 and applied before crawler emergence began.

## Discussion

Some protocols were effective in improving HWA settlement. In particular, both the use of mesh sleeves and the loose attachment of HWA-infested branches to the target branch substantially increased settlement rates. This finding has several possible explanations. The bag may have favourably altered microclimatic conditions. It is also possible that by confining crawlers to a small fraction of the available foliage, the mesh sleeves may have eliminated dispersal, a major factor in the population dynamics of other hemipterans such as aphids (Hazell *et al*., 2005), in favour of local settlement. The bag may also have kept out HWA predators, although studies have suggested that predation losses are minimal (Montgomery & Lyon, 1996).

Altered dispersal may also explain why attaching infested branches to the top vs. the underside of branches slightly increased HWA settlement. If HWA crawlers disperse by being blown or falling off foliage, crawlers leaving infested foliage attached to the top of the target branch would be far more likely to encounter suitable foliage than those crawlers dispersing from infested foliage attached to the bottom of the branch.

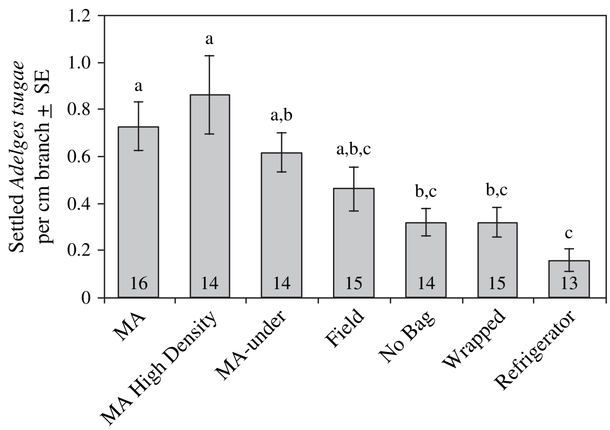


**Figure 1** Settled *Adelges tsugae* per cm branch (mean ± SE) in the seven different inoculation treatments begun on 22 or 25 April 2005 and collected on 15 August 2005. Treatments as referred to in text. Treatments with the same lowercase letter above them are not significantly different at $P < 0.05$ (Tukey's HSD test). The number at the base of each bar indicates replicates per treatment.

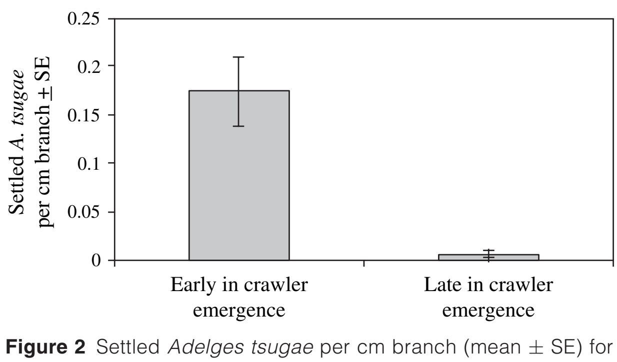


**Figure 2** Settled *Adelges tsugae* per cm branch (mean ± SE) for branches inoculated with infested hemlock foliage collected during the early phase of crawler emergence (2 May, 9 and 16 May 2005; $n = 14$) vs. the late phase of crawler emergence (23 and 30 May 2005; $n = 8$). Settlement was significantly higher ($F_{1,20} = 12.1$, p = 0.002) in the early vs. late-emergence treatment; note difference in scale of *y*-axis between Figs 1 and 2.

The seemingly paradoxical result that tightly attaching infested foliage to target branches decreased HWA settlement is probably due to increased mortality of adult adelgids. Although the woolly mass surrounding mature adelgids may provide protection from predators, it does not protect the soft-bodied adults from being crushed or dislodged. It is probable that the 'Wrapped' treatment's combination of increased handling time and greater contact between the infested and target branches both killed and dislodged adult HWA, decreasing the portion of adults producing crawlers and lowering the settlement rate.

There are several non-exclusive explanations for our finding that greater numbers of HWA-infested branches did not significantly increase crawler settlement. The first explanation is that multiple branches of infested foliage produced more crawlers than the available resource could support. However, settlement rates of up to 48 crawlers per $cm^2$ foliage has been reported on new uninfested foliage in Connecticut (McClure, 1991). Because our high-density treatment was produced by putting on more infested branches, we can also reject the explanation that the reduced fecundity typical of high density HWA populations may have offset the increase in adult densities (McClure, 1991). A second explanation for our finding may be that crawlers in the high-density three-branch treatment settled on other HWA-infested branches rather than on the uninfested foliage. Because hemlock branches spread out horizontally, only one or two of the three HWA-infested branches in the high-density treatment were in full contact with the uninfested branch. A high density of infested branches makes it more likely that crawlers will encounter infested foliage before finding the uninfested branch. If they choose to settle on the already-infested foliage, the sessile adult phase cannot later move to more suitable foliage.

We were surprised that HWA overwintered in relatively benign conditions had a lower settlement rate than did HWA exposed to much colder conditions, which are likely to decrease adelgid survival (Parker *et al*., 1998; Parker *et al*., 1999). Comparisons of HWA overwintering survival between populations in northern and southern locations show that harsh winter climates lead to low overwintering survival (Skinner *et al*., 2003; Butin *et al*., 2005). The most likely explanation for our result may be unavoidably confounding experimental procedures. Branches in the 'Refrigerator' treatment were removed from the parent tree for nearly 4 months, and the nutritional value of the foliage may have decreased over this period. An experimental design similar to ours that employed potted hemlocks infested with HWA rather than cut branches would address this concern, and might be a more effective means of testing this protocol than our approach.

Our finding that HWA-infested branches collected during the first stages of crawler emergence had high settlement rates was consistent with what we know about adelgid biology. Crawlers generally settle on vegetation within a few days of their emergence (McClure, 1992), and our observations of HWA-infested foliage indicated that the vast majority of crawler emergence took place over an approximately 1-month period. Because hemlock branches kept hydrated in aquapics remain green and flexible for up to 2 weeks after being cut, foliage cut early in the period of crawler emergence provide the opportunity for virtually all of the potential crawlers to emerge and settle. Early-emerging crawlers on branches cut later will already have settled, reducing the number of crawlers able to inoculate new foliage. Although determining when crawlers are about to emerge requires regular examination of the infested foliage, our results indicate that branches cut approximately 1 week before crawler emergence begins are maximally effective at inoculation.

In summary, the present study suggests that a standardized protocol using mesh sleeves, carefully handled and attached branches, and branches with a high infestation density will yield the best results in experimental inoculations of this invasive pest. Spring inoculations using the sistens generation is suggested because the mean fecundity is much higher than for that of the progrediens generation. In addition, regular pre-experiment monitoring of the 'infested' foliage is recommended to start the experimental inoculations either immediately before or as soon as crawlers begin to emerge. Use of a standard infestation protocol involving these elements should increase the overall success of experimental manipulations of HWA presence/absence, and increase the amount and complexity of research addressing the population dynamics of this harmful invasive pest.

## Acknowledgements

This research would not have been possible without G. Boettner, S. Flaherty, A. Paradis and G. Witkus. We thank M. Montgomery for comments on the manuscript, and the editor and two anonymous reviewers for editing and formatting suggestions. This research was funded via a cooperative agreement with the USDA Forest Service, no. 04-CA-11244225-275.

## References

Anonymous (2001) Holding the beetle border: the struggle continues to block adelgids from spreading into northern states. *Natural New England*, **6**, 16–17.

Butin, E., Porter, A. & Elkinton, J. (2005) Adaptation during biological invasions and the case of *Adelges tsugae*. *Evolutionary Ecology Research*, **87**, 887–900.

Esham, B., Schlarbaum, S., Burnham, C. *et al*. (2005) Preserving eastern hemlock gene pools through *ex situ* plantings. *Third Symposium on the Hemlock Woolly Adelgid in the Eastern United States* (ed. by R. Reardon, B. Onken and J. Lashomb), pp. 191–198. New Jersey Agricultural Experiment Station, Asheville, North Carolina.

Hazell, S.P., Gwynn, D.M., Ceccarelli, S. & Fellowes, M.D.E. (2005) Competition and dispersal in the pea aphid: clonal variation and correlations across traits. *Ecological Entomology*, **30**, 293–308.

Johnson, K., Taylor, G. & Remaley, T. (2005) Managing hemlock woolly adelgid and balsam woolly adelgid at Great Smoky Mountains National Park. *Third Symposium on the Hemlock Woolly Adelgid in the Eastern United States* (ed. by R. Reardon, B. Onken and J. Lashomb), pp. 232–233. New Jersey Agricultural Experiment Station, Asheville, North Carolina.

McClure, M. (1989a) Evidence of a polymorphic life cycle in the hemlock woolly adelgid *Adelges tsugae* (Homoptera: Adelgidae). *Annals of the Entomological Society of America*, **82**, 50–54.

McClure, M. (1989b) Importance of weather to the distribution and abundance of introduced adelgid and scale insects. *Agricultural and Forest Meteorology*, **47**, 291–302.

McClure, M. (1991) Density-dependent feedback and population cycles in *Adelges tsugae* (Homoptera: Adelgidae) on *Tsuga canadensis*. *Environmental Entomology*, **20**, 258–264.

McClure, M. (1992) Hemlock woolly adelgid. *American Nurseryman*, 3/15/1992, 82–89.

Montgomery, M. & Lyon, S. (1996) Natural enemies of adelgids in North America: their prospect for biological control of *Adelges tsugae*. *First Hemlock Woolly Adelgid Review* (ed. by S. Salom, T. Tignor and R. Reardon), pp. 89–102. USDA Forest Service, Morgantown, West Virginia.

Parker, B., Skinner, M., Gouli, S., Ashikaga, T. & Teillon, B. (1998) Survival of hemlock woolly adelgid (Homoptera: Adelgidae) at low temperatures. *Forest Science*, **44**, 414–420.

Parker, B., Skinner, M., Gouli, S., Ashikaga, T. & Teillon, H.B. (1999) Low lethal temperature for hemlock woolly adelgid (Homoptera: Adelgidae). *Environmental Entomology*, **28**, 1085–1091.

Reardon, R., Onken, B. & Lashomb, J., eds. (2005) *Third Symposium on the Hemlock Woolly Adelgid in the Eastern United States,* p. 384. New Jersey Agricultural Experiment Station, Asheville, North Carolina.

Rodriguez-Saona, C. & Thaler, J.S. (2005) Herbivore-induced responses and patch heterogeneity affect abundance of arthropods on plants. *Ecological Entomology*, **30**, 156–163.

SAS (2004) JMP-IN, Version 5.1. Duxbury Learning, Pacific Grove, California.

Skinner, M., Parker, B., Gouli, S. & Ashikaga, T. (2003) Regional responses of hemlock woolly adelgid (Homoptera: Adelgidae) to low temperatures. *Environmental Entomology*, **32**, 523–528.

Souto, D., Luther, T. & Chianese, B. (1996) Past and current status of hemlock woolly adelgid in eastern and Carolina hemlock stands. *First Hemlock Woolly Adelgid Review* (ed. by S. Salom, T. Tignor and R. Reardon), pp. 9–15. USDA Forest Service, Morgantown, West Virginia.

Tighe, M., Dvorak, W., Whittier, W., Romero, J. & Rhea, R. (2005) The *ex situ* conservation of Carolina hemlock. *Third Symposium on the Hemlock Woolly Adelgid in the Eastern United States* (ed. by R. Reardon, B. Onken and J. Lashomb), pp. 180–190. New Jersey Agricultural Experiment Station, Asheville, North Carolina.

Young, R., Shields, K. & Berlyn, G. (1995) Hemlock woolly adelgid (Homoptera: Adelgidae): stylet bundle insertion and feeding sites. *Annals of the Entomological Society of America*, **88**, 827–835.

Accepted 28 December 2006
First published online 24 April 2007