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Sunghoon Baek

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Spatial and Temporal Distribution of Hemlock Woolly Adelgid (Hemiptera: Adelgidae) Ovisac and Its Associations with the Environment

by

Sunghoon Baek

Thesis submitted to the Davis College of Agriculture, Natural Resources and Design
at West Virginia University
in partial fulfillment of the requirements
for the degree of

Master of Science
in
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SADIE, population dynamics

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ABSTRACT

Spatial and Temporal Distribution of Hemlock Woolly Adelgid (Hemiptera: Adelgidae) Ovisac and Its Associations with the Environment

Sunghoon Baek

Eastern hemlock, *Tsuga canadensis* (Pinales: Pinaceae) is an important tree species in eastern North America ecosystems. During the past 65 years, eastern hemlock stands have been threatened by the hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae). The goal of this research was to determine the spatial and temporal distribution of *A. tsugae* populations. First, the appropriate sample unit for *A. tsugae* was determined based on within-branch and -tree distribution of *A. tsugae*. The results of this study showed that *A. tsugae* ovisacs were more frequently found at the tip of a branch and no significant ($P > 0.05$) differences in *A. tsugae* ovisac density were found among four cardinal directions (i.e. north, south, east, and west). However, *A. tsugae* ovisacs were found more frequently on upper third branches ($P < 0.05$). Based on coefficient of variation values, a 50-cm branch sample approximately 3 m above the ground would be the optimal unit for sampling *A. tsugae* ovisacs. Using the optimal sample unit, spatial associations between *A. tsugae* ovisacs and surrounding environmental factors were investigated. The results showed that trees infested with *A. tsugae* ovisacs were spatially aggregated regardless of three study sites and two *A. tsugae* generations. Elevation, plant species diversity and basal area showed inconsistent spatial relationships with *A. tsugae* ovisac populations. This study provides fundamental information to develop efficient sampling plans and management strategies for *A. tsugae*.

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CHAPTER 1: INTRODUCTION

Thesis Organization

This thesis is organized into four chapters. Chapter 1 is a general introduction to the study and a literature review. Chapter 2 suggests an efficient sample unit for hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae) ovisacs based on their within-tree distribution pattern. Chapter 3 addresses spatial associations between hemlock woolly adelgid ovisacs and surrounding environmental factors. Chapter 4 provides a general conclusion for this study. This thesis was prepared according to the publication guidelines established by the Entomological Society of America.

General introduction

Eastern hemlock, *Tsuga canadensis* (Pinales: Pinaceae), is an important tree species in the eastern North America ecosystem. It provides not only esthetic beauty but also unique micro-environments for other organisms. Hemlock stands in the eastern United States are now threatened by *A. tsugae*, causing needle loss, shoot and branch dieback, reduced tree vigor, and eventual death of the tree. The absence of natural enemies, parthenogenetic reproduction of *A. tsugae*, and susceptibility of eastern hemlock allow *A. tsugae* populations to build quickly. Chemical control of *A. tsugae* is sometimes impossible or difficult due to the high costs of pesticides and potential leaching of pesticide to nearby streams. Currently, no sampling methods are available to estimate density of *A. tsugae* even though sequential sampling plans for chemical control have been proposed. Moreover, limited information is available to understand the population dynamics, spatial distribution, and dispersal of *A. tsugae*.

Objectives of Study

The goal of this research was to determine spatial and temporal distribution of *A. tsugae* populations. The objectives of this research were:

1. To determine optimal sample unit for *A. tsugae* ovisac (Chapter 2).
2. To investigate spatial associations between *A. tsugae* ovisacs and their surrounding environment (Chapter 3).

Literature Review

Distribution and Importance of Hemlock. Eastern hemlock, *Tsuga canadensis* (Pinales: Pinaceae), is distributed in the northeastern and Appalachian regions of North America. *T. canadensis* is a shade-tolerant and the longest living tree in North America; it can live well beyond 400 years and up to 900 years. Therefore, *T. canadensis* can become a dominant plant species in the forest system of North America (Evans 2002). Currently, *T. canadensis* is growing on ca. 19 million acres in the eastern United States (Schmidt and McWilliams 1996) and is the predominant species on 2.3 million acres (McWilliams and Schmidt 2000).

In a hemlock-dominated forest, about 5% sunlight can reach the understory (Battles et al. 2000). Soils under hemlock dominated stands exhibit low pH, high carbon to nitrogen ratio, and low rate of nitrogen mineralization and nitrification (Mladenoff 1987, Jenkins et al. 1999, Yorks 2000). These conditions create unique micro-climates providing favorable habitats for certain animals, understory plant species composition, biomass, and productivity among various hemlock stands (Evans 2002). Therefore, continuous loss of hemlock can cause a change in ecosystem properties of hemlock-dominant forests (Evans 2002).

Damage by A. tsugae. *Adelges tsugae* is a major pest of *T. canadensis* and *T. caroliniana* (Carolina hemlock) in the eastern United States (Fidgen et al. 2002, Flowers et al. 2006). *T. caroliniana* is limited to the Blue Ridge Mountains of the Southern Appalachians (Ward et al. 2004). Heavy infestations of *A. tsugae* cause poor crown health and reduced shoot growth that can result in rapid tree decline and death with other environmental stresses (McClure 1987, Mayer et al. 2002, Orwig et al. 2002). Trees left untreated can die in less than four years (McClure 1991).

Adelges tsugae populations can build quickly because *T. canadensis* exhibits little resistance to *A. tsugae* and there are limited natural enemies that can suppress *A. tsugae* populations in the eastern United States (Havill et al. 2014). Because *T. canadensis* is a keystone species across most of its native range especially near streams, its loss from the ecosystem could be devastating (Quimby 1996). Currently, *A. tsugae* control tactics focus on treating individual trees with insecticide through foliar sprays, trunk or soil injections,

and ground soaks (McClure 1992). In ornamental and nursery settings, systemic insecticides have proven effective in managing *A. tsugae* populations (McClure 1992, Rhea 1995, Webb et al. 2003); however, many hemlocks in the forest are found in many inaccessible locations or along riparian zones where chemical controls are not feasible (Flowers et al. 2006). Also, it is economically infeasible to control whole hemlocks in hemlock dominated stands.

Introduction of *A. tsugae*. *Adelges tsugae* has been reported to be introduced from Japan (McClure 1992, Havill et al. 2006). It was first found in British Columbia in 1924 (Annand 1924) and later in Oregon in 1928 (Annand 1928); it was thought to be a native species at that time (Havill et al. 2006). In the eastern United States, *A. tsugae* was found in Richmond (VA) in 1951 (Ward et al. 2004) and has spread to new regions at a speed of 8.9 to 20.4 km each year (Morin et al. 2009), and is still spreading. When *A. tsugae* becomes established at any location within a tree crown, it typically can be found everywhere within a year (McClure 1990). Evans and Gregoire (2007) found that within-tree spatial distribution of *A. tsugae* varied depending on the density of *A. tsugae*. However, Whitmore (2009) showed that *A. tsugae* was found on the lower branches because crawlers fell downward through the canopy after a few generations.

Life Cycle of *A. tsugae*. In the eastern United States, *A. tsugae* produces two parthenogenetic generations: progrediens and sistens (McClure 1987) (Fig. 1). The sistens are present from summer to the next spring. In June and July, sistens nymphs emerge as crawlers from eggs and settle at the base of young needles where they immediately enter aestivation (i.e. summer diapause) (McClure 1987, Salom et al. 2001). They resume development in October and complete their development in late winter. Adult sistens produce eggs from March until May and their progenies are determined to become either winged (sexuparae) or wingless (progrediens) forms based on the density of *A. tsugae* per twig. Winged sexuparae disperse to spruce, but no suitable alternate hosts are known in the eastern United States. Therefore, winged sexuparae of *A. tsugae* die without reproduction (McClure 1992). The wingless progrediens remain on hemlock by completing their development and depositing sistens eggs in June (McClure 1989).

Current Management of *A. tsugae*. Even though several chemicals (e.g., imidacloprid, dinotefuran, acetamiprid, and thiamethoxam) are available for *A. tsugae*

management, neonicotinoids are popularly used due to their systemic activity (Havill et al. 2014). Neonicotinoids can be applied by soil drench, soil injection, and trunk injection. However, chemical application can cause negative effect on other arthropods, secondary pest outbreaks (e.g., spruce spider mites and hemlock rust mites), and contamination of aquatic resources (Havill et al. 2014).

Significant efforts have been made to identify and utilize biological control agents against *A. tsugae*. Native predators of *A. tsugae* have been surveyed and identified in the eastern United States, but were mostly generalists (Wallace and Hain 2000) and not effective to control *A. tsugae* populations (Montgomery and Lyon 1996). Therefore, a few predators have been introduced from other countries. *Sasajiscymnus tsugae* (Coleoptera: Coccinellidae) was introduced from Japan and has been released in the eastern United States. Research revealed that this predator is thought to be established successfully (Blumenthal 2002, Cheah and McClure 2002) even though it took five to seven years to reach a detectable level (Havill et al. 2014). *Scymnus* lady beetles such as *S. camptodromus*, *S. sinuanodulus*, and *S. ningshanensis* (Coleoptera: Coccinellidae) native to China were tested within cages in the field. These lady beetles significantly reduced the density of *A. tsugae* even though the control efficiency was affected by the density of *A. tsugae* (Butin et al. 2002). Among them, *S. sinuanodulus* and *S. ningshanensis* have not been established after releasing in the field and *S. camptodromus* is still being mass reared for release (Havill et al. 2014). Another predatory beetle species, *Laricobius nigrinus* (Coleoptera: Derodontidae), native to western North America, is phenologically synchronized with *A. tsugae* (Zilahi-Balogh et al. 2003). Field studies indicated that it significantly reduced *A. tsugae* population within temporary branch enclosures (Lamb et al. 2005). *L. osakensis*, recently identified as a potential predator from Japan, has a few advantages compared to *L. nigrinus*: higher survivorship in more northern habitats, greater fecundity, and a higher predation rate (Havill et al. 2014). *L. osakensis* was first released in 2012 and is still being studied for mass rearing, large-scale releases, and efficient monitoring techniques (Havill et al. 2014).

Generalist entomopathogenic fungi, such as *Beauveria bassiana*, *Lecanicillium lecanii*, *Metarbizium anisopliae*, *Verticillium lecanii*, and *Paecilomyces* sp., were identified

as potential biological control agents (Wallace and Hain 2000, Havill et al. 2014). These pathogens are still being tested in the field (Havill et al. 2014).

Spatial analysis to characterize spatial pattern. Each species has its own, readily recognizable distribution pattern resulting from the interaction between its selected environment and the behavior that has evolved for survival in it (Taylor 1984). Plants or animals in any geographical area are scattered about in one of three spatial patterns: uniform, random, or aggregated (Krebs 1999). A classical method of quantifying such spatial patterns in ecological data is to use indices of aggregation such as the variance to mean ratio. The most popular methods among them are Taylor's power law (Taylor 1961) and Iwao's patchiness regression indices (Iwao 1972). Both methods use linear regression; Taylor's power law uses the relationship between mean and variance of a population and Iwao's patchiness regression uses the relationship between mean crowding index and mean population density. In both methods, a slope greater than one suggests an aggregated pattern, a slope equal to one suggests a random pattern, and a slope is less than one suggests a uniform pattern. These methods generally agree with mathematical distribution models. If an insect population shows random distribution, the data follows the Poisson distribution; if an insect population shows aggregation distribution, the data follows the negative binomial distribution; if an insect population shows uniform distribution, the data follows the binomial distribution (Southwood and Henderson 2000).

One of the popular methods for spatial analysis is Ripley's K and the L function. The K function is the average number of individuals within a circle of radius d centered on a randomly chosen individual (Ripley 1977). The L function is more popularly used instead of K function because K value becomes too large as d increases (O'Sullivan and Unwin 2010). The L function can be defined as the square root of the expected value of $K(d)$ divided by π . A Monte Carlo procedure is used randomly to locate n individuals in the study area. Results from analysis of these randomly located data can then be used to construct confidence envelopes (Diggle 1983). If observed pattern is above the upper envelope, the population might have statistically significant clustering and if observed pattern is below the lower envelope, the population might have significantly dispersed pattern (O'Sullivan and Unwin 2010).

Another popular method is Moran's I , which is an index of spatial autocorrelation (Perry et al. 2002). If the data are positively autocorrelated, then most pairs of adjacent locations will have values on the same side of the mean and Moran's I will have a positive value (O'Sullivan and Unwin 2010). If the data are negatively autocorrelated, then most pairs of adjacent locations will have values on the opposite sides of the mean and Moran's I will have a negative value (O'Sullivan and Unwin 2010), indicating uniform pattern.

Spatial analysis by distance indices (SADIE) measures the degree of clustering forming patches and gaps and tests the statistical significance of spatial aggregation, randomness, and uniformity (Perry 1995). A patch is a region of relatively large insect counts close to one another, and a gap is a region of relatively small insect counts close to one another (Perry 1995). To quantify the degree of clustering in insect counts, SADIE calculates the overall aggregation index, I_a , described by Perry (1995). A value of $I_a = 1$ suggests a spatially random pattern, $I_a > 1$ suggests a more aggregated pattern, and $I_a < 1$ suggests a more regular pattern (Perry et al. 1999).

Geostatistical methods of quantifying spatial patterns are variogram and kriging (Perry et al. 2002). A variogram estimates the average degree of similarity between the values as a function of their separation distance and direction (Isaaks and Srivastava 1989, Rossi et al. 1992). For random data, all values of variance are essentially the same, and thus the variogram appears nearly horizontal. Patterned data produce a variogram that has small values for short lags, then increases with increasing distance, but levels off and even decreases after a certain lag distance. Kriging is a method of interpolation that predicts unknown values from data observed at known locations. This method uses the variogram to express the spatial variation, and it minimizes the error of predicted values which are estimated by spatial distribution of the predicted values (Cressie 1993).

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Figure 1. Hemlock woolly adelgid annual life cycle on hemlock in North America (Ward et al. 2004).

CHAPER 2: Determination of Sampling Unit for Hemlock Woolly Adelgid (Hemiptera: Adelgidae) Ovisacs Based on Their Within-Tree Distribution Pattern

Abstract. Within-tree distribution of an insect pest can help determine where to sample and apply control measures. This study was conducted to characterize the within-branch and within-tree distribution pattern and determine sample units suitable for hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae) ovisacs on eastern hemlock. A total of 46 eastern hemlock trees infested with *A. tsugae* were selected from hemlock stands in Blackwater Falls State Park (WV), Cathedral State Park (WV), and Buchannan State Forest (PA). In each tree, 24 branches were cut with regard to six vertical heights and four cardinal directions. The cut branches were separately put into plastic bags and brought to the lab to count the number and map the location of ovisacs within a branch. The spatial distribution of *A. tsugae* ovisacs within a branch was analyzed with spatial analysis by distance indices (SADIE). Coefficients of variation (CV) were calculated and used to determine optimal sample unit for *A. tsugae* ovisacs. The results of this study showed that *A. tsugae* ovisacs were more frequently found at the tip of a branch but no significant ($P > 0.05$) differences in *A. tsugae* density were found among the four cardinal directions. *A. tsugae* ovisacs were found more on upper third branches ($P < 0.05$). Based on CV values, 50-cm branch samples approximately 3 m above the ground would be the optimal sample unit for *A. tsugae* ovisacs.

Keywords: *Adelges tsugae*, eastern hemlock, coefficient of variation, geostatistics

The hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), is a major pest of *Tsuga canadensis* (Eastern hemlock) in the eastern United States (Fidgen et al. 2002, Flowers et al. 2006). *T. canadensis* is distributed in the northeastern and Appalachian regions of North America (Evans 2002). *T. canadensis* is shade-tolerant and the longest living tree in North America, and becomes a dominant plant species in the forest system of North America (Evans 2002). In a hemlock-dominated forest, about 5% sunlight can reach the understory (Battles et al. 2000). Soils under hemlock dominated strands exhibit low pH, high carbon to nitrogen ratios, and low rate of nitrogen mineralization and nitrification (Mladenoff 1987, Jenkins et al. 1999, Yorks 2000). These conditions create unique micro-climates providing favorable habitats for certain animals, understory plant species composition, biomass, and productivity among various hemlock stands (Evans 2002). Therefore, continuous loss of hemlock can cause a change in ecosystem properties of hemlock-dominant forests (Evans 2002). These important hemlock-dominant forests are now threatened by *A. tsugae*. Heavy infestations of *A. tsugae* cause poor crown health and reduced shoot growth that, in combination with other environmental stresses, can result in rapid tree decline and death (McClure 1987, Mayer et al. 2002, Orwig et al. 2002). Limited natural enemies, parthenogenetic reproduction of *A. tsugae*, and susceptibility of *T. canadensis* allow *A. tsugae* populations to build quickly (McClure and Cheah 2002). Currently, *A. tsugae* control tactics focus on treating individual trees with insecticide (McClure 1992) and biological control agents including predators and entomopathogenic fungi (Cheah et al. 2004).

One of the most important steps in integrated pest management (IPM) is sampling to estimate the population density, delimit its distribution, and determine where to apply control measures. Because counting individual *A. tsugae* on an entire tree is infeasible, population density of *A. tsugae* needs to be estimated or compared from the direct count on a certain sample unit which can be defined as a unit-area of the habitable space (Pedigo and Buntin 1994). The selected sampling units not only determine the nature of a population but also affect the components of sampling plans such as sampling techniques, sampling number, spatial pattern of samples, and timing of sampling (Pedigo and Buntin 1994); different sampling plans would be needed based on the selection of sample units. In general, sample units are selected based on multiple criteria (Morris 1955) including

stability and possibility of delineation in the field, consistency, the balance between cost (e.g., labor and time) and variation (e.g., precision), and suitable size.

Currently, there are a few sample units suggested for *A. tsugae* populations. Costa and Onken (2009) recommended a 100-cm branch in the lowest crown as a sampling unit. However, there is no scientific reason to support the selected sample unit in their recommendation. Evans and Gregoire (2006) suggested using different sample units depending on tree heights and population levels; for example, suitable sample unit with *A. tsugae* high infestation would be branches located < 8.5 m above the ground. Joseph et al. (2011) suggested both upper and lower branches as a sample unit to characterize the infestation of *A. tsugae*. However, both suggestions by Evans and Gregoire (2006) and Joseph et al. (2011) ignored consistency and precision of the sample unit. In addition, these previous studies did not consider the sample size of the selected sampling unit and within-branch distribution pattern of *A. tsugae*.

Previous studies investigated within-tree distribution of *A. tsugae*, which is critical information that directly affects selection of sample units and applying control measures. Fidgen et al. (2006) hypothesized that the density of *A. tsugae* from the middle crown would not be different from that of the lower crown. Evans and Gregoire (2006) found the density of *A. tsugae* was slightly higher on the lower branches than on the upper branches in the case of high density of *A. tsugae*, but higher on the upper branches in trees with low *A. tsugae* population density. Whitmore (2009) concluded that more *A. tsugae* were found on lower branches based on his hypothesis that the crawlers became established at any place within a tree crown, but *A. tsugae* would usually be found on the lower branches as the crawlers fell downward through the canopy after a few generations. Joseph et al. (2011) reported that *A. tsugae* ovisacs were more abundant on the higher branches in insecticide untreated trees, but their within-tree distribution changed over time. All of these studies indicate inconsistency of *A. tsugae* distribution within a hemlock tree. Moreover, there have been no detailed studies about within-branch distribution of *A. tsugae* which might be the most important factor in deciding the suitable sample size and unit. Therefore, a two-year study was conducted in three locations to determine optimal sample unit of *A. tsugae* ovisac by investigating within-branch and -tree distribution and characterizing spatial distribution patterns.

Materials and methods

Study sites. This study was conducted in Blackwater Falls State Park (N39°6'25", W79°30'13") and Cathedral State Park (N39°19'44", W79°32'24") in West Virginia and Buchanan State Forest (N39°57'32", W77°57'0") in Pennsylvania. No *A. tsugae* management was done in any of three sites during the period of this study although intensive managements had been done in the past. In Blackwater Falls State Park, the infestation of *A. tsugae* started in 2003, management of *A. tsugae* using a predatory beetle (*Sasajiscymnus tsugae*) was initiated on June 4, 2004, and imidacloprid was applied as both soil treatment and trunk injection every year from 2004 to 2010. Cathedral State Park has been infested with *A. tsugae* since 2003, imidacloprid has been applied since 2004, and 1,500 *S. tsugae* were released on July 7, 2004 (Tim Tomon, Personal communication, November 1, 2012). In Buchanan State Forest, 5,000 *S. tsugae* were released on April 1, 2005 (James Smith, Personal communication, October 15, 2012) to manage *A. tsugae* which was introduced in 1995 (James Unger, Interoffice memorandum of Pennsylvania Department of Conservation and Natural Resources Bureau of Forestry, August 24, 1995).

Sampling. Sampling *A. tsugae* ovisacs in this study was conducted inside hemlock stands to avoid any edge effect. Before collecting samples, trees were examined and marked to assure that all sampled trees had at least one ovisac. A total of 46 trees (3.5-7 m in height) were randomly selected from 75 marked trees. In each tree, two different lengths of branches (i.e. a 100-cm branch and a whole branch) were cut with a 10-m pole pruner. In Blackwater Falls State Park, 504 branches in 21 trees were sampled on April 7, 2011. These samples represent ovisacs of progrediens generation of *A. Tsugae*. Ovisacs of sistens generation were sampled by collecting 120 branches from five trees in Cathedral State Park on June 29, 2011. A total of five tall (15.5-18.3 m) trees also were sampled by felling trees in Buchanan State Forest on June 1, 2012. A total of 24 branches per tree were collected by randomly selecting and cutting a branch within a tree which had 24 possible combinations with six different heights (subdivided six sections of tree height from the lowest to the highest) and four horizontal directions (East, West, South, and North). In case that hemlock branch was shorter than 100 cm, entire branches were collected.

For the whole-branch sampling, an additional 360 branches from 15 trees were collected at all three sites. Tree size was 3.5-7 m in height in Cathedral and Blackwater

Falls State Park and 15.5-18 m in Buchanan State Forest. The sampling dates were March 29, 2013 in Cathedral State Park, June 11, 2013 in Buchanan State Forest, and June 18, 2013 in Blackwater Falls State Park. All samples were transported to a cold room in the Division of Plant and Soil Science at West Virginia University. The room was maintained at approximately 4°C.

Characterizing within-branch distribution of *A. tsugae*. To map the distribution of *A. tsugae* ovisacs within a branch, each cut branch was laid on a grid (1 cm by 1 cm) (see Fig. 1 for details). The number of ovisacs on a 100-cm branch was counted in each node with allocated coordinates; hatched ovisacs were also included in the count. The length of branch with needles was also measured to estimate habitable spaces. All branches at six different heights (i.e., the lowest, low-high, middle-low, middle-high, high-low, and the highest) in each site were pooled to show the distribution of *A. tsugae* ovisacs within a branch as a within-branch distribution map because the number of ovisacs was not statistically different in each study site (Table 1). The distribution of *A. tsugae* ovisacs within branches was visualized with ArcGIS 9.3 (Environmental Systems Research Institute, Redlands, CA) by using ordinary kriging as an interpolation method.

To characterize the spatial distribution of *A. tsugae* ovisacs within a branch, spatial analysis by distance indices (SADIE) (Perry 1995) was used. SADIE measures the degree of clustering with patches and gaps. A patch is a region with relatively large number of ovisacs, and a gap is a region with relatively small number of ovisacs. To quantify the degree of clustering of ovisacs within a branch, the overall aggregation index, I_a expressed as $I_a = D / E_a$, was used. D is distance to regularity which is defined as the minimum total distance that individual samples in an observed arrangement would need to move to result in uniform or regular spatial distribution. Therefore, the higher D value, the higher degree of clustering of ovisacs in distribution. E_a is the mean expected distance to regularity. $I_a = 1$ means a spatially random pattern, $I_a > 1$ means a more aggregated pattern, and $I_a < 1$ means a more regular pattern (Perry et al. 1999). The significance test for spatial patterns was executed with formal randomization tests (Perry et al. 1999, Perry and Dixon 2002) under the null hypothesis: the observed ovisacs were arranged randomly among the given sample locations.

Estimating within-branch abundance of A. tsugae ovisacs. The cumulative percentage of *A. tsugae* ovisac counts on the whole branch was modeled using a Weibull function. The function usually well describes insect occurrence patterns that have a single peak (Kim et al. 2000).

$$f(x) = 100 [1 - \exp(-[x - a]^b)], \quad (\text{Eq. 1})$$

where $f(x)$ is the cumulative percentage of ovisac occurrence, x is branch length from the tip, and a and b are model parameters.

Frequency data from ovisac counts per 25-cm branch were converted to cumulative proportions. The parameters of the Weibull function were estimated with PROC NLIN (SAS Institute 1999).

Characterizing within-tree distribution of A. tsugae. The effect of branch height and direction on the number of *A. tsugae* ovisacs was analyzed by using ANOVA followed by Tukey's HSD test (SAS Institute 1999). The statistical analyses were conducted for two types of data: total ovisac numbers per branch and ovisac numbers per 10-cm twig with needles. The ovisac number per 10-cm twig with foliage was calculated by dividing total number of ovisacs on a branch by total length of twigs with needles on the same branch. All data were square root or double-square root transformed to make data normally distribute before statistical analysis. To determine the optimal sample unit, the coefficients of variation (CV) of *A. tsugae* ovisacs were compared among six branch heights and three branch lengths (i.e., 25, 50, and 100 cm); CV is the ratio of the standard deviation to the mean. In sampling, the value of CV has important meanings. In a case of using the sample unit with the lowest CV value, the possibility of finding target insects can be increased compared to other sample units with similar variance (i.e. higher mean density). Moreover, the estimated number becomes more precise compared to other sample units with similar mean.

Because it is very difficult to measure twig lengths with needles in the field, data for ovisac number per 10-cm twig with needles was not included to calculate CV values. Moreover, the effect of the cardinal direction was not considered for CV calculation because there was no significant ($P > 0.05$) difference in the number of *A. tsugae* ovisacs

among the directions. For the calculation of CV values, both data from two sample units (i.e., 100-cm and whole branches) were pooled together to determine sample units that can be more applicable to diverse field situations

All statistical analyses were done separately for each site and sample unit (i.e. whole branch and 100-cm branch) because the total number ($F = 4.85$, $df = 2$, $P = 0.02$) and numbers of *A. tsugae* ovisacs per 10-cm twig with needles ($F = 4.25$, $df = 2$, $P = 0.02$) were statistically different among the sites. Also, statistical differences were found among the sample units in total ovisac numbers ($t = 2.43$, $df = 42$, $P = 0.01$) and ovisac numbers per 10-cm twig with needles ($t = 2.10$, $df = 42$, $P = 0.02$) per branch.

Decision of optimal sample size. The optimal sample size is the smallest number of sample units that would satisfy the objective of sampling program and achieve the desired precision of estimates (Pedigo and Buntin 1994). The optimal sample size was developed using the parameters of Taylor's power law expressed as:

$$\ln(s^2) = \ln(a) + b \times \ln(m), \quad (\text{Eq. 2})$$

where a depends mainly on the sampling unit and b is an index of dispersion (Taylor 1961). Data was pooled for each of four horizontal directions in each site. Linear regression was conducted using PROC REG in SAS (SAS Institute 1999) and the fit of Taylor power law model to the observed data was evaluated based on r^2 values.

Although there are several methods to estimate the optimal sample size, the methods of Elliott (1971), Ruesink (1980), and Pedigo and Buntin (1994) were used in this study because of the aggregation pattern of *A. tsugae* ovisacs and the popularity of these equations. The equations are:

$$N = [1 / m + 1 / k] / D^2, \quad (\text{Eq. 3})$$

$$N = [a \times m^{(b-2)}] / D^2, \quad (\text{Eq. 4})$$

$$N = (t_{\alpha/2} / D)^2 (S^2 / m^2) \quad (\text{Eq. 5})$$

where k is the aggregation factor of the negative binomial ($k = m^2 / [S^2 - m]$), N is sample size, D is precision which is the proportion of mean to standard error, a and b are the parameters of Taylor power law, $t_{\alpha/2}$ is t value for a given probability (α), and S^2 and m are the sample variance and the mean number of *A. tsugae* ovisacs, respectively, on the lowest branches 3 m above the ground.

Results

Determination of optimal sample unit. The lower CV values were mostly found in samples from the middle-high or high-low branches in Cathedral and Blackwater Falls State Parks and from the lowest branches in Buchanan State Forest (Table 2). The lower CV values also were found in 50-cm or 100-cm branch samples in Cathedral and Blackwater Falls State Park and 50-cm branches in Buchanan State Forest (Table 2). Considering both small (< 8 m in height) and tall (> 15 m in height) trees, 50-cm branches might be a suitable sample unit for *A. tsugae* ovisacs. The middle-high branches (2.9 m above the ground with a range of 2-4 m) among 50-cm branches had the lowest CV values in Cathedral and Blackwater Falls State Parks. In Buchanan State Forest, branches were collected at an average height of 2.8 m from the ground. Therefore, 50-cm branches approximately 3-m in height had the lowest CV values.

When the required sample number was compared at a fixed-precision level of 0.1 between the current sampling unit (100-cm, lowest branches) and the results in this study (50-cm branches 3 m above the ground), the required sample number could be decreased in all study sites. In Cathedral State Park, the optimal sample number was dramatically decreased with the proposed sample unit compared with the current one (Table 3).

Within-branch distribution of A. tsugae ovisacs. In this study, approximately 85% of 744 branches showed significant ($P < 0.05$) spatial aggregation of ovisacs. More *A. tsugae* ovisacs were found and aggregated at the tip of branches except when ovisac density was very low; the branches with less than 0.75 ovisacs per 10-cm twig showed random distribution. Ovisacs in upper branches were aggregated not only at the tip of a branch but also at tips of twigs (Fig. 2).

Estimation model for within-branch abundance of A. tsugae ovisacs. The cumulative percentage of *A. tsugae* ovisacs on a whole branch of the selected vertical

location (i.e. 3 m above the ground) fit well with the Weibull function (Table 4, $F = 1801.1$; $df = 2, 118$; $P < 0.0001$; $r^2 = 0.97$). The selected sample unit (i.e. 50-cm branch) in this study contained about 71% ovisacs compared with the 100-cm branch sample at the same vertical location. A total of 50% ovisacs could be found within 37 cm from the tip of a branch and 99% ovisacs within 200 cm (Fig. 3).

Characteristics of *A. tsugae* ovisacs within-tree distribution. Both the number and density of *A. tsugae* ovisacs were not different among four cardinal directions ($P > 0.05$), but showed differences among six heights in all three sites except for two cases ($P < 0.05$) (Tables 1 and 5). Upper branches generally had more ovisacs than middle and lower branches (Tables 5 and 6). The variation of ovisac numbers increased as the average numbers of ovisacs increased. The average and variation of ovisac numbers generally increased from the lowest to highest branches for small trees (< 8-m height), but the density was the lowest in the middle branches for tall trees (> 15-m height) (Table 5 and 6).

Discussion

Within-branch distribution of *A. tsugae* ovisacs. The density of *A. tsugae* ovisacs showed spatial aggregation on the tip of branches except for the highest branches when density was very low (0.75 ovisacs per 10-cm branch). This pattern was consistent with the finding of Joseph et al. (2011) who also reported the density was significantly higher on the tip of branches in the lower and middle crown but not in the upper crown. This pattern is related with the distribution of new shoot growth because *A. tsugae* settles at the base of needles on new growth and their movement is limited (Young et al. 1995). The reason for nymphal settlement at the base of needles on new growth is that *A. tsugae* can maximize their access to the vascular connections with adjacent leaves (Fay et al. 1996) and the amount of monoterpenes from new shoots is different than that from previous year's shoots (Broeckling and Salom 2003). However, *A. tsugae* can also establish on older twigs as its population increases (McClure 1992).

A sistens adult produces a single cottony ovisac from March to May while a progrediens adult produces an ovisac during June and July (McClure 1992, Gray and Salom 1996). These white wooly ovisacs of *A. tsugae* are currently used as the first sign of infestation on the underside of twigs often on the most recent growth (Whitmore 2009).

Such within-branch distribution of *A. tsugae* ovisacs might lead to the use of branch tip as a sample unit for *A. tsugae* studies and management. One problem of branch-tip sampling would be ambiguity of how to define the tip of a branch: length and number of tips from new shoots. Another problem of branch-tip sampling is that the size of new shoots on each branch tip could vary, which can affect the carrying capacity for *A. tsugae* on each new shoot.

Estimation model for within-branch abundance of A. tsugae ovisacs. Joseph et al. (2011) tried to estimate *A. tsugae* ovisacs number on 10-cm and 30-cm branch tips from 60-cm branches. They used a linear regression and found that 75% and 95% of the variation in the data was explained with the regressions of 10-cm and 30-cm branch tips, respectively. However, this method may not estimate the total number of *A. tsugae* ovisacs on whole branches because they developed the model based on only 60-cm branches. Moreover, using a non-linear regression in our study, we found that 97% variation of data could be explained when Weibull function was fitted to the cumulative percentage of *A. tsugae* ovisacs, which would be very useful to predict the total ovisac number by counting a part of a branch.

Within-tree distribution of A. tsugae ovisacs. The number and density of *A. tsugae* ovisacs didn't show any significant differences in all cardinal directions in this study. Evans and Gregoire (2007) reported that *A. tsugae* density was higher on the north facing branches probably due to increased predation or desiccation of *A. tsugae* crawlers on the south facing branches. However, light is a limiting factor in individual trees inside hemlock stands. Battles et al. (2000) reported only ca. 5% sunlight could reach the understory. The effect of higher light condition might affect only trees at the edge of hemlock stands and the branches on the highest crown. The trees in this study were selected inside of hemlock stands to minimize the edge effect.

In our study, significantly and consistently more *A. tsugae* ovisacs were found on branches in the upper crown. Joseph et al. (2011) reported that the density of *A. tsugae* ovisacs were more abundant on the upper crown branches in untreated trees even though ovisacs were more abundant on the lower crown branches in insecticide treated trees. In our study, imidacloprid was applied in Blackwater Falls and Cathedral State Park, but not applied in Buchanan State Forest. However, the vertical distribution of *A. tsugae* ovisacs

within-tree was not affected by pesticide application. Evans and Gregoire (2007) also observed that *A. tsugae* density was higher in the upper crown when trees had low *A. tsugae* population even though this trend was reversed when *A. tsugae* population was high. Such inconsistent pattern of vertical distribution of *A. tsugae* ovisacs might be caused by the small sample unit (30-cm branches) and locations of sampled trees (i.e. near roads). In our study, a 25-cm branch sampling showed relatively higher variation compared to the mean (Table 4).

The trees near a road are inevitably affected more by environmental conditions rather than the trees inside forests. The higher light conditions at the higher branches may explain the vertical distribution pattern of *A. tsugae* ovisacs because one important characteristic of a hemlock dominant forest is limited light condition within the understory (Battles et al. 2000). Moreover, *A. tsugae* enters aestivation during summer (McClure 1987, Salom et al. 2001). Therefore, more light in the upper canopy might be a favorable condition for the survivorship and development of *A. tsugae*. This phenomenon was reported in *A. cooleyi* on Engelmann spruce, *Picea engelmanni* (Fay and Whitham 1990). They found that the growth, fecundity, and growth of progenies of *A. cooleyi* on the highest branches were at least double that of the lowest branches. Rentch et al. (2009) also found that more *A. tsugae* were found on south-facing trees due to increased amount of light. The higher density of adelgids on higher branches was also reported in similar species, *A. abietis* (Fidgen et al. 1994) and *A. piceae* (Greenbank 1970, Bryant 1976).

Determination of optimal sample unit based on CV value. The number and density of *A. tsugae* ovisacs generally increased from the lowest branches to highest branches in small (< 8 m) trees. However, the number and density was the lowest in the middle crown and the highest on branches in the upper crown in tall (> 15 m) trees. The standard deviation of *A. tsugae* ovisac number increased as the mean of ovisacs increased. Therefore, branches with a moderate mean had the lowest CV values, and these were selected as the optimal sample unit from this study. Even though the density of *A. tsugae* ovisacs varied by site, the lowest CV value among six different vertical heights was found when the average density was not too high or low. Joseph et al. (2011) suggested sampling from both low and high crown height due to inconsistent vertical distribution of *A. tsugae* ovisac within a hemlock tree. The highest branches might not be a good sample unit

because the mortality of *A. tsugae* population could be very high during winter and sampling in such season is hard to accomplish. Fay and Whitham (1990) reported that the fitness of *A. cooleyi* was the highest on higher branches but *A. cooleyi* was rarely found on the highest branches due to high mortality caused by cold temperature. Therefore, the selection of higher branches may cause high variation during winter. However, higher fitness of *A. tsugae* on the highest branches allows the possibility to use aerial application of pesticides and predatory beetles during summer because *A. tsugae* might increase their population quickly at the highest branches. Moreover, upper crown could be a good sample unit for *A. tsugae* initial detection during early summer.

The branches with the lowest CV values could be selected as a suitable sample unit because the lower CV value indicates lower variation of *A. tsugae* populations. The proposed sample unit, taking 50-cm branch samples 3 m above the ground, needs 16-22 samples in the optimal sample number at $D = 0.25$ in the sites of this study. The current sample unit, taking 100-cm branches from the lowest branch, needs 8-100 samples at $D = 0.25$ and 75% reliability (Costa and Onken 2006). Moreover, the sample unit of 50-cm branches can save time for searching and counting *A. tsugae* ovisacs compared to 100-cm samples. The amount of habitable areas and the number of ovisacs could be decreased about 32% and 29% by using 50-cm branches instead of 100-cm branches in our study, respectively. However, using 25-cm samples to save searching and counting time may not work because new shoots of *T. canadensis* can grow more than 25 cm. The average of new shoots in the highest branches in *P. engelmanni* was more than 23 cm (Fay and Whitham, 1990). Even though the crawlers of *A. tsugae* settle down at the base of new shoots, their ovisacs can be found just after new shoots because *A. tsugae* are sessile after settlement and *T. canadensis* develops new shoots during the developmental time of *A. tsugae* from crawler's settlement to adult's oviposition. Therefore, 25-cm branch sample might not be large enough to find *A. tsugae* ovisacs. By using a standard and smaller sample unit, the sampling of *A. tsugae* could be more precise, economical, and less destructive.

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Table 1. Results of ANOVA for the number and density of *A. tsugae* ovisacs per 10-cm twig with needles at four different horizontal directions (east, west, south, and north) and six different heights

100-cm Branches				Whole Branches			
Cathedral State Park							
Horizontal locations							
	<i>F</i> value	<i>DF</i>	<i>P</i> value		<i>F</i> value	<i>DF</i>	<i>P</i> value
Number	1.51	3	0.22	Number	0.08	3	0.97
Density	1.06	3	0.37	Density	0.06	3	0.98
Vertical locations							
	<i>F</i> value	<i>DF</i>	<i>P</i> value		<i>F</i> value	<i>DF</i>	<i>P</i> value
Number	6.95	5	< 0.01	Number	1.35	5	0.25
Density	6.60	5	< 0.01	Density	2.84	5	0.02
Blackwater Falls State Park							
Horizontal locations							
	<i>F</i> value	<i>DF</i>	<i>P</i> value		<i>F</i> value	<i>DF</i>	<i>P</i> value
Number	0.32	3	0.81	Number	0.02	3	0.99
Density	0.14	3	0.94	Density	0.13	3	0.94
Vertical locations							
	<i>F</i> value	<i>DF</i>	<i>P</i> value		<i>F</i> value	<i>DF</i>	<i>P</i> value
Number	17.01	5	< 0.01	Number	24.71	5	< 0.01
Density	14.10	5	< 0.01	Density	41.67	5	< 0.01
Buchanan State Forest							
Horizontal locations							
	<i>F</i> value	<i>DF</i>	<i>P</i> value		<i>F</i> value	<i>DF</i>	<i>P</i> value
Number	0.61	3	0.61	Number	1.39	3	0.25
Density	0.94	3	0.42	Density	0.61	3	0.61
Vertical locations							
	<i>F</i> value	<i>DF</i>	<i>P</i> value		<i>F</i> value	<i>DF</i>	<i>P</i> value
Number	2.84	5	0.02	Number	1.65	5	0.15
Density	2.94	5	0.02	Density	13.33	5	< 0.01

Table 2. Coefficients of variance (CV) for the number of *A. tsugae* ovisacs in six different vertical locations

Site	Vertical Location	Branch Length		
		25cm	50cm	100cm
Cathedral State Park	Lowest	1.82	1.75	1.85
	Low-higher	1.17	1.64	2.13
	Middle-lower	1.49	1.57	1.37
	Middle-higher	1.10	1.14	1.00
	High-lower	1.00	1.17	1.03
	Highest	1.12	0.89	0.81
Blackwater Falls State Park	Lowest	1.47	1.30	1.17
	Low-higher	1.24	1.24	1.21
	Middle-lower	1.48	1.31	1.14
	Middle-higher	1.24	1.17	1.22
	High-lower	1.21	1.21	1.09
	Highest	1.29	1.32	1.13
Buchanan State Forest	Lowest	1.28	1.01	1.03
	Low-higher	1.77	1.34	1.27
	Middle-lower	2.01	1.78	1.51
	Middle-higher	1.40	1.49	1.49
	High-lower	1.22	1.09	1.24
	Highest	1.35	1.16	1.37

Table 3. Comparison of coefficients of variance (CV) and optimal sample size in fixed-precision-levels (D) of 0.1 between “current” (i.e. the lowest 100 cm branches) and “improved” sample unit from this study (i.e. 50-cm branches 3 m above the ground)

Site	Sample Unit	CV value	Sample Size		
			Eq. 3	Eq. 4	Eq. 5
Cathedral State Park	Current	1.85	394	77	1,574
	Improved	1.14	131	46	524
Blackwater Falls State Park	Current	1.17	157	96	630
	Improved	1.17	138	70	552
Buchanan State Forest	Current	1.03	107	89	428
	Improved	1.01	103	69	412

Table 4. Parameter estimates ($\pm$ SE) of Weibull function (Eq.1) and Taylor's power law (TPL) (Eq. 2)

Model	Site	Selected Sample Unit*	Parameter	Estimate $\pm$ SE	r^2
Weibull function		Branches 3-m	a	50.94 ± 2.964	0.97
		above ground	b	1.14 ± 0.122	
	Cathedral	Current	$\ln(a)$	-0.65 ± 0.846	0.95
			b	2.08 ± 0.185	
TPL	State Park	Improved	$\ln(a)$	-4.02 ± 3.693	0.68
			b	2.76 ± 0.779	
	Blackwater	Current	$\ln(a)$	-1.47 ± 3.601	0.61
			b	2.30 ± 0.753	
	Falls State Park	Improved	$\ln(a)$	-0.31 ± 0.870	0.96
			b	1.94 ± 0.164	
	Buchanan	Current	$\ln(a)$	0.96 ± 0.902	0.95
			b	1.76 ± 0.159	
	State Forest	Improved	$\ln(a)$	-0.83 ± 0.492	0.98
			b	1.77 ± 0.094	

* Current sample unit indicates the lowest 100-cm branch and improved sample unit indicates 50-cm branch 3 m above the ground.

Table 5. The number and density of *A. tsugae* per 10-cm twig with needles (mean $\pm$ standard error) in whole branches collected from six different vertical locations

Vertical location	Cathedral State Park		Blackwater Falls State Park		Buchanan State Forest	
	N / branch	Density	N / branch	Density	N / branch	Density
Lowest	88 $\pm$ 36.6 ^a	0.5 $\pm$ 0.21 ^b	6 $\pm$ 2.1 ^c	0.1 $\pm$ 0.03 ^c	656 $\pm$ 111.2 ^a	2.5 $\pm$ 0.34 ^c
Low-higher	167 $\pm$ 76.8 ^a	0.7 $\pm$ 0.21 ^{ab}	4 $\pm$ 1.6 ^c	0.1 $\pm$ 0.03 ^c	966 $\pm$ 159.6 ^a	2.6 $\pm$ 0.37 ^c
Middle-lower	297 $\pm$ 86.7 ^a	1.1 $\pm$ 0.27 ^{ab}	37 $\pm$ 16.8 ^{bc}	0.2 $\pm$ 0.06 ^c	993 $\pm$ 162.3 ^a	3.9 $\pm$ 0.73 ^c
Middle-higher	200 $\pm$ 52.0 ^a	1.4 $\pm$ 0.38 ^{ab}	86 $\pm$ 21.8 ^{abc}	0.7 $\pm$ 0.11 ^{bc}	847 $\pm$ 142.7 ^a	5.2 $\pm$ 0.88 ^{bc}
High-lower	179 $\pm$ 48.1 ^a	1.9 $\pm$ 0.48 ^{ab}	177 $\pm$ 48.5 ^a	1.5 $\pm$ 0.29 ^b	1,075 $\pm$ 184.9 ^a	7.2 $\pm$ 0.77 ^{ab}
Highest	104 $\pm$ 33.9 ^a	2.2 $\pm$ 0.58 ^a	108 $\pm$ 23.9 ^{ab}	3.3 $\pm$ 0.66 ^a	662 $\pm$ 135.1 ^a	9.3 $\pm$ 0.96 ^a

Means followed by the same letter within a column are not significantly different at $\alpha = 0.05$, Tukey HDS.

Table 6. The number and density of *A. tsugae* per 10-cm twig with needles (mean $\pm$ standard error) in 100-cm branches collected from six different vertical locations

Vertical location	Cathedral State Park		Blackwater Falls State Park		Buchanan State Forest	
	N / branch	Density	N / branch	Density	N / branch	Density
Lowest	32 $\pm$ 7.2 ^c	0.6 $\pm$ 0.10 ^b	145 $\pm$ 16.7 ^c	3.0 $\pm$ 0.47 ^d	106 $\pm$ 26.9 ^{ab}	2.2 $\pm$ 0.69 ^{ab}
Low-higher	42 $\pm$ 7.4 ^c	0.5 $\pm$ 0.07 ^b	282 $\pm$ 29.4 ^c	3.9 $\pm$ 0.41 ^d	53 $\pm$ 18.8 ^b	0.6 $\pm$ 0.20 ^{ab}
Middle-lower	71 $\pm$ 13.5 ^c	0.5 $\pm$ 0.07 ^b	397 $\pm$ 44.6 ^{abc}	5.7 $\pm$ 0.67 ^{cd}	22 $\pm$ 6.8 ^b	0.3 $\pm$ 0.14 ^b
Middle-higher	117 $\pm$ 15.5 ^{bc}	0.8 $\pm$ 0.09 ^b	630 $\pm$ 73.2 ^{ab}	8.5 $\pm$ 0.99 ^{bcd}	13 $\pm$ 6.0 ^b	0.3 $\pm$ 0.24 ^b
High-lower	268 $\pm$ 61.8 ^a	2.3 $\pm$ 0.63 ^b	701 $\pm$ 84.7 ^a	10.2 $\pm$ 1.06 ^a	201 $\pm$ 100.2 ^{ab}	2.3 $\pm$ 1.42 ^{ab}
Highest	237 $\pm$ 29.6 ^{ab}	4.7 $\pm$ 1.15 ^a	648 $\pm$ 88.4 ^a	12.4 $\pm$ 1.45 ^{ab}	401 $\pm$ 172.6 ^a	3.8 $\pm$ 1.40 ^a

Means followed by the same letter within a column are not significantly different at $\alpha = 0.05$, Tukey HDS.

Figure 1. Coordination method used in this study. Relative coordinates of nodes within a branch were assigned to map the distribution of *A. tsugae* ovisacs within a branch. The tip of terminal part of a branch was indicated by (0, 0). For example, the coordinate of the point 1 was (5, 0) and the coordinate of the point 2 was (7, 3).

Figure 2. Example within-tree distribution of *A. tsugae* ovisacs at three different sites. Darker color indicates higher density of *A. tsugae* ovisacs. I_a is aggregation index ($I_a < 1$ regular, $I_a = 1$ random, $I_a > 1$ aggregated) and P_a is associated probability.

Figure 3. Cumulative ovisac counts (mean $\pm$ SE) relative to sample location on the branch 3 m above the ground only when the ovisacs of *A. tsugae* are present

CHAPER 3: Spatial Associations between Hemlock Woolly Adelgid (Hemiptera: Adelgidae) Ovisacs and Surrounding Environment

Abstract. Understanding spatial associations between an insect pest and its environment can help develop management and sampling plans. This study was conducted to characterize the distribution pattern of the hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae) on eastern hemlock and its spatial association with the surrounding environment. A systematic-fixed sampling was conducted on each site. Each site was divided into 100 grids (10 m by 10 m) which were laid out in three eastern hemlock stands located in Blackwater Falls State Park (WV), Cathedral State Park (WV), and Buchanan State Forest (PA). In each grid, two 50-cm branches from pre-selected trees in each grid were cut at peak of ovisac occurrence in 2012-2014. Environmental and biological factors (i.e., elevation, aspect, species, diameter at breast height (DBH), tree height, basal area, and plant species diversity in each grid) also were measured to investigate their spatial associations with *A. tsugae*. Spatial distribution patterns of *A. tsugae* and its spatial associations with the surrounding environments were analyzed with Spatial Analysis by Distance Indices (SADIE). The results of this study showed that *A. tsugae* ovisacs were spatially aggregated regardless of sites and generations. The spatial associations of aspect, DBH, and hemlock tree height with *A. tsugae* ovisac population showed some significant spatial associations even though elevation, plant species diversity and basal area showed inconsistent spatial relationship with *A. tsugae* ovisac populations. These results can support to plan the sampling and management of *A. tsugae* populations.

Keywords: *Adelges tsugae*, eastern hemlock, SADIE, spatial distribution, population dynamics

The hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae) is an invasive insect that causes serious damage on eastern hemlock, *Tsuga canadensis* (Pinales: Pinaceae) in the eastern United States (Fidgen et al. 2002, Flowers et al. 2006). *A. tsugae* produces two parthenogenetic generations (i.e., progrediens and sistens) annually on hemlock and a winged form (i.e., sexupara form) occurs with progrediens in late spring (McClure 1987). The sistens are present from summer to the following spring. In June and July, sistens nymphs emerge as crawlers from eggs and settle at the base of young needles where they immediately enter aestivation (summer diapause) (McClure 1987, Salom et al. 2001). They resume development in October and complete their development in late winter. Adult sistens produce eggs from March until May and their progenies are determined to become either winged (sexuparae) or wingless (progrediens) forms based on the density of *A. tsugae* per twig (McClure 1991). Winged sexuparae disperse to spruce, but no suitable alternate hosts are known in the eastern United States. Therefore, winged sexuparae are known to die without reproduction (McClure 1991). The wingless progrediens remain on hemlock and complete their development, depositing sistens eggs in June (McClure 1991).

In the eastern United States, *A. tsugae* was first found in Richmond, VA in 1951 (Ward et al. 2004) and has been spreading to new areas at a speed of 8.9 to 20.4 km each year (Morin et al. 2009). According to Havil et al. (2014), almost 50% of the geographic range of eastern hemlock has been contaminated with *A. tsugae*, suggesting that approximately 9.5 million acres in the eastern United State need to be managed for *A. tsugae* (Schmidt and McWilliams 1996). Moreover, some hemlock stands are located in non-accessible areas such as along ridge tops, steep hillsides and narrow valleys (Orwig et al. 2002). Therefore, understanding spatial distribution of *A. tsugae* and its relationship with environmental factors is the key to manage *A. tsugae* efficiently and thoroughly.

Each insect species has its own, distinguishable spatial distribution which was determined from evolution between its environmental condition and behavior (Taylor 1984). Insect distribution in any geographical area can be categorized with three spatial patterns: uniform, random, or aggregated (Krebs 1999). Earlier studies used aggregation indices (e.g., Taylor's power law and Iwao's patchiness) to describe population distribution patterns. Currently, geostatistics, spatial analysis with distance indices (SADIE), and the

geographic information system are popularly used to describe spatial patterns with the advantage of visualization of insect distributions (Park and Tollefson 2006). If pest distributions are consistent from year to year, or if pest distributions are dependent on certain biological or physical environmental factors, it should be helpful to predict these pest distributions (Park and Tollefson 2006). These results of association analyses can help determine priority of sampling for extensive hemlock management areas.

There have been a few studies to predict *A. tsugae* damage based on environmental factors. Evans and Gregoire (2007) considered plant hardiness zone, elevation, forest cover type, urbanization, precipitation, temperature, and geographic range of eastern hemlock to predict the spread of *A. tsugae*. The speed of *A. tsugae* spread was approximately 12.5 km per year (Evans and Gregoire 2007). Koch et al. (2006) found that roads, major trails, and riparian corridors were good indicators to predict *A. tsugae* infestation. Orwig et al. (2002) reported latitude among a few environmental factors was most strongly correlated with *A. tsugae* infestation. Rentch et al. (2009) studied the relationship between *A. tsugae* and environmental factors with dendrochronology and found that their study sites and aspect were significantly related with *A. tsugae*. Although these studies are useful to detect and predict infestation of *A. tsugae*, spatial distribution of *A. tsugae* has not been characterized and spatial association between *A. tsugae* population and its environmental factors has not been explained. Therefore, this study was conducted to characterize spatial distribution patterns of *A. tsugae* in eastern hemlock stands, to investigate spatial relationships among different generations of *A. tsugae*, and to determine spatial association between *A. tsugae* ovisacs and environmental and biological factors.

Materials and methods

Study Sites. This study was conducted in three 100-m² eastern hemlock dominant stands in Blackwater Falls State Park (N39°6'26", W79°30'13") and Cathedral State Park (N39°19'26", W79°32'35") in West Virginia and Buchanan State Forest (N39°57'30", W77°57'01") in Pennsylvania. No *A. tsugae* management had been done in all three sites during the period of this study although intensive management was done in the past. In Blackwater Falls State Park, the infestation of *A. tsugae* started in 2003, management of *A. tsugae* using a predatory beetles (*Sasajiscymnus tsugae*) was initiated on June 4, 2004, and

imidacloprid was applied as both soil treatment and trunk injection every year from 2004 to 2010. Cathedral State Park has been infested with *A. tsugae* since 2003, imidacloprid has been applied since 2004 and 1,500 *S. tsugae* were released on July 7, 2004 (Tim Tomon, Personal communication, November 1, 2012). In Buchanan State Forest, 5,000 *S. tsugae* were released on April 1, 2005 (James Smith, Personal communication, October 15, 2012) to manage *A. tsugae* which was introduced in 1995 (James Unger, Interoffice memorandum of Pennsylvania Department of Conservation and Natural Resources Bureau of Forestry, August 24, 1995).

Sampling. A systematic fixed sampling was conducted on each site. Each site was divided into 100 grids (10 m by 10 m) which were laid using a compass, a tape measure, and flags. Then a tree located in the middle of each grid was selected for sampling. The sampling of *A. tsugae* ovisacs was conducted at the peak of ovisac number by checking the ovisac population dynamics every week. *A. tsugae* ovisacs were collected from a selected tree using a 3-m pole pruner to cut two 50-cm branches approximately 3 m above the ground; a total of 200 branch samples were collected at each site. If there was no branch within 3 m above the ground in the selected trees, the lowest branches were randomly selected and cut. If the selected trees were dead or fell during the events of winter storms, the nearest trees were selected for sampling. The sampling dates were June 6 in 2012, April 5 and June 25 in 2013, and May 2 in 2014 in Cathedral State Park, June 8 in 2012, March 26 and June 11 in 2013, and April 9 in 2014 in Buchanan State Forest, and June 13 in 2012, April 17 and July 3 in 2013, and April 16 in 2014 in Blackwater Falls State Park. All branch samples were transported to a cold room in the Division of Plant and Soil Science at West Virginia University. The room was maintained at approximately 4°C.

Environmental factors in each grid were sampled to investigate their spatial association with *A. tsugae* ovisacs. Elevation was measured using a differentially-corrected global positioning system (DGPS, Mobile Mapper pro, Megellan, Santa Clara, CA) and aspect was measured using a compass (Silva Expedition 4, Silva, Sweden) at the selected tree of each grid. In Buchanan State Forest, elevation and aspect were not measured because the site was flat. The diameter at breast height (DBH) and tree height of selected trees were measured by a tape-measure and a clinometer (Haglöf Electronic Clinometer, Haglöf Sweden, Långsele, Sweden), respectively. The clinometer auto-calculated tree

heights by measuring a straight-line distance from the target tree and angles for the top and base of target tree. Basal area of each grid was estimated by measuring DBH of all trees more than sapling stage (Eq. 1). All trees and shrubs were identified to determine diversity using Simpson's diversity index (Eq. 2, Simpson 1949).

$$\text{Basal area} = 0.00007854 \times \text{DBH}^2, \quad (\text{Eq. 1})$$

$$D = \sum_{i=1}^s \frac{n_i(n_i - 1)}{N(N - 1)}, \quad (\text{Eq. 2})$$

where s is the number of species, n_i is the total number of individuals in species i , and N is the total number of individuals in all species.

Characterization of spatial distributions. To characterize the spatial distribution of *A. tsugae* ovisacs within an eastern hemlock stand, spatial analysis by distance indices (SADIE) (Perry 1995) was used. SADIE measures the degree of clustering with patches and gaps. A patch is a region with relatively large number of ovisacs, and a gap is a region with relatively small number of ovisacs. To quantify the degree of clustering of ovisacs within a branch, the overall aggregation index, I_a expressed as $I_a = D / E_a$, was used. D is distance to regularity which is defined as the minimum total distance that individual samples in an observed arrangement would need to move to result in uniform or regular spatial distribution (Perry et al. 1999). Therefore, the higher D value, the higher degree of clustering of ovisacs in distribution. E_a is the mean expected distance to regularity. $I_a = 1$ means a spatially random pattern, $I_a > 1$ means a more aggregated pattern, and $I_a < 1$ means a more regular pattern (Perry et al. 1999). The significance test for spatial patterns was executed with formal randomization tests (Perry et al. 1999, Perry and Dixon 2002) under the null hypothesis, the observed ovisacs were arranged randomly among the given sample locations. SADIE also quantifies the contribution of each ovisac count at each tree to a patch or a gap with unitless clustering indices (Perry et al. 1999). $v_i = 1$ or $|v_j| = 1$ means random distribution of ovisacs within a stand, $v_i > 1$ means a unit belongs to a patch, and $v_j < -1$ means a unit belongs to a gap. In this study, two thresholds, 1.5 for v_i and -1.5 for v_j , were used to map patches and gaps of *A. tsugae* ovisacs within a stand, representing

clustering half as large as that expected by chance alone (Perry et al. 1999). All SADIE statistics were calculated with SADIEShell version 1.22 (Rothamsted Experimental Station, Harpenden, Herts, UK). ArcGIS version 9.3 (ESRI, Redlands, CA) was used to map the patches and gaps.

Spatial associations between population peaks of *A. tsugae ovisacs*. SADIE was used for spatial association analysis in which SADIE measures spatial associations between *A. tsugae* generations. The overall spatial association (X) is the mean of the local correlation coefficient between the clustering indices of the two sets; $X > 0$ for positive spatial association, $X = 0$ for no spatial association, $X < 0$ for negative spatial association (Perry and Dixon 2002); positive X values indicate the coincidence of a patch cluster for one set with a patch cluster for the other or the coincidence of two gaps and negative X values indicated by a patch coinciding with a gap (Perry and Dixon 2002). The associated possibility (P) also was calculated based on randomization tests (Perry and Dixon 2002) as the spatial aggregation analysis (Perry et al. 1999). The null hypothesis is that the spatial arrangement of the count data between two data sets is random (Park et al. 2006). In this study, $X > 0$ indicates consistent spatial association, $X = 0$ indicates no spatial association, $X < 0$ indicates an opposite change of distribution because samplings were conducted at same sites during four generations in two years.

Spatial association of *A. tsugae ovisacs* with environmental factors. The measurement of spatial association, a SADIE index X , was used as described above to measure the spatial association of *A. tsugae ovisacs* and geographic factors (i.e., elevation and aspect), tree measurements (i.e., DBH and height), and environmental factors (i.e., basal area and diversity of each grid).

Results

Population dynamics. In Buchanan State Forest, *A. tsugae ovisacs* population was 1.5–3 times higher in summer than spring (Fig. 1). This pattern was shown in other sites and years except between summer 2012 and spring 2013 in Cathedral State Park. When data from all three sites were pooled, the population density of *A. tsugae ovisacs* generally decreased from the first sampling (53.6 ± 2.61 per 50-cm branch) to the last (8.0 ± 1.04 per 50-cm branch) (Fig. 1).

Characteristics of spatial distribution of *A. tsugae*. The spatial distribution of *A. tsugae* ovisacs generally showed aggregation patterns even though random patterns were found in Cathedral State Park (spring 2013) and in Buchanan State Forest (spring 2014) (Table 1 and Fig. 2). When all the data in each site were pooled, significant ($P < 0.05$) spatial aggregation of *A. tsugae* ovisacs was found (Table 1).

Spatial associations between population peaks of *A. tsugae* ovisacs. Spatial associations between generations of *A. tsugae* ovisacs showed positive or no spatial relationship: 33% of significant ($P < 0.025$) positive spatial association and 67% of no spatial association (Table 2). Significant positive spatial association was found between summer 2012 and spring 2013 and between spring 2013 and summer 2013 in Buchanan State Forest, and between spring 2013 and summer 2013 in Blackwater Falls State Park. However, negative spatial associations were found between summer and spring generations in Blackwater Falls State Park even though the spatial associations were not statistically ($P < 0.975$) significant (Table 2).

Spatial association of *A. tsugae* ovisacs with environmental factors. The spatial associations of *A. tsugae* ovisacs with DBH were significantly ($P < 0.025$) positive (33%) or no relationship (67%) (Table 3). The significantly positive relationships with DBH were found in summers of 2012 and 2013 and spring 2014 in Cathedral State Park and in summer 2012 in Buchanan State Forest. The association between ovisacs and tree height also was consistently significant ($P < 0.025$) with positive (42%) or no spatial relationships (58%) (Table 3). The positive relationships of tree height occurred in spring 2013 in Blackwater Falls State Park, summers of 2012 and 2013, spring 2014 in Cathedral State Park, and summer 2012 in Buchanan State Forest. The relationship between *A. tsugae* ovisacs and plant species diversity was not consistent: 25% positive associations, 25% negative associations, and 50% no relationships (Table 3). Both elevation and basal area showed significantly positive ($P < 0.025$) associations with *A. tsugae* ovisacs only in summer 2012 in Blackwater Falls State Park and showed negative ($P > 0.975$) spatial association in spring 2014 in Blackwater Falls State Park (Table 3); the other cases showed no spatial relationships. Aspect showed 50% significantly ($P < 0.025$) positive relationships with *A. tsugae* ovisacs (spring 2014 in Blackwater Falls State Park, and summer 2012 and spring of 2013 and 2014 in Cathedral State Park).

Discussion

Population dynamics of *A. tsugae* ovisacs. The population of *A. tsugae* ovisacs decreased over the course of this study. Sussky and Elkinton (2014) reported that survivorship to progrediens adult stage was always <10% under their experimental conditions. Sistens generation experiences two important biological events, aestivation and overwintering. Therefore, the survivorship to sistens adult stage is generally much lower compared to the progrediens generation. It was reported that the average aestivation survival was 16.2% in forest conditions (Sussky and Elkinton 2015) and the mortality during overwintering was between 60% and 96% based on winter temperature (McClure and Cheah 1999). Moreover, adult progrediens can produce 20-30 eggs in early summer or late spring whereas sistens produce 50-100 eggs in spring (Paradis 2011). Therefore, *A. tsugae* ovisac number might be higher in early summer (progredientes' ovisacs) than spring (sistentes' ovisacs). In our study, the *A. tsugae* population we observed in Buchanan State Forest agreed with this pattern (Fig. 1). The Cathedral State Park population showed the opposite case, spring population was higher than summer population (Fig. 1). This might be due to unusual storm damage which caused approximately 50% of selected trees to fall even though the damage in the other sites was trivial. Fay and Whitham (1990) reported that *Adelges cooleyi* showed almost 0% survivorship on top branches and at least 60% survivorship on lower branches due to harsh winter conditions. *A. tsugae* populations in Blackwater Falls State Park continuously decreased (Fig. 1). According to Skinner et al. (2003), only 14% of *A. tsugae* could survive after exposure to -15°C in March. In our study, the lowest March temperature was consistently lower than -15°C, and it might have contributed to the population crash of *A. tsugae*. It also was reported that when a population was established from a small number of *A. tsugae*, the population had less fitness compared to one from a large number (Tobin and Turcotte 2013). Therefore, the Blackwater Falls State Park population may show continuous population decrease.

Characteristics of spatial distributions of *A. tsugae*. Spatial distribution of insect populations commonly shows some degree of aggregation because available resources and environmental factors are not uniform (Taylor 1984). The populations of *A. tsugae* also showed aggregation except for only two cases in spring 2013 in Cathedral State Park and spring 2014 in Buchanan State Forest. The aggregation of *A. tsugae* in most cases might be

caused by not only heterogeneities of local environmental conditions but also limited movement ability of *A. tsugae*. Because mobile *A. tsugae* crawlers settle at the base of needles on nearby new twig growth (Young et al. 1995), *A. tsugae* population may disperse gradually from the infested trees. The random distribution of *A. tsugae* in Cathedral State Park might be caused by unusual storm damage which caused approximately 50% of trees to fall. The random distribution of *A. tsugae* in Buchanan State Forest might be caused by high mortality of *A. tsugae* during winter. Skinner et al. (2003) reported that only 14% of the adelgids could survive after exposure to -15°C.

Spatio-temporal associations between population peaks of A. tsugae ovisacs. This study showed 67% of no spatial association between generations of *A. tsugae* ovisacs and 33% of significantly ($P < 0.025$) positive spatial associations. It is known that *A. tsugae* is sessile except for the crawler stage and passively disperses with the help of wind, birds, deer, and humans (McClure 1990). Limited movement or dispersal abilities might explain positive spatial association between two consecutive sampling periods. However, spatial association may be *A. tsugae*-density dependent. McClure (1991) reported that *A. tsugae* populations showed peaks every two years; during the first year of the infestation with *A. tsugae*, new shoot development of eastern hemlocks is limited. As a result, *A. tsugae* populations might decrease in the second year. In the third year, *A. tsugae* population might increase a little bit due to newly developed shoots. This population cycle is determined by the density of *A. tsugae* (McClure 1991).

Spatial association of A. tsugae ovisacs with environmental factors. Basal area of eastern hemlock trees and elevation in each grid show no spatial association with *A. tsugae* ovisacs in most cases. This result agreed with the finding by Orwig et al. (2002) who investigated the association between elevation and *A. tsugae* populations. Environmental factors such as latitude and distance to the closest stream, trail, and road were not studied in this study, but these factors are important to predict the occurrence of *A. tsugae*. It was revealed that latitude was most strongly correlated with *A. tsugae* population even though aspect and stand size were also correlated with *A. tsugae* (Orwig et al. 2002). Distance to closest stream, trail, and road were also useful to predict occurrence of *A. tsugae* related with dispersal of *A. tsugae* (Koch et al. 2006).

Our study showed that the spatial associations of DBH, tree height, and aspect with *A. tsugae* ovisacs showed some significant spatial associations according to sites and sampling times. Codominant trees and east- or south-facing trees receive more light, and then maximize new shoot growth (Rentch et al. 2009). The increased amount of new shoots might accommodate more *A. tsugae* populations. These results were consistent with previous studies. Orwig et al. (2002) reported that aspect was significantly correlated with *A. tsugae* population and Rentch et al. (2009) found that aspect and overtopped trees were correlated with *A. tsugae*. They also found that occurrence of *A. tsugae* populations was not related with decline of eastern hemlock due to compensation of trees. These results imply that large hemlock trees on east-facing slopes might have possibilities of higher *A. tsugae* populations. Therefore, DBH, tree height, and aspect need to be considered for sampling and management of *A. tsugae*.

The results of this study suggest two important considerations in *A. tsugae* sampling and management. First, *A. tsugae* ovisacs showed spatial aggregation in most cases, which allows for planning site-specific management for *A. tsugae*. Site-specific insect pest management is a management strategy based on local insect densities within an area rather than uniform management of insect pests based on average densities throughout the area (Park et al. 2007). Because *T. canadensis* is mainly distributed along streams (Evans 2002) and located in broad areas, the site-specific pest management strategy may provide a solution. However, a major factor limiting the application of site-specific pest management is high sampling cost (Fleisher et al. 1997, Krell et al. 2003). If an efficient sampling plan is developed for *A. tsugae*, site-specific pest management may be more feasible to control *A. tsugae* in eastern hemlock stands. Second, we found that *A. tsugae* ovisacs were spatially associated with some environmental factors. DBH, tree height, and aspect are key factors determining *A. tsugae* distribution, so these factors should be considered for sampling and management of *A. tsugae*.

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Table 1. Spatial distribution of *A. tsugae* ovisacs measured with SADIE index, I_a . Overall degree of clustering (I_a) with its associated P value (P_a) in parenthesis. $I_a = 1$ suggests a random, $I_a > 1$ suggests an aggregated, and $I_a < 1$ suggests a regular spatial pattern. Significant ($P < 0.05$) associations are in bold

Place	Summer, 2012	Spring, 2013	Summer, 2013	Spring, 2014	Pooled
Blackwater Falls State Park	1.35 (0.03)	1.49 (0.01)	1.73 (0.01)	1.91 (0.01)	1.56 (0.01)
Cathedral State Park	1.55 (0.01)	0.99 (0.46)	1.32 (0.04)	1.61 (0.01)	1.35 (0.03)
Buchanan State Forest	2.59 (0.01)	2.33 (0.01)	2.61 (0.01)	0.95 (0.46)	2.97 (0.01)

Table 2. Spatial association between two generations of *A. tsugae* based on ovisac counts. Index of association (X) with its associated P value (P_t) in parenthesis. For a two-tail test at 95% confidence level, $P_t < 0.025$ indicates significant positive association and $P_t > 0.975$ indicates significant negative association. Significant associations are in bold

Place	Summer 2012 vs. Spring 2013	Spring 2013 vs. Summer 2013	Summer 2013 vs. Spring 2014
Blackwater Falls State Park	-0.09 (0.79)	0.36 (< 0.01)	-0.02 (0.59)
Cathedral State Park	0.06 (0.28)	0.12 (0.13)	0.13 (0.11)
Buchanan State Forest	0.36 (< 0.01)	0.49 (< 0.01)	0.18 (0.04)

Table 3. Spatial associations between *A. tsugae* ovisacs and surrounding environmental factors. Index of association (X) with its associated P value (P_t) in parenthesis. For a two-tail test at 95% confidence level, $P_t < 0.025$ indicates significant positive association and $P_t > 0.975$ indicates significant negative association. Significant associations are in bold

Place	Time	DBH*	Tree height	Basal area	Diversity **	Elevation	Aspect
Black-water Falls State Park	2012 Summer	-0.03 (0.61)	0.05 (0.30)	0.02 (0.41)	0.09 (0.19)	0.28 (< 0.01)	0.14 (0.09)
	2013 Spring	-0.11 (0.85)	0.22 (0.02)	0.19 (0.03)	0.18 (0.04)	-0.04 (0.66)	-0.28 (0.99)
	2013 Summer	-0.18 (0.96)	0.12 (0.12)	0.01 (0.47)	0.32 (< 0.01)	-0.03 (0.62)	-0.08 (0.74)
	2014 Spring	-0.01 (0.52)	-0.02 (0.56)	-0.25 (0.99)	-0.24 (0.99)	0.11 (0.14)	0.72 (< 0.01)
	2012 Summer	0.29 (< 0.01)	0.25 (< 0.01)	-0.07 (0.77)	-0.37 (1.00)	0.18 (0.04)	0.46 (< 0.01)
	2013 Spring	-0.07 (0.76)	0.01 (0.47)	0.08 (0.21)	-0.03 (0.61)	0.12 (0.13)	0.29 (0.01)
Cathedral State Park	2013 Summer	0.30 (< 0.01)	0.34 (< 0.01)	0.12 (0.13)	-0.27 (0.99)	0.07 (0.24)	0.18 (0.05)
	2014 Spring	0.35 (< 0.01)	0.31 (< 0.01)	0.05 (0.68)	-0.05 (0.67)	0.05 (0.31)	0.37 (< 0.01)
	2012 Summer	0.28 (< 0.01)	0.21 (0.01)	-0.11 (0.85)	0.21 (0.02)	-	-
Buchanan State Forest	2013 Spring	0.09 (0.17)	0.08 (0.20)	0.09 (0.17)	0.35 (< 0.01)	-	-
	2013 Summer	0.20 (0.04)	0.18 (0.05)	0.20 (0.04)	0.09 (0.19)	-	-
	2014 Spring	-0.03 (0.63)	-0.10 (0.84)	-0.18 (0.93)	-0.03 (0.61)	-	-

* Diameter at breast height

** Simpson's diversity index

Figure 1. Population dynamics of *A. tsugae* ovisacs. Error bars indicate standard error of the mean ovisac counts.

(A) Blackwater Falls State Park

(B) Cathedral State Park

(C) Buchanan State Forest

Figure 2. Spatial distribution maps with patches and gaps of *A. tsugae* ovisacs in three study sites, (A) Blackwater Falls State Park, (B) Cathedral State Park, and (C) Buchanan State Forest. Red areas indicate patches with clusters of relatively large counts (i.e., $v_i > 1.5$) and blue area indicate gaps with clusters of relatively small counts (i.e., $v_i < -1.5$).

CHAPTER 4: Conclusion

The hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae) is an invasive pest of *Tsugae canadensis* which creates a unique environment for wildlife in the eastern United States. Currently, *T. canadensis* is growing on ca. 19 million acres in the eastern United States and is the predominant species on 2.3 million acres. These important hemlock-dominant forests are now threatened by *A. tsugae*. Almost 50% of eastern hemlock already has been infested with *A. tsugae*. Therefore, efficient sampling plans are required to manage *A. tsugae*.

The results of this study (Chapter 2) help determine the sample unit for *A. tsugae* ovisacs. Taking 50-cm branch samples might be an efficient and stable sampling unit of *A. tsugae* because means of *A. tsugae* ovisacs were relatively higher and variances were lower than sampling units being used currently. However, even smaller sample units might be achievable by using the non-linear equation developed in this study to estimate the number of *A. tsugae* ovisacs. Vertical locations of branches needed to be considered to determine a sample unit of *A. tsugae* ovisacs because *A. tsugae* ovisacs were more frequently found on higher branches. By using a standard and efficient sample unit, i.e. 50-cm branches 3 m above the ground, the sampling of *A. tsugae* could be more precise, economical, and less destructive.

This study (Chapter 3) also considers the implications of sampling and management for *A. tsugae* populations. The populations of *A. tsugae* ovisacs decreased in all three study sites and might continue to decrease if early spring temperature is lower than -15°C. Also, it may be feasible to apply site-specific management of *A. tsugae* by analyzing the relationship between *A. tsugae* population and its environmental factors because the spatial distribution of *A. tsugae* ovisacs showed spatial aggregation in most cases. A few environmental factors need to be considered when planning the sampling and management of *A. tsugae* because hemlock stands on east-facing slopes and with large trees showed the positive relationships with *A. tsugae* ovisacs.

In conclusion, this study showed two important findings. First, *A. tsugae* ovisacs were spatially aggregated and associated with DBH, tree height, and aspect. Second, the optimal sample unit for *A. tsugae* ovisacs was 50-cm branch 3 m above the ground. These

findings suggest the possibility of development of sampling plans and site-specific management of *A. tsugae*.