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Regional Comparison of Overwintering Mortality, Fecundity, and Virulence in the Hemlock Woolly Adelgid

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**REGIONAL COMPARISON OF OVERWINTERING MORTALITY, FECUNDITY, AND
VIRULENCE IN THE HEMLOCK WOOLLY ADELGID**

A Thesis Presented

by

ARTEMIS DEMAS ROEHRIG

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

MASTER OF SCIENCE

February 2013

Organismic and Evolutionary Biology

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Dedication

In memory of my dog Darwin (2008-2012), my constant field and travel companion, protector, and best friend. Without his innate ability to find the car, I might still be lost in the hemlock stands of rural Appalachia.

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I would like to extend my thanks to the countless individuals without whom this project would not have been possible. First and foremost, my advisor, Joe Elkinton, provided me with valuable help, support, and hours of his time. Ben Normark and Matt Kelty served on my graduate committee and were frequently available to help at short notice. John Buonaccorsi provided valuable advice on statistical analyses. Beth Jakob and the other OEB faculty, staff, and students offered their advice and encouragement. Penny Jaques dealt with endless paperwork and logistical headaches on my behalf.

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ABSTRACT

REGIONAL COMPARISON OF OVERWINTERING MORTALITY, FECUNDITY, AND VIRULENCE IN THE HEMLOCK WOOLLY ADELGID

FEBRUARY 2013

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Throughout the eastern United States, the spread of the hemlock woolly adelgid, *Adelges tsugae* Annand, has caused high mortality of eastern hemlocks *Tsuga canadensis* (L.). We recorded overwintering survival and fecundity of *A. tsugae*, and tree new growth at sites in the northeastern and southeastern United States and in a common garden experiment in Massachusetts.

Overwintering mortality of *A. tsugae* was much higher in the north (87%) than the south (37%) in 2009, and showed significantly positive density-dependence in the north only. In 2010, overwintering mortality decreased in both regions but remained higher in the north (54%) than the south (34%), and, unlike 2009, density-dependent mortality was strongly negative in the north, and positive in the south.

In both years, sistens fecundity was significantly higher in the south than the north, but we observed no density-dependent trends, and fecundity measurements were similar in the two years.

The regional discrepancies in fecundity suggested the possibility of an evolutionary trade-off between overwintering mortality and sistens fecundity. However, when we reared samples in a common garden, we found that source region had no effect on either sistens fecundity or overwintering mortality, which suggests observed regional differences can be attributed to environmental factors rather than genetic differences.

In our regional studies, branch samples from the north had significantly more new growth than those from the south in both 2009 and 2010, even though *A. tsugae* densities were comparable. This difference persisted in the common garden wherein branches inoculated with northern-derived *A. tsugae* had significantly higher new growth than those infested with southern-derived *A. tsugae*. These findings raise the possibility that *A. tsugae* may be evolving towards reduced virulence. If true, these findings may help explain why *A. tsugae* is killing hemlocks much more slowly now in New England than it did when it first invaded this region more than 20 years ago, or as it is doing now in more recently invaded regions in the southern United States.

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CHAPTER I
REGIONAL COMPARISON OF OVERWINTERING MORTALITY FECUNDITY, AND
VIRULENCE IN THE HEMLOCK WOOLLY ADELGID

Introduction

The hemlock woolly adelgid, *Adelges tsugae* Annand, is a sap-feeding insect from Japan that has been attacking and killing large numbers of eastern (*Tsuga canadensis*) and Carolina (*T. caroliniensis*) hemlocks in the eastern United States. In this region, it was first reported in Virginia in 1951 (Gouger 1971), and has since spread to northern Georgia, southern Maine, and westward into Tennessee, New York and Michigan (Figure 1). Recent analyses predict that in the next thirty years most of the hemlock stands in the eastern United States will be affected by *A. tsugae* (Albani et al. 2010). *A. tsugae* has two generations per year: the sistens, or overwintering generation, and the progrediens, or spring generation, both of which are parthenogenetic. A sexual generation of *A. tsugae* is initiated by sexuparae, which develop at the same time as the progrediens. These migrate to and lay eggs on spruce trees, which hatch into sexuales, the sexual generation of *A. tsugae*. However the sexuales do not survive on the North American species of spruce trees, so in the United States only parthenogenic reproduction occurs. (McClure 1989a,1995).

McClure (1991) described the population dynamics of *A. tsugae* in relation to its hemlock host. Peak densities of the adelgid occurred after rapid population growth during the first year of infestation, causing an inhibition in tree new growth, which induced a population crash in *A. tsugae* populations the following year. In the third year new growth on the trees recovered somewhat, allowing a resurgence in *A. tsugae* density. New growth and *A. tsugae* densities then

declined again in the fourth year, after which, both insects and trees were all dead. According to McClure (1991) this process occurred on both wild and cultivated hemlock trees.

Mortality of hemlocks by *A. tsugae* has had widespread effects on the forest ecosystem. Forest stands consisting of a mix of hemlock and hardwood have a denser understory than stands with just hardwoods (Kelty 1989), and *A. tsugae* invasions create canopy gaps, altering forest understory dynamics (Orwig and Foster 1998). This increases bryophyte species richness (Cleavitt et al. 2008), alters overall vegetation composition (Spaulding and Rieske 2010), and impacts rates of litter decomposition (Cobb 2010) and nutrient cycling (Stadler et al. 2006). Hemlocks often grow along streams and provide intense shade, which is lost when hemlocks die and are replaced by hardwoods (Orwig and Foster 1998). This results in higher stream temperatures and major changes in stream flora and fauna (Snyder et al. 2002), such as shifts in species composition (Huddleston et al. 2010). Other hemlock-associated species are also affected by loss of hemlocks. For instance, breeding pair density of the Acadian flycatcher was found to be negatively correlated with *A. tsugae*-caused tree defoliation (Allen et al. 2009). Thus, well beyond causing hemlock tree deaths, *A. tsugae* has serious effects on the entire ecosystem.

Regional disparities exist in the impact of *A. tsugae* on tree mortality. Forest managers and researchers who work on *A. tsugae* in the southern United States report that tree death occurs within two to four years after initial infestation. A 2009 study on the impact of *A. tsugae* on the carbon cycle, done in western North Carolina, also suggested that the decline of hemlock trees is occurring more quickly in the southern region. In fact, in this study, declines in tree basal area increment, very fine root biomass, and soil CO₂ efflux occurred as quickly as one year post *A. tsugae* infestation (Nuckolls et al. 2009).

Hemlock researchers who work in New England, however, report that infested trees persist for ten years or more, and relatively little tree death occurs (Paradis 2011). For example, Paradis (2011) investigated *A. tsugae* in Massachusetts and at sites in Connecticut near those studied by McClure (1991). In her study, *T. canadensis* produced some new growth every year

despite densities of *A. tsugae* that were just as high as those documented 20 years earlier by McClure (1991), and, in contrast to the McClure's study, all of the trees remained alive after six years.

Temperature is considered a defining factor for the regional disparities in *A. tsugae* population density, with colder and more variable winter temperatures dictating lower population densities in the Northern range. In the southern range, warmer winter temperatures dictate lower *A. tsugae* mortality, and thus higher population densities, which in turn increase tree mortality (Costa and Onken 2006). Simulations by Fitzpatrick et al. (2012) found winter temperature variation to be a more important factor than tree abundance for *A. tsugae* spread dynamics. Other previous research shows that overwintering mortality is a major determinant of *A. tsugae* population trends. Various studies have shown that cold winter temperatures cause high mortality in northern populations and in some years is sufficient to cause major declines in *A. tsugae* population density (Parker et al. 1998, Butin et al. 2005, Paradis et al. 2008). However, it has been found that *A. tsugae* from the northern region are actually able to survive lower temperatures than those from the Central and Southern regions, and that cold-hardiness varies over the course of the year (Skinner et al. 2003).

The aim of our study is to further investigate the differences between *A. tsugae* populations in the northern and southern regions. Although it may be obvious to an observer that there are indeed regional differences in the impact of the *A. tsugae* invasion, it is not clear what factors are the driving forces behind these differences. Both acknowledging and understanding these differences may play an important role in how we choose to control the invasion in different areas, and also in our predictions of future spread and impact. Spread rate is affected by any process that impacts growth or movement (Morin et al. 2009), so our investigations into overwintering mortality and fecundity may be important to future spread models. In this study, we quantify *A. tsugae* density, mortality, and fecundity in southern populations (Virginia, Tennessee, and Georgia), for which no complete study has yet been done, and continue studies of

New England populations of *A. tsugae* initiated by Paradis et al. (2008) and Paradis (2011) in order to extend our understanding of the population dynamics of *A. tsugae* to include both regions of the invasion.

Another invasive insect on *T. canadensis* is the elongate hemlock scale *Fiorinia externa*, which has invaded the New England states along with the adelgid (Preisser et al 2008). The impact of the scale on tree health is not as dramatic as that caused by *A. tsugae* (Miller-Pierce, Orwig, and Preisser 2010). Paradis (2011) found no significant relationship between *F. externa* densities and the density, fecundity, or mortality of *A. tsugae*. However, Preisser et al (2008) found a positive correlation between *F. externa* and *A. tsugae* densities, while Preisser and Elkinton (2008) showed that the occurrence of this second invasive insect can actually reduce the density of *A. tsugae* (Preisser and Elkinton 2008) and found that *A. tsugae* showed a 44% lower settlement rate on branches already infested by *F. externa* (Miller-Pierce and Preisser 2012). Thus, we chose to measure *F. externa* density in our study.

Butin et al. (2005) used a common garden experiment to show that the greater cold hardiness of northern versus southern *A. tsugae* populations has a genetic basis. Therefore, in our common garden experiment, we expected to see higher overwintering mortality among *A. tsugae* from southern populations, as previously demonstrated by Skinner et al (2003) and Butin et al. (2005). This would indicate that either southern populations have genetically diminished in their ability to withstand cold temperatures, or that northern populations have gained a genetic basis for increased cold tolerance. If this is true, theories of life-history evolution suggest that some sort of evolutionary trade-off may be present. (Stearns 1989).

Evolutionary trade-offs cause the evolution of a beneficial trait change to correlate with a detrimental trait change. The most common trade-off occurs when increased survival is gained by a decrease in reproduction (Stearns 1989). Thus, we included measures of fecundity, a factor which few investigators have examined in previous studies of this species. We predicted that in addition to higher densities and decreased mortality, we would find higher fecundity in the

southern sites. If this is the case, in the common garden we expect to see lower fecundity measures in those *A. tsugae* derived from northern populations than those from southern populations. In turn, we expected to see higher overwinter mortality in southern-derived *A. tsugae* than those from the north. If no genetic differences exist between the northern and southern populations in our common garden experiment, we should observe no differences between the two populations in terms of overwintering mortality, fecundity, or densities.

Materials and Methods

In this study we examined regional populations of *A. tsugae* over the course of two years, and ran an experiment in which we reared *A. tsugae* derived from the two regions in a common garden in Massachusetts. Samples were collected from a total of ten different southern locations and twelve different northern locations during the course of both regional studies and used as sources for common garden inoculations, however not all locations were included in all analyses due to unsatisfactory sample quality for some sampling periods (Figure 1). Eight of these northern sites were the same as those used by Paradis (2011).

Northern sites ranged in elevation from 25 to 372m, while southern sites ranged from 266 to 1173m. All of the northern sites, and our southern site in Virginia, were located in counties where *A. tsugae* infestations were first reported prior to 1997. In the other southern locations, our site in North Carolina was located in a county where *A. tsugae* was first reported in 2001, and the county in Tennessee had *A. tsugae* first reported in 2002. Five sites in Georgia were located in two different counties in which *A. tsugae* was first reported in 2004. The final two Georgia sites were located in a county where *A. tsugae* was first reported only three years prior to the start of our sampling, in 2006.

In the early spring, samples were collected from all sites in March 2009 and March 2010. Late spring samples were collected from southern sites in April 2009 and May 2010 and from northern sites in June 2009 and late May 2010, taking into account the later hatch times in the north. All of our sample collections and measurements followed the same protocol, and general collection timing, used by Paradis (2011).

At each site we selected two to seven trees, and collected one to eight branches of approximately 30 cm in length from chest-height. Branch selection was haphazard; however we made attempts to select branches from different sides of the trees and representing a range of infestation levels. The ends of the freshly-cut branches were placed in hydrated florist foam and transported to the University of Massachusetts, Amherst, where they were processed immediately.

To process each branch, we counted the total number of branchlets and recorded the proportion of branchlets with new growth and the proportion of branchlets with *A. tsugae* present. A branchlet was defined as the length of branch growth from the past year, measured from the most distal node. New growth was defined as the growth from the current season, distinguished by its much brighter coloration.

From each branch, we then haphazardly selected anywhere from one to all of the infested branchlets. For those branches from which we used only a subset of branchlets, we tried to select branchlets representing a range of *A. tsugae* densities. More branchlets were processed when *A. tsugae* densities were low, in order to assure that we had sufficient numbers to estimate density, fecundity, and survival accurately. On the branchlets, we then measured the length of the last year's growth, counted the total number of mature sistens, and calculated the numbers of live and dead mature sistens. *F. externa* density was measured by counting the number of individuals present on the first ten needles of each sampled branchlet. In 2010, we also calculated the density of dead immature sistens. Overwintering sistens mortality was determined using hemolymph response as outlined by both Trotter & Shields (2009) and Paradis et al. (2008). In our analyses

we used data from six northern sites, and 10 southern sites in 2009; and five northern sites and nine southern sites in 2010.

For late spring sampling, we selected and collected sample branches following the same protocols as in the early spring. After transportation, samples were bagged and frozen until they could be analyzed, which does not affect the variables we were measuring. For processing, we counted the total number of branchlets on each branch and recorded the proportion of branchlets with new growth and the proportion with *A. tsugae* present, as done in overwintering mortality measurements. Branchlets were selected from each branch following the same protocol as used in the early spring samples. For each branchlet we calculated sistens density, *F. externa* density, and, in addition, we measured the length of the new growth on each branchlet. We then measured sistens fecundity by counting chorion, following the same methods as Paradis et al. (2008) and Paradis (2011). We used data from six northern sites and eight southern sites in 2009; and four northern sites and eight southern sites in 2010.

All data for the regional studies were analyzed at the branch level, using JMPIN4 for regional effects, and SAS 9.13 to analyze density dependence. We used $P < 0.05$ as our baseline for determining statistical significance. We measured new growth in only the late spring sampling period because the early spring samples preceded new growth in hemlocks.

To investigate potential genetic causes for regional disparities, we supplemented our regional studies with a common-garden experiment. We set up our common-garden plot in the Cadwell Research Forest in Pelham, MA, near the locations used by Butin et al. (2005), Butin et al. (2007), and Preisser and Elkinton (2008), and using similar protocol. *A. tsugae* was present in this area prior to our study. Source adelgid were taken from healthy-appearing trees at nine southern sites (ranging 266 to 1176 meters elevation) and 11 northern sites (Figure 1). In order to reduce tree effects, we took samples from multiple trees at each site, but specifically chose branches with higher *A. tsugae* densities.

To prepare our common garden plot, we haphazardly chose 48 mature trees in late April 2009, all of which were taller than 10 m, had little or no detectable adelgid infestation at human height, appeared to be of similar health, and were growing in similar conditions. On each tree, one to four branches were then selected for inoculation. Branches were located at heights of approximately one to two meters off the ground, and were spaced so no selected branches were adjacent. Each branch was checked thoroughly for infestation, and all branchlets containing adelgid were removed. Adjacent branches were also checked and stripped of any branchlets containing adelgid.

Each pre-selected branch was inoculated in late April 2009 with adelgid from a single source site, using approximately 0.3 to 0.9 meters of the branch. Unlike previous inoculation studies done at this site (Butin et al 2007, Preisser and Elkinton 2008), we did not use aquapics on the source branches during the inoculation process. Branches were then confined inside mesh bags to improve the rate of inoculation and to prevent adelgid spread to adjacent branches or trees. For each site, we inoculated one to three branches, which were assigned haphazardly but spaced away from other branches inoculated with adelgid from the same source site. Early spring branch samples were collected in March 2010 and late spring samples in May 2010. These samples contained adelgids two generations after inoculation. We collected branches of approximately 30 cm in length, as in the regional study, from each bag. Branches were processed, and measurements were analyzed following the same protocol as those in the regional study, except results were analyzed at the bag level. At the conclusion of the study, all inoculated branches were destroyed, and all trees used in the experiment were checked to ensure there were no *A. tsugae* near the inoculation sites.

Results

The proportion of branchlets with new growth and the mean length of new growth on sampled branchlets was significantly higher in the North than the South in both 2009 (Fig. 2a, 2b) and 2010 (Fig. 3a, 3b). Both of these measurements were also significantly higher in the North than the South when the two years were looked at together. In the common garden, both proportion and length of new growth were significantly higher in samples with northern-derived populations than southern-derived populations (Fig. 4a, 4b) (Table 1).

In the late spring samples, *A. tsugae* density was significantly higher in the north than the south in 2010 and significantly higher in the northern-derived samples in the common garden, but did not show a regional difference in either 2009 or when 2009 and 2010 were looked at together. The proportion of branchlets with *A. tsugae* was greater in the South than the North in 2009, and did not differ by region in 2010, the two years combined, or the common garden. *Sistens* fecundity was consistently higher in the South than the North in 2009, 2010, and when both years were looked at together, but showed no regional difference in the common garden. *F. externa* density was significantly higher in the North than the South in all regional analyses, but there were no source region effects in the common garden (Table 2). Most of our southern sites had little or no infestation of *F. externa*, an observation that matches a study by Abel and Van Driesche (2012), who revealed lower densities of *F. externa* in their study site in North Carolina than their sites in Pennsylvania and Connecticut.

We analyzed the combined effects of *F. externa* and *A. tsugae* densities on proportion and length of new growth by way of multiple regression (Proc REG and Proc GLM, SAS 9.2). *Adelges tsugae* was positively correlated with proportion of new growth in the South in 2009, but was unrelated in any other case, and showed no correlation with length of new growth. *Fiorinia externa* showed a significant negative correlation with both proportion and length of new growth in the North in 2010, and when both years were looked at together, but showed no effect in 2009.

Expectedly, since abundance was so low, *F. externa* had no effect on new growth in the southern region. (Table 3a). In the common garden, *A. tsugae* density was negatively correlated with proportion of new growth when the two regions were combined; otherwise neither *A. tsugae* nor *F. externa* had any correlation with either proportion or length of new growth. There were no significant interactions between these two variables (SAS, Proc -GLM).

In the early spring, region had no effect on *A. tsugae* density in 2009, 2010, or the common garden. Proportion branchlets with *A. tsugae* was higher in the South than the North in 2009, but was unaffected by region in either 2010 or the common garden. As in late spring measurements, *F. externa* densities were significantly higher in the North than the South in both 2009 and 2010, but unaffected by source site in the common garden. (Table 4).

As expected, overwintering mortality in 2009 was much higher in the North than the South (Table 4). In 2009, based on a logistic model, there was a strong relationship between sistens density and overwintering mortality in the North, while no density dependent trend was evident in the South. (Fig. 5a, Table 5).

In 2010, overwintering mortality was still higher in the North than the South. However, mortality in the North showed a significant drop from 85% in 2009 to 54% in 2010, while mortality in the South had an insignificant drop from 36% in 2009 to 34% in 2010 (Table 4). These results are likely explained by the differences in winter temperature (Table 4). Unlike in 2009, overwintering mortality showed a strongly positive density-dependent trend in the South and a strongly negative density-dependent trend in the North. (Fig. 5b, Table 5).

In the common garden experiment, overwintering mortality was not significantly different between northern-derived and southern-derived samples (Table 4). There also was no relationship apparent between sistens density and overwintering mortality in the southern-derived samples. However, as in the 2010 northern regional samples, the northern-derived samples in the common garden showed a strongly negative relationship between density and overwintering mortality (Fig. 5c, Table 5).

At the regional sites, in 2010, we additionally counted dead immature sistens representing those that failed to put on wool prior to winter and thus died in summer or fall. . The mean density of dead immature sistens in the North was not significantly different than in the South ($F=1.396$, $df=197$, $p=0.239$). However, the density of dead immature sistens was significantly higher in the South than the North ($F=6.816$, $df=197$, $p=0.010$).

There was no significant relationship between sistens density and fecundity in either the North or the South in 2009 (Fig. 6a), 2010 (Fig. 6b), or when both years were looked at together (Table 6). In the common garden, there was no significant density-dependence in either northern or southern-derived samples (Fig. 6c), or when both regions were looked at together (Table 6). Proportion of branchlets with *A. tsugae* were positively correlated with sistens fecundity in the North in 2009 and when both years were looked at together (Table 7). In 2009 there was also a negative correlation between proportion of branchlets with *A. tsugae* and sistens fecundity in the south, but no relationship was seen in any other instance (Table 6).

In samples from the North, there was no correlation between *F. externa* density and *A. tsugae* density in either 2009 or 2010 (Fig. 7a), or when both years were looked at in combination (Table 7). Nor were these related in either the northern-derived or southern-derived samples in the common garden (Fig. 7b). *Fiorinia externa* had no effect on *A. tsugae* density or proportion branchlets with *A. tsugae* except for a positive correlation in the North in 2010 (Table 7).

Discussion

In our regional study, infested branches in the North had significantly higher new growth than those in the South in both years. Northern branches had 3 to 4 times greater proportions of new growth than those in the south, and 31 to 133 times the centimeters of new growth at the branchlet level. It is definitely possible that these regional differences were produced by differences in the timing of our sampling with respect to hemlock budburst and twig growth,

especially in 2009 when we sampled the northern plots much later than in the South. However, this pattern persisted when we reared *A. tsugae* populations from both regions in the common garden experiment, with approximately 9 times the proportion new growth, and 35 times the length new growth on branches inoculated with source populations from the North (Table 1). Differences in hemlock phenology cannot explain these results because all these samples were taken at the same time.

This regional disparity in new growth cannot be explained by *A. tsugae* densities, which were comparable between regions (Table 2). These findings raise the possibility that *A. tsugae* populations in the North have evolved lower virulence, defined as harm inflicted on the host (Anderson and May 1981, May and Anderson 1983). If true, this could help explain why tree mortality has been reported to occur much more quickly and at higher rates in the southern Appalachians than in New England, especially in recent years (Orwig et al. 2012).

A decline in virulence of northern *A. tsugae* populations could explain the discrepancy between the findings of Paradis (2011) and those of McClure (1991), both of whom collected data on *A. tsugae* fecundity and mortality from populations in New England. McClure's study was conducted right after *A. tsugae* first invaded his study sites in the 1980s. He documented a boom--bust cycle of adelgid density and tree decline, wherein high densities of adelgid completely eliminated new growth in hemlocks the following year. This produced a decline in *A. tsugae* density that allowed the tree to recover and put on some new growth again in the third year. In the fourth year all trees died along with their *A. tsugae* populations. The studies by Paradis (2011) were conducted 20 years later in hemlock stands not far from those studied by McClure (1991) and that had harbored populations of *A. tsugae* for an unknown period. In her study, she documented fluctuations in *A. tsugae* density of a similar magnitude to the densities McClure reported. High densities of adelgid caused reductions in hemlock growth the following year, but unlike McClure's study, trees experienced some level of new growth every year, even those

following years of peak *A. tsugae* density. Probably for that reason, none of the 60 trees she looked at died during the six-year duration of her study.

All hemipterans, including *A. tsugae*, extract nutrients from their hosts and can deplete these nutrients at high density, but they gain no other advantage from killing or harming their hosts in the process. Many hemipterans persist at very high densities on their hosts without obvious ill effects. *Fiorinia externa*, for example, rarely causes hemlock death, even though it attains densities that are much higher than those of *A. tsugae* (Preisser et al. 2008). It appears that *A. tsugae* interacts with its host in a complex physiological way that extends beyond nutrient extraction.

Much has been learned in recent years about the physiological basis of this interaction. Radville et al. (2011) showed that *A. tsugae* induces a hypersensitive response in hemlock that causes false ring production and tissue necrosis on infested twigs. Gomez et al. (2012) showed that *A. tsugae* induces increases in the amino acid concentrations of its host. Various studies have found that *A. tsugae* has a much more detrimental impact on *T. canadensis* than does *F. externa*. Radville et al (2011) found a positive correlation between *A. tsugae* densities and hydrogen peroxide levels in *T. canadensis* needles, which indicates a hypersensitive response. They also found that unlike with *F. externa*, which only induced a localized hypersensitive response, *A. tsugae* induced increased levels of hydrogen peroxide in new growth as well. Gonda-King et al (2012) found that false ring formation, an indicator of decreased tree health, was 30% greater in trees infested with *A. tsugae* than with *F. externa*.

Adelges tsugae exists on its native hemlock species in the Far East and in the Pacific Northwest without apparent ill effect, although densities in these regions are much lower due either to tree resistance or natural enemy impacts (Havill and Footit 2007). It is thus conceivable that the detrimental effect of *A. tsugae* on eastern hemlock is largely a product of the newness of

its association with this species and a process that is amenable to rapid selection given the two generations per year and the vast numbers of individual *A. tsugae* that are present in the eastern United States.

These considerations do not explain why adelgid virulence should be higher in the North than in the South. Populations of *A. tsugae* in both regions originated from an introduction to Richmond, Virginia in 1951 (Gouger 1971) and have persisted on eastern hemlock for an equal number of generations. *Adelges tsugae* spread more rapidly to the North than it did to the South, perhaps carried by migrating birds that fly north during the period when adelgid crawlers are active. For this reason, all of our southern sampling sites, except the one in Virginia, had been invaded by *A. tsugae* much more recently than those in the North. Perhaps there is some unexplained correlation between virulence and propensity to disperse, so that higher virulence occurs on the invasion front. Further studies will be needed to investigate this idea and to verify the possibility that populations of *A. tsugae* vary in virulence.

Higher virulence of southern *A. tsugae* populations could explain why there was a correlation between *A. tsugae* densities and new growth in the South but not the North (Table 3), but in fact these correlations were very weak and only marginally significant. The opposite was true for *F. externa*, which was correlated with new growth in the North but not in the South. The regional disparity in this finding makes sense, since *F. externa* was either nonexistent or present in very low densities in our southern sites.

Preisser and Elkinton (2008) found decreased foliage growth on trees that were infested with *A. tsugae* alone versus those with *F. externa* present. As we found significantly higher *F. externa* densities in the North than the South this could possibly be the case in our study. However, in our common garden experiment we found significantly higher new growth in branches infested with northern-derived populations, but saw no regional difference in *F. externa* densities.

In contrast to Preisser et al (2008), but as with Paradis (2011), we found no relationship between *A. tsugae* and *F. externa* densities in either year of regional studies or in the common garden experiment. These findings are surprising given the conclusion in Preisser and Elkinton (2008) that the presence of *F. externa* helps protect the tree from *A. tsugae*. However, it is important to note that multiple studies have suggested that it makes a significant difference in what order the two invaders colonize the tree (Gomez et al 2012, Miller-Pierce & Preisser 2012). In our common garden experiment both species had invaded the stand some years before we did our study but the trees we chose had little or no infestation of *A. tsugae*.

In both 2009 and 2010 we observed higher sistens fecundity in the South than in the North. These findings raised the possibility that an evolutionary tradeoff had occurred, where the increased cold tolerances of the northern *A. tsugae* populations may come at the expense of lower fecundity, as suggested by Butin et al. (2005). These are the observations that caused us to initiate our common garden experiment. However, in 2010, we did not observe any difference in fecundity between *A. tsugae* from northern and southern populations reared in the common garden setting, and fecundities were similar to those of the northern samples from our regional studies. This indicates that the differences we observed between northern and southern fecundity were most likely attributable to environmental variables, and are an example of phenotypic plasticity rather than genetic divergence. For our northern sites, our average fecundity measurements were lower than those in other studies in this region, which often exceeded 100 eggs per adult (McClure 1991, Paradis 2011). Paradis (2011) observed that sistens fecundity declined with lower winter temperatures, so this is the likely explanation of the lower fecundity we observed in the North in our study, and why the difference disappeared in the common garden experiment.

McClure (1991) reported pronounced density-dependent reductions in fecundity in forest stands newly infested with *A. tsugae*. In contrast we found no relationship between sistens fecundity and density in the North or South in either 2009 or 2010, or in common garden samples derived from either region. Paradis (2011) found a density-dependent decline in fecundity of the progrediens stage, but she, like us, found no such effects on sistens fecundity. The density-dependence in sistens fecundity observed by McClure (1991) occurred during the declining phase of the *A. tsugae* density cycle, during which *A. tsugae* at higher densities are forced to feed on older, rather than new growth. We chose populations of *A. tsugae* at southern sites that had only recently been infested, so that we could document the entire course of the expected rise and fall of *A. tsugae* populations. Thus, we may have quantified fecundity during the increasing phase of the *A. tsugae* populations at these sites before the expected decline in density and the associated reduction in fecundity. We also looked only at *A. tsugae* sistens that were on the last year's growth, while McClure (1991) looked at several previous years' growth as well.

We were surprised to find strong density-dependent mortality in the overwintering populations in the North in 2009, since in many species this is a density-independent population dynamics factor, and is simply related to temperature or winter severity. Density-dependent mortality could be caused by interactions between cold temperatures and density-related factors such as population quality or physiological stress. If this finding were a general phenomenon in *A. tsugae*, it would help explain the evident stability of *A. tsugae* in northern populations (Paradis 2011). Other investigators have reported inconsistent relationships between *A. tsugae* density and overwintering mortality as well (Paradis 2011, Trotter and Shields 2009). Previous studies in Connecticut (McClure 1991) and Massachusetts (Paradis 2011) demonstrated density-dependent mortality and fecundity effects operate in the summertime progrediens generation. These effects alone can account for the observed stability in *A. tsugae* populations (Elkinton et al 2012).

The average level of overwintering mortality we observed in the North in 2009 (87%) was much higher than that observed by Paradis et al (2008) at some of the same sites from 2003

to 2008. In previous studies, at other similar northern sites, overwintering mortality was 20% in 1998 (Skinner et al. 1998). In other sites in New England and New York, mean mortality was 86% in 2003 and 93.6% in 2004 (Shields and Cheah 2005). Mortality at Pennsylvania sites was 73.8% in 2003, and 78.4% in 2004 (Shields and Cheah 2005). At sites in North Carolina, mortality was 11.2% in 2003, and 21.1% in 2004 (Shields and Cheah 2005).

In our common garden experiment we found no significant difference in overwintering mortalities of northern-derived and southern-derived samples. This contrasts with findings from a common garden study by Butin et al (2005). However, in that study, *A. tsugae* samples were collected from Maryland to represent the southern region, while we used samples collected much farther south, in Virginia, Georgia, South Carolina, and Tennessee, but at much higher elevations. As our source sites ranged in elevation, it is possible that the environmental temperatures of our southern sites reached much colder lows than those in Butin et al (2005), who at her site in Maryland reported a monthly low temperature in January of -14.8° C. Trotter and Shields (2009) reported increased survival with decreased elevation. Another explanation for the lack of difference in overwinter mortality in the common garden may be that the winter temperatures never reached a low enough temperature for differences to be noticeable in either region.

Tables

Table 1. ANOVA of the effect of region on proportion of new growth and mean length of new growth. Means are real values, statistics are based on $\text{Log}_{10}(x+1)$ transformed dependent variable). All degrees of freedom=1. Standard errors are in parentheses.

Dependent variable	Study	Yr	North			South			F	p
			n	mean	(SE)	n	mean	(SE)		
Proportion new growth	Regional	2009	99	0.205	(0.032)	240	0.060	(0.022)	17.901	<.001
		2010	67	0.589	(0.031)	176	0.128	(0.019)	161.913	<.001
		Both yrs	99	0.465	(0.025)	240	0.110	(0.016)	142.766	<.001
	Common Garden	2010	31	0.107	(0.017)	33	0.012	(0.017)	16.685	<.001
cm new growth	Regional	2009	32	1.198	(0.265)	64	0.009	(0.187)	25.195	<.001
		2010	58	4.279	(0.328)	173	0.137	(0.190)	231.504	<.001
		Both yrs	90	3.1835	(0.251)	237	0.103	(0.154)	195.740	<.001
	Common Garden	2010	32	0.383	(0.110)	45	0.011	(0.093)	10.725	0.002

Table 2. ANOVA of the effect of region on sistens fecundity and late spring *A. tsugae* and *F. externa* densities on branch samples collected in the late spring. All degrees of freedom=1. Standard errors are in parentheses.

Dependent Variable	Study	Yr	North			South			ANOVA	
			n	Mean	(SE)	n	Mean	(SE)	F	p
<i>A. tsugae</i> density (sistens per cm)	Regional	2009	34	1.036	(0.252)	66	1.460	(0.181)	1.867	0.175
		2010	58	0.792	(0.057)	173	0.642	(0.033)	5.214	0.023
		Both yrs	92	0.882	(0.098)	239	0.868	(0.060)	0.015	0.902
	Common Garden	2010	32	0.548	(0.057)	43	0.724	(0.049)	5.501	0.022
Proportion branchlets with <i>A. tsugae</i>	Regional	2009	33	0.404	(0.044)	64	0.547	(0.032)	6.953	0.010
		2010	63	0.229	(0.026)	165	0.256	(0.016)	0.818	0.367
		Both yrs	96	0.289	(0.026)	229	0.337	(0.017)	2.553	0.111
	Common Garden	2010	31	0.043	(0.013)	33	0.074	(0.013)	2.859	0.096
<i>A. tsugae</i> fecundity (no. chorion per sistens)	Regional	2009	33	58.859	(10.378)	70	100.281	(7.125)	10.827	0.001
		2010	50	47.605	(8.106)	112	116.149	(5.416)	49.437	<0.001
		Both yrs	82	52.258	(6.464)	182	110.046	(4.339)	55.095	<0.001
	Common Garden	2010	31	61.779	(6.227)	46	54.991	(5.112)	0.710	0.402
<i>F. externa</i> density (no. per 10 needles)	Regional	2009	30	1.925	(0.315)	63	0.015	(0.217)	24.913	<0.001
		2010	58	0.865	(0.176)	173	0.008	(0.102)	17.687	<0.001
		Both yrs	88	1.226	(0.158)	236	0.009	(0.096)	43.260	<0.001
	Common Garden	2010	32	0.361	(0.141)	45	0.702	(0.119)	3.393	0.069

Table 3a. Multiple regression of the effect of *F. externa* and *A. tsugae* densities on new growth in the regional north and south. Statistics are based on Log₁₀ (x+1) transformed dependent variable. All degrees of freedom = 1. Standard errors are in parentheses.

Yr	Region	Dependent variable	n	R ²	Independent variable							
					<i>A. tsugae</i> density (sistens per cm)				<i>F. externa</i> density (no. per 10 needles)			
					Slope	SE	t value	Pr> t	Slope	SE	t value	Pr> t
2009	North	Proportion new growth	26	0.137	-0.006	(0.011)	-0.059	0.558	0.007	(0.004)	1.820	0.082
		cm new growth	28	0.039	-0.057	(0.057)	-1.000	0.325	0.000	(0.020)	-0.010	0.995
	South	Proportion new growth	55	0.415	0.018	(0.003)	6.040	<.001	-0.060	(0.054)	-1.120	0.266
		cm new growth	56	0.016	-0.001	(0.002)	-0.900	0.374	-0.005	(0.030)	-0.180	0.856
2010	North	Proportion new growth	58	0.157	-0.002	(0.021)	-0.110	0.915	-0.014	(0.004)	-3.140	0.003
		cm new growth	58	0.120	0.023	(0.091)	0.250	0.804	-0.052	(0.019)	-2.740	0.008
	South	Proportion new growth	173	0.023	-0.026	(0.015)	-1.720	0.087	-0.111	(0.099)	-1.130	0.262
		cm new growth	173	0.022	-0.037	(0.020)	-1.830	0.069	-0.104	(0.134)	-0.780	-0.437
Both yrs	North	Proportion new growth	84	0.081	-0.017	(0.014)	-1.210	0.229	-0.009	(0.004)	-2.250	0.028
		cm new growth	86	0.098	-0.065	(0.054)	-1.200	0.235	-0.039	(0.015)	-2.650	0.010
	South	Proportion new growth	228	0.014	0.006	(0.005)	1.330	0.186	-0.082	(0.068)	-1.210	0.227
		cm new growth	229	0.018	-0.011	(0.006)	-1.870	0.062	-0.054	(0.086)	-0.630	0.530

Table 3b. Multiple regression of the effect of *F. externa* and *A. tsugae* densities on new growth in a common garden. Statistics are based on $\text{Log}_{10}(x+1)$ transformed dependent variable. All degrees of freedom = 1. Standard errors are in parentheses.

Source Region	Dependent variable	n	R ²	Independent variable							
				<i>A. tsugae</i> density (sistens per cm)				<i>F. externa</i> density (no. per 10 needles)			
				Slope	(SE)	t Value	Pr> t	Slope	(SE)	t Value	Pr> t
North	Proportion new growth	31	0.123	-0.064	(0.032)	-1.980	0.058	-0.004	(0.018)	-0.240	0.809
	cm new growth	32	0.081	-0.184	(0.117)	-1.580	0.126	-0.002	(0.055)	-0.030	0.977
South	Proportion new growth	30	0.024	-0.003	(0.004)	-0.770	0.450	0.000	(0.002)	0.180	0.859
	cm new growth	43	0.008	0.002	(0.005)	0.400	0.693	0.001	(0.002)	0.420	0.677
Combined regions	Proportion new growth	61	0.127	-0.041	(0.016)	-2.610	0.012	-0.010	(0.007)	-1.340	0.186
	cm new growth	75	0.058	-0.087	(0.043)	-2.060	0.044	-0.010	(0.018)	-0.540	0.590

Table 4. ANOVA of the effect of region on *A. tsugae* overwintering mortality and *A. tsugae* and *F. externa* densities on branch samples collected in the early spring. All degrees of freedom=1. Standard errors are in parentheses.

Dependent Variable	Study	Yr	North			South			F	p
			n	Mean	(SE)	n	Mean	(SE)		
<i>A. tsugae</i> overwintering mortality (proportion dead)	Regional	2009	51	0.869	(0.027)	35	0.368	(0.032)	140.690	<0.001
		2010	119	0.543	(0.026)	162	0.342	(0.022)	35.737	<0.001
	Common Garden	2010	9	0.794	(0.060)	20	0.864	(0.040)	0.944	0.340
<i>A. tsugae</i> density (sistens per cm)	Regional	2009	51	3.492	(0.392)	35	3.075	(0.473)	0.460	0.499
		2010	120	1.429	(0.141)	160	1.192	(0.121)	1.621	0.204
	Common Garden	2010	9	1.432	(0.229)	20	1.188	(0.154)	0.780	0.385
Proportion branchlets with <i>A. tsugae</i>	Regional	2009	45	0.281	(0.033)	34	0.606	(0.038)	41.384	<0.001
		2010	121	0.300	(0.020)	159	0.280	(0.018)	0.568	0.452
	Common Garden	2010	9	0.107	(0.024)	19	0.117	(0.017)	0.112	0.740
<i>F. externa</i> density (no. per 10 needles)	Regional	2009	45	10.129	(1.259)	19	0.000	(1.937)	19.227	<0.001
		2010	120	4.411	(0.441)	160	0.000	(0.382)	57.121	<0.001
	Common Garden	2010	9	0.491	(0.330)	20	1.120	(0.222)	2.499	0.126

Note: In 2009, the average minimum temperature for NOAA weather stations near our sites was -14.4°C in the south and -20°C in the north and in 2010 was -14.2°C in the south and -17.8°C in the north.

Table 5. Logistic analysis of the effect of *A. tsugae* density on *A. tsugae* overwintering mortality. All degrees of freedom=1. Standard errors are in parentheses. PROC Logistic SAS 9.13; logit p= a*log₁₀(x+1) +b.

Independent variable	Dependent variable	Study	Yr	Region	n	F	p	Slope (SE)		Intercept (SE)	
<i>A. tsugae</i> density (sistens per cm)	Overwintering mortality (proportion dead mature sistens)	Regional	2009	North	51	306.259	<.001	3.027	(0.173)	0.985	(0.082)
				South	34	0.998	0.318	0.117	(0.117)	-0.654	(0.068)
		Common Garden	2010	North	119	63.131	<.001	-0.627	(0.079)	0.0175	(0.035)
				South	162	54.582	<.001	0.562	(0.076)	-0.853	(0.039)
			2010	North	9	47.407	<.001	-9.745	(1.415)	5.468	(0.712)
				South	20	1.151	0.283	1.290	(1.150)	1.251	(0.446)

Table 6. Linear regression analysis of the effect of *A. tsugae* density on *A. tsugae* sistens fecundity. All degrees of freedom=1. Standard errors are in parentheses.

Independent variable	Dependent variable	Study	Yr	Region	n	F	p	R ²	Slope (SE)		Intercept (SE)	
<i>A. tsugae</i> density (sistens per cm)	Sistens fecundity (no chorion per sistens)	Regional	2009	Combined	97	1.989	0.162	0.021	5.054	(3.584)	76.408	(7.173)
				North	31	0.116	0.736	0.004	2.827	(8.314)	54.586	(13.078)
				South	66	1.131	0.292	0.017	3.992	(3.753)	89.363	(8.158)
			2010	Combined	162	0.071	0.791	0.000	-3.167	(11.914)	97.026	(9.217)
				North	50	0.348	0.558	0.007	5.468	(9.270)	43.218	(9.259)
				South	112	2.985	0.087	0.026	33.380	(19.321)	97.126	(12.528)
		Both yrs	North	81	0.623	0.432	0.008	4.542	(5.753)	47.303	(7.192)	
			South	178	0.092	0.762	0.001	1.238	(4.073)	107.265	(5.784)	
		Common Garden	2010	Combined	72	0.543	0.464	0.008	9.246	(12.549)	52.827	(9.263)
				North	31	0.062	0.806	0.002	6.179	(24.879)	58.374	(15.313)
				South	41	1.021	0.319	0.026	15.333	(15.173)	45.395	(12.423)
				2009	Combined	89	0.126	0.724	0.001	-9.699	(27.369)	95.800
North	26				5.103	0.033	0.175	73.216	(32.412)	28.831	(17.162)	
South	63				9.893	0.003	0.140	-110.89	(35.257)	164.184	(20.787)	
Regional	2010	Combined	155	0.421	0.517	0.003	16.845	(25.962)	92.803	(7.967)		
		North	47	0.244	0.624	0.005	13.513	(27.363)	45.388	(8.740)		
		South	108	0.668	0.416	0.006	25.235	(30.867)	111.890	(9.298)		
	Both yrs	North	73	6.012	0.017	0.078	47.309	(19.295)	38.291	(7.850)		
		South	171	3.434	0.066	0.020	-35.068	(18.925)	124.419	(8.148)		
	Common Garden	2010	Combined	59	0.028	0.867	0.000	-9.718	(57.724)	57.024	(5.752)	
North			30	0.715	0.405	0.025	-138.00	(163.712)	67.932	(10.024)		
South			29	0.526	0.475	0.019	39.738	(54.794)	47.442	(7.001)		

Table 7. Linear regression of the effect of *F. externa* density on *A. tsugae* density. All degrees of freedom=1. Standard errors are in parentheses.

Independent variable	Dependent variable	Study	Yr	Season	Region	n	F	p	R ²	Slope (SE)	Intercept (SE)
<i>F. externa</i> density (no. per 10 needles)	<i>A. tsugae</i> density (sistens per cm)	Regional	2009	Early spring	North	45	1.974	0.167	0.044	-0.065 (0.047)	4.370 (0.660)
				Late spring	North	30	0.016	0.901	0.001	-0.008 (0.067)	1.042 (0.238)
			2010	Early spring	North	120	1.7713	0.186	0.015	-0.023 (0.017)	1.53 (0.148)
				Late spring	North	58	1.526	0.222	0.027	0.034 (0.028)	0.762 (0.078)
			Both yrs	Late spring	North	88	0.649	0.423	0.007	0.024 (0.029)	0.842 (0.091)
					Combined	28	1.115	0.300	0.040	-0.134 (0.127)	1.388 (0.173)
		Common Garden	2010	Early spring	North	9	0.180	0.684	0.025	0.166 (0.391)	1.350 (0.359)
					South	20	2.057	0.169	0.103	-0.182 (0.127)	1.392 (0.193)
				Combined	75	0.274	0.603	0.004	-0.025 (0.048)	0.663 (0.047)	
			Late spring	North	32	0.938	0.340	0.030	-0.082 (0.085)	0.578 (0.057)	
				South	43	0.439	0.511	0.011	-0.040 (0.060)	0.751 (0.068)	
				Combined	75	0.274	0.603	0.004	-0.025 (0.048)	0.663 (0.047)	
	Proportion of branchlets with <i>A.</i> <i>tsugae</i>	Regional	2009	Early spring	North	39	0.076	0.784	0.002	0.001 (0.003)	0.285 (0.050)
					North	26	0.021	0.886	0.001	-0.003 (0.019)	0.415 (0.070)
			2010	Early spring	North	83	0.000	0.986	0.000	0.000 (0.007)	0.316 (0.029)
					North	54	5.391	0.024	0.094	0.023 (0.010)	0.205 (0.029)
			Both yrs	Late spring	North	80	3.274	0.074	0.040	0.017 (0.010)	0.264 (0.031)
					Combined	28	1.184	0.287	0.044	-0.015 (0.014)	0.127 (0.018)
Common Garden		2010	Early spring	North	9	0.005	0.946	0.001	0.002 (0.026)	0.106 (0.024)	
				South	19	1.735	0.205	0.093	-0.024 (0.018)	0.142 (0.026)	
			Combined	61	0.003	0.955	0.000	0.001 (0.014)	0.061 (0.013)		
		Late spring	North	31	0.060	0.809	0.002	-0.004 (0.016)	0.044 (0.009)		
			South	30	0.266	0.610	0.009	-0.012 (0.023)	0.090 (0.025)		
			Combined	61	0.003	0.955	0.000	0.001 (0.014)	0.061 (0.013)		

Figures

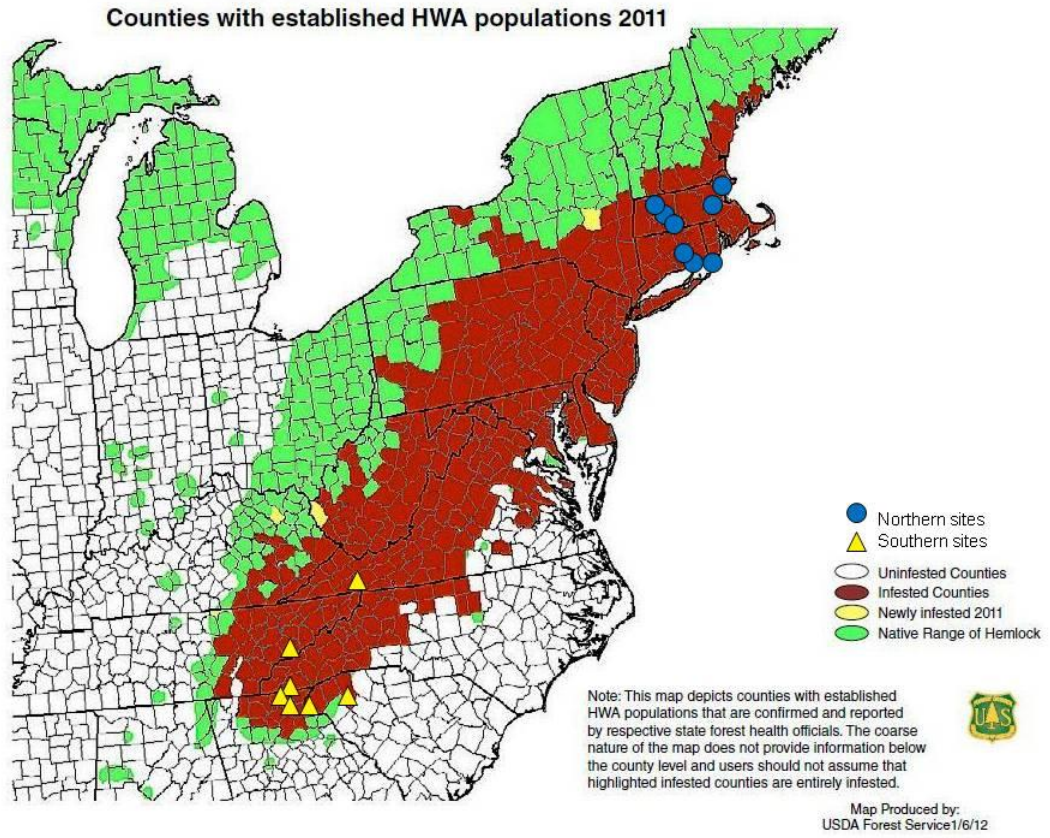


Fig. 1. Locations of *A. tsugae* sampling sites.

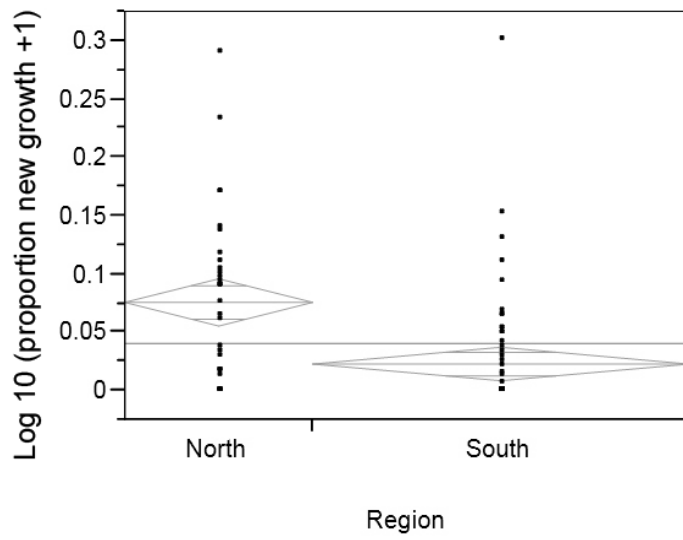


Fig. 2a. Boxplot of the effect of region on proportion of new growth in 2009 regional samples.

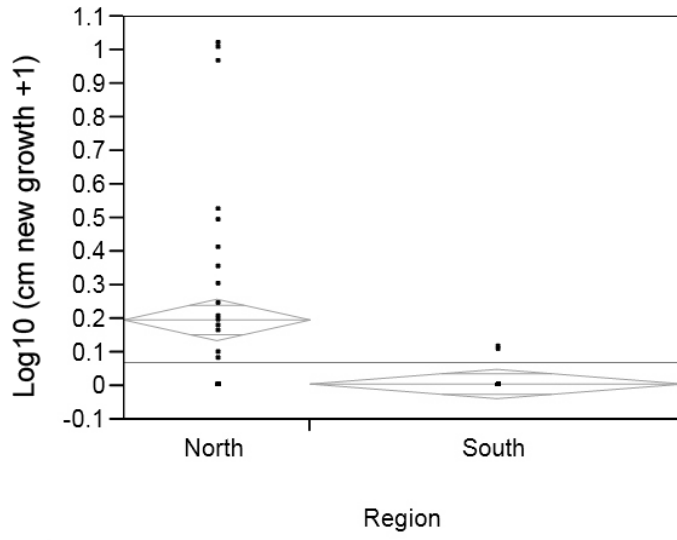


Fig. 2b. Boxplot of the effect of region on length of new growth in 2009 regional samples.

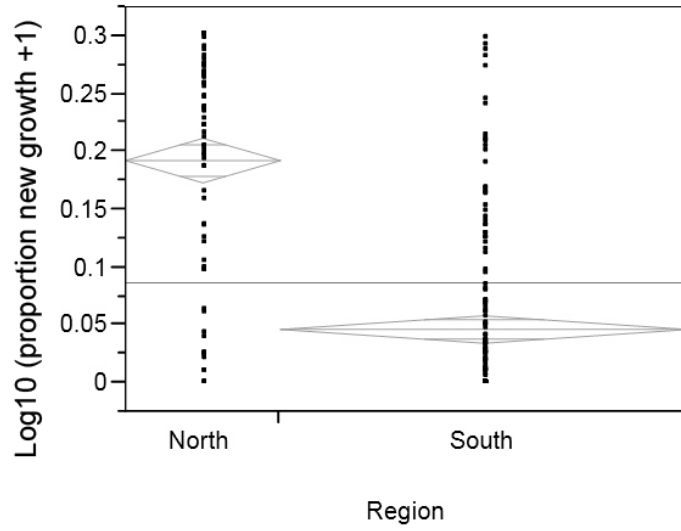


Fig. 3a. Boxplot of the effect of region on proportion of new growth in 2010 regional samples.

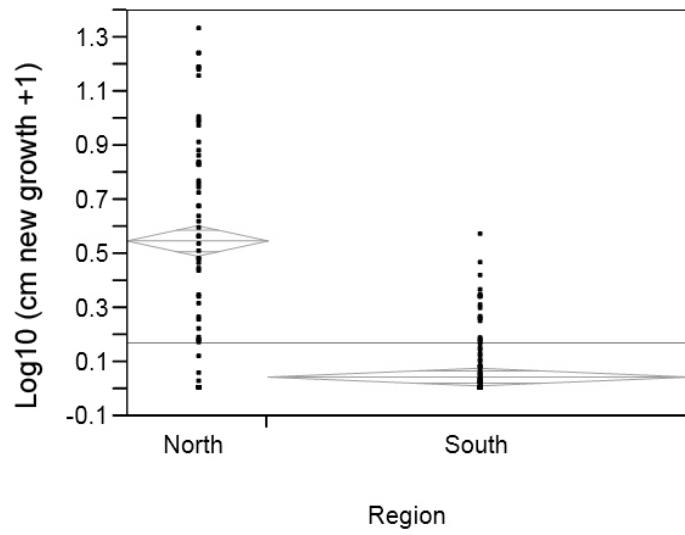


Fig. 3b. Boxplot of the effect of region on length of new growth in 2010 regional samples.

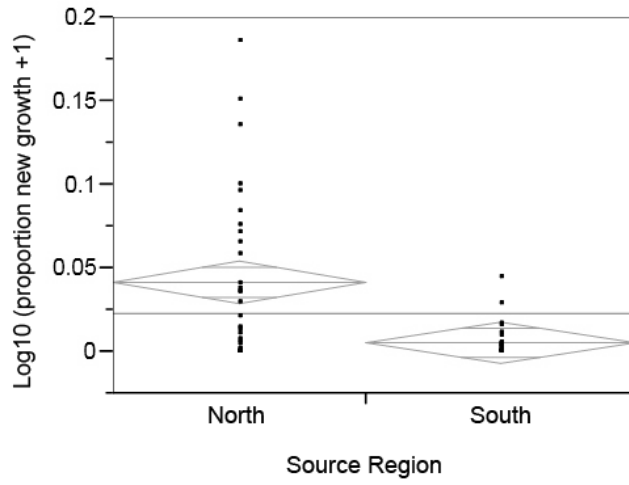


Fig. 4a. Boxplot of the effect of region on proportion of new growth in common garden samples. Most samples from the south had no new growth.

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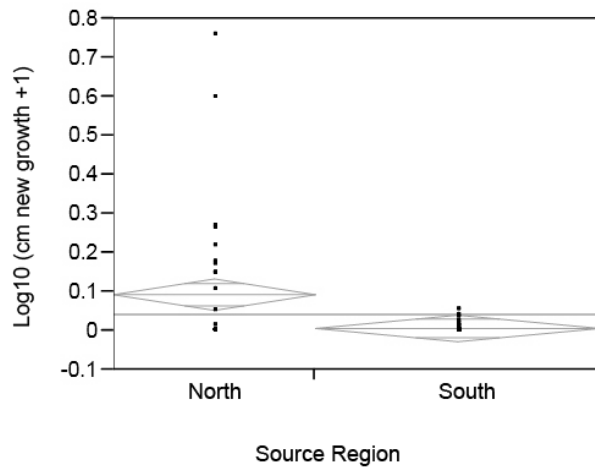


Fig. 4b. Boxplot of the effect of region on length of new growth in a common garden samples. Most samples from the south had no new growth.

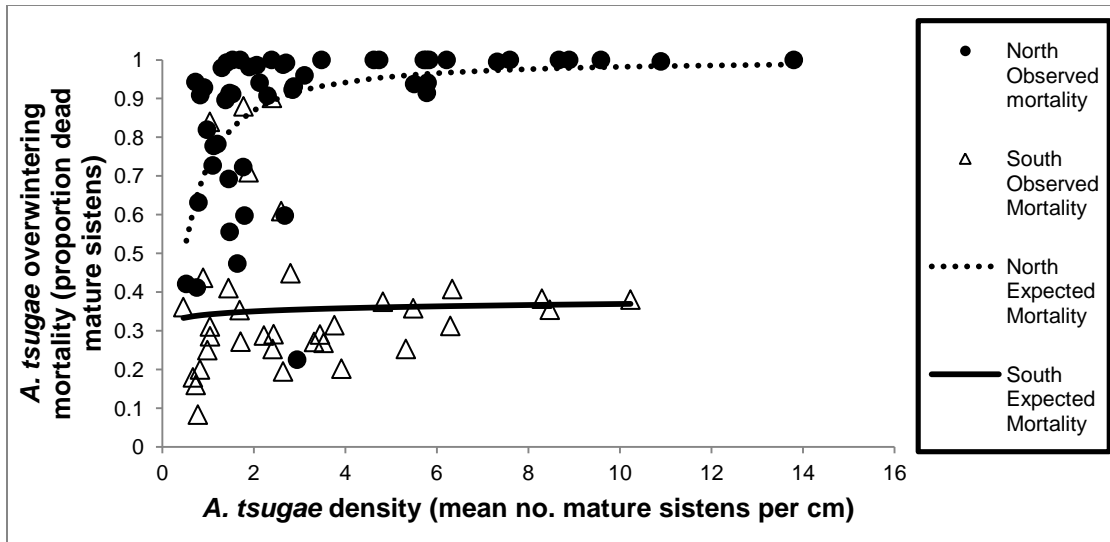


Fig. 5a. The effect of *A. tsugae* density on *A. tsugae* overwintering mortality in 2009 regional samples. Based on a logistic model, there was a strong relationship between density and overwintering mortality in the north (PROC Logistic SAS 9.13; logit $p = 3.0266 \cdot \log_{10}(x+1) + 0.9853$, $df=1$, Wald $\chi^2 = 306.2594$, $P < 0.001$), while no density dependent trend was evident in the south (PROC Logistic SAS 9.13; logit $p = 0.1168 \cdot \log_{10}(x+1) - 0.6536$, $df=1$, Wald $\chi^2 = 0.9983$, $P = 0.3177$).

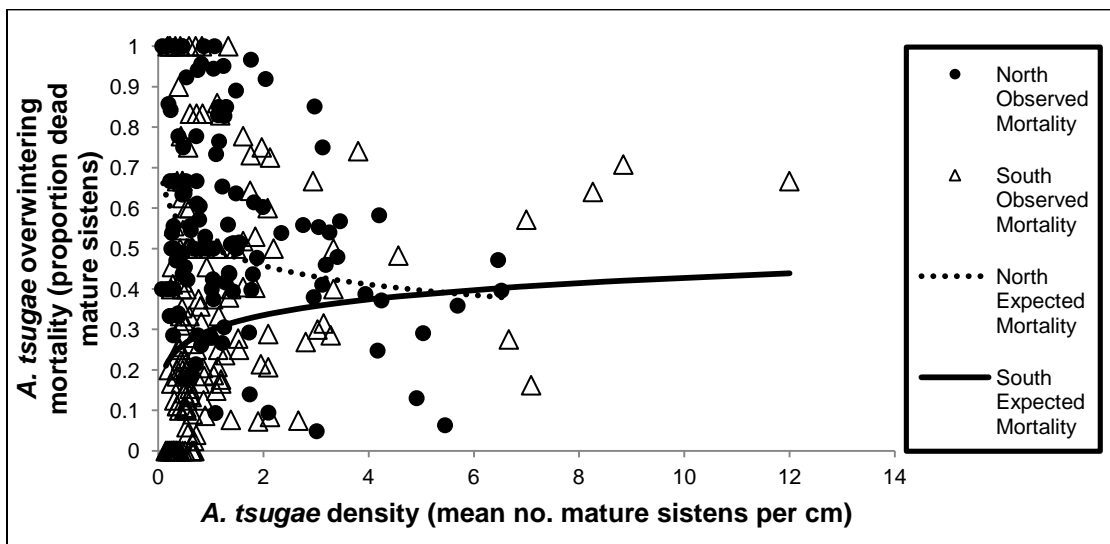


Fig. 5b. The effect of *A. tsugae* density on *A. tsugae* overwintering mortality in 2010 regional samples. Based on a logistic model, overwintering mortality was strongly density dependent in both the north (PROC Logistic SAS 9.13; logit $p = 0.0175 \cdot \log_{10}(x+1) - 0.6273$, $df=1$, Wald $\chi^2 = 63.1305$, $p < 0.001$) and the south (PROC Logistic SAS 9.13; logit $p = 0.5619 \cdot \log_{10}(x+1) - 0.8533$, $df=1$, Wald $\chi^2 = 54.5823$, $p < 0.0001$).

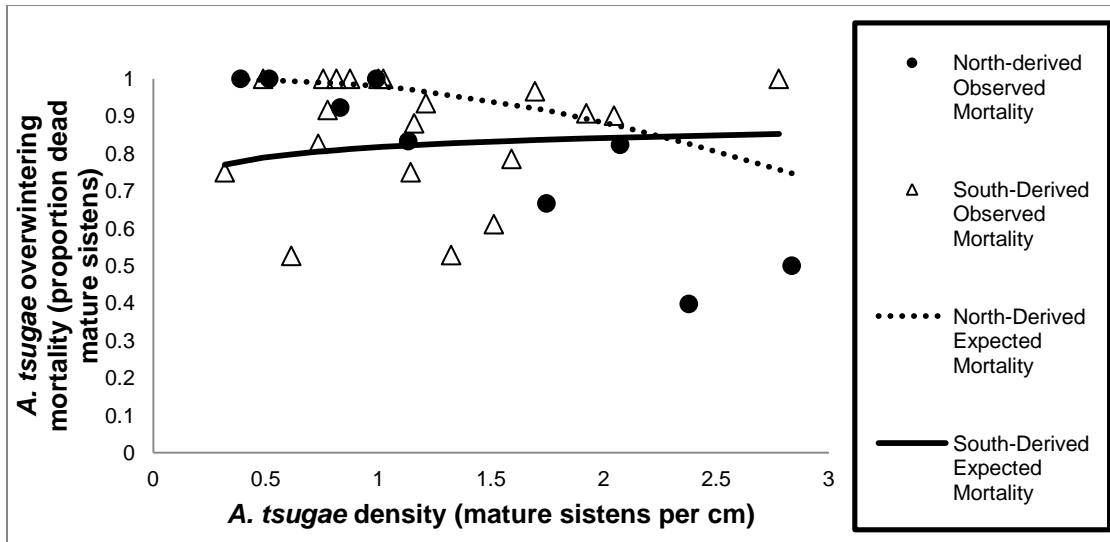


Fig. 5c. The effect of *A. tsugae* density on *A. tsugae* overwintering mortality in common garden samples. Based on a logistic model, overwintering mortality was strongly density dependent in the north (PROC Logistic SAS 9.13; logit $p = -6.201 \cdot \log_{10}(x+1) + 3.888$, $df=1$, Wald $\chi^2 = 47.587$, $p < 0.001$) but not the south (PROC Logistic SAS 9.13; logit $p = 0.574 \cdot \log_{10}(x+1) + 1.496$, $df=1$, Wald $\chi^2 = 0.249$, $p = 0.618$).

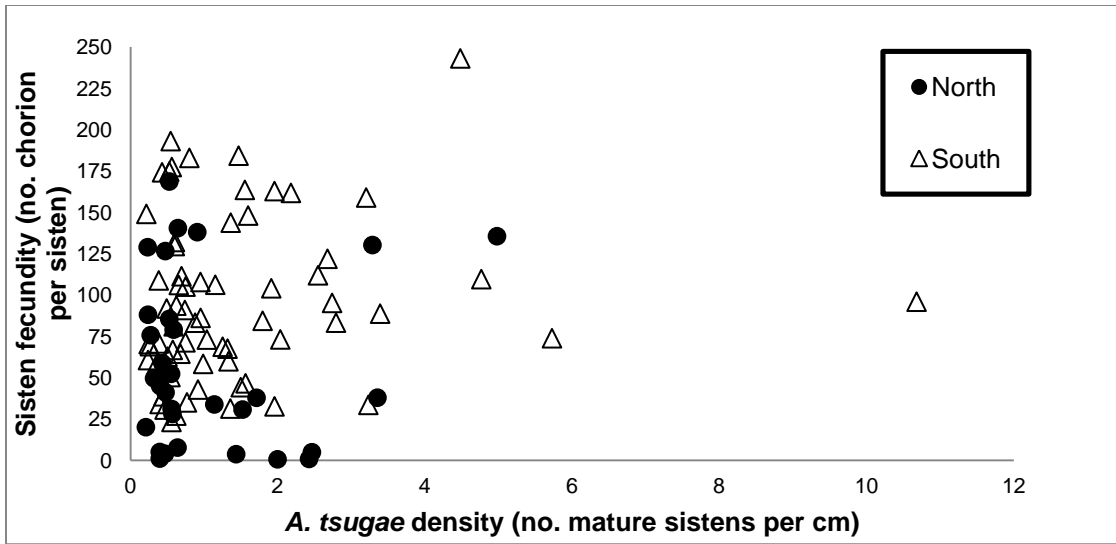


Fig. 6a. The effect of *A. tsugae* density on *A. tsugae* sistens fecundity in 2009 regional samples.

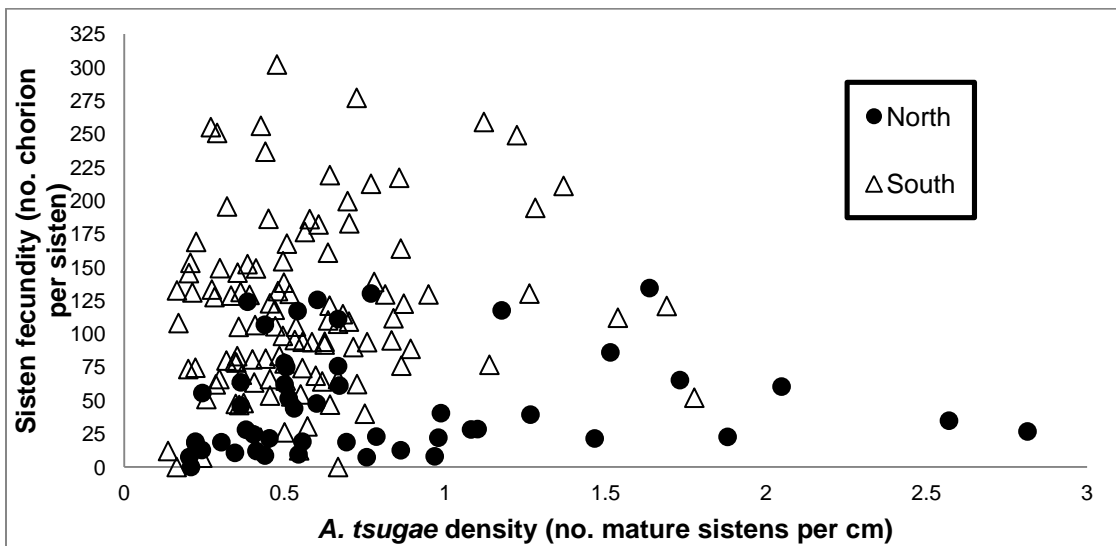


Fig. 6b. The effect of *A. tsugae* density on *A. tsugae* sistens fecundity in 2010 regional samples.

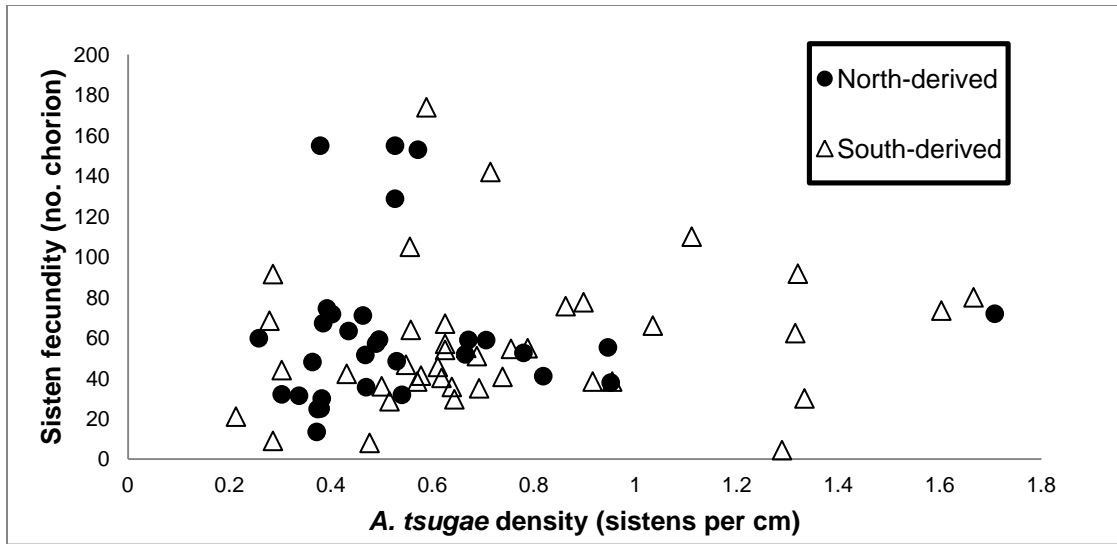


Fig. 6c. The effect of *A. tsugae* density on *A. tsugae* sistens fecundity in common garden samples.

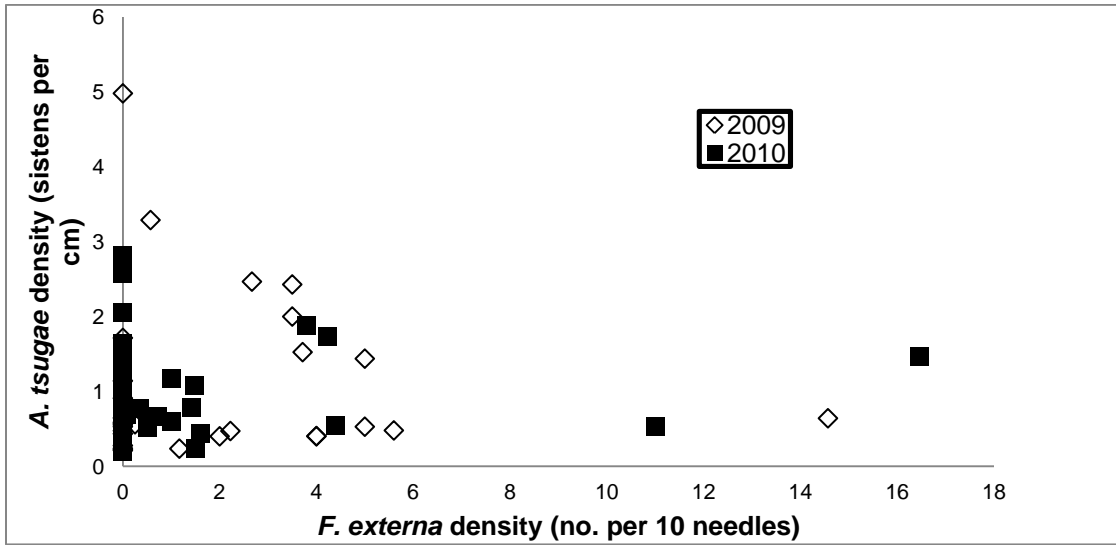


Fig. 7a. The effect of *F. externa* density on *A. tsugae* density in late spring 2009 and 2010 northern regional samples.

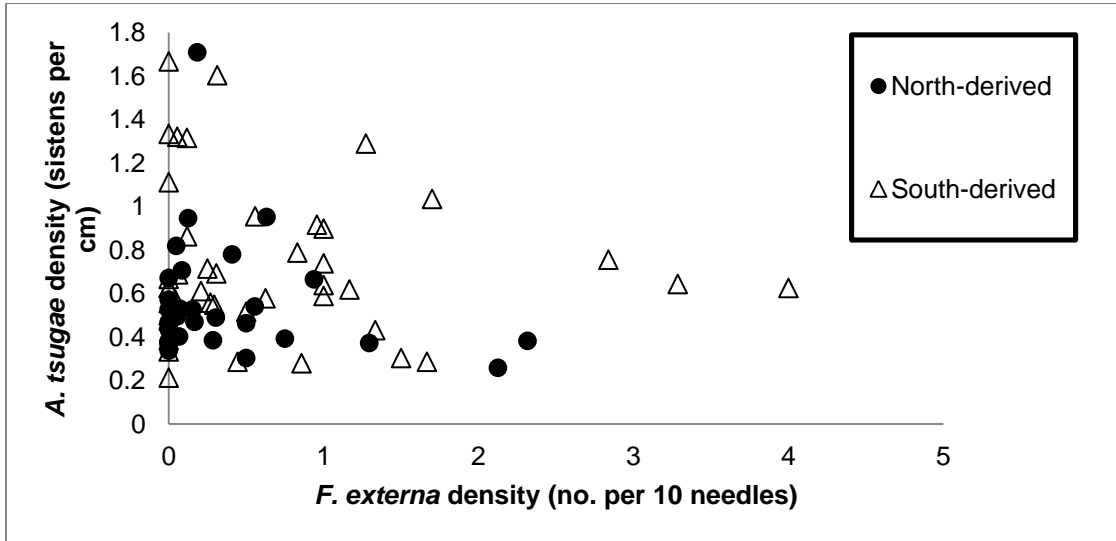


Fig. 7b. The effect of *F. externa* density on *A. tsugae* density in late spring common garden samples.

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