MANAGEMENT STRATEGIES TO REDUCE HEMLOCK WOOLLY ADELGID (HEMIPTERA: ADELGIDAE) DAMAGE

by

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(Under the direction of S. KRISTINE BRAMAN and JAMES L. HANULA)

ABSTRACT

The goals of this work were to improve control of hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae). The first project focused on manipulating tree health and adelgid populations using low rates of imidacloprid insecticide and fertilizer to provide an adequate, uninterrupted supply of high quality adelgids to support long-term predator population growth. After two years, more ovisacs and eggs were found on trees that received imidacloprid in the order 0% > 10% > 25%. Fertilized trees had greater adelgid fecundity which was positively correlated with total foliar N in both winter generations. Trees treated with low rates of imidacloprid were healthier than untreated trees, but only trees treated with 10% of the recommended rate of imidacloprid and fertilizer had sufficient adelgids to possibly sustain predators over extended periods of time. The second project looked at how *A. tsugae* is distributed within hemlock trees and how their distribution changes over time. We found that adelgid density tended to be high in the upper tree crown after multiple generation sampling. The third study evaluated host tree resistance to hemlock woolly adelgid among various hemlock species, whether or not fertilizers affected resistance, and to determine whether fertilizer and host tree species affected prey quality. Adelgids were denser on *Tsuga mertensiana* (Bong.) Carrière than on other hemlock species (*T. canadensis*, *T. heterophylla* (Raf.) Sarg. or *T. chinensis*

(Franch.) E. Pritz). Application of fertilizer did not increase adelgid density on reportedly resistant *T. heterophylla*; density was higher on unfertilized plants. The fourth project focused on evaluating eight insecticides for controlling hemlock woolly adelgid on eastern hemlock. Although dinotefuran-treated trees demonstrated an average range of 63-100% adelgid suppression, residual activity diminished 26 months after spring application as adelgids re-colonized treated trees. Clothianidin and imidacloprid were slower acting and provided more long-term adelgid suppression. The fifth study looked at how odors of *T. canadensis* branches affect the behavior of the adelgid predators, *Laricobius nigrinus* Fender (Derodontidae) and *Sasajiscymnus tsugae* (Sasaji & McClure) (Coccinellidae). Neither *S. tsugae* nor *L. nigrinus* demonstrated attraction to *T. canadensis* branches in Y-tube olfactometer studies.

INDEX WORDS: hemlock woolly adelgid, *Adelges tsugae*, eastern hemlock, *Tsuga canadensis*, imidacloprid, tree health, western hemlock, *Tsuga heterophylla*, mountain hemlock, *Tsuga mertensiana*, Chinese hemlock, *Tsuga chinensis*, dinotefuran, clothianidin, Acetamiprid, Y-tube olfactometer, *Laricobius nigrinus*, *Sasajiscymnus tsugae*

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Hemlock woolly adelgid (HWA) *Adelges tsugae* Annand (Hemiptera: Adelgidae) is an invasive pest causing widespread mortality of eastern hemlock, *Tsuga canadensis* (L.) Carrière, and Carolina hemlock, *Tsuga caroliniana* Engelmann in (McClure 1987a). It was first reported in eastern North America in Richmond, VA in 1951 (Stoetzel 2002) and by the 1960s, HWA had spread to Pennsylvania (McClure 1987a). In 1986, its range, which extended to Connecticut along the southwest coastline, elevated in inlands, and banks of Housatonic and Connecticut River suggests that perhaps hurricane Gloria in September 1985 played an important part in its spread (McClure 1987a). By 2004, *A. tsugae* was documented in Georgia (Johnson 2005), and it is currently found in more than 11 northern counties in the state spreading at 15 km/yr (Evans and Gregoire 2007). Now, HWA occurs in 17 states from North Georgia and Alabama to New England (Cheah and McClure 2000, Havill et al. 2006, Lamb et al. 2006, McClure 1991b, McClure 2001). Carolina hemlock is endemic to the southern Appalachian Mountains within the boundaries of Virginia, Tennessee, Georgia and North and South Carolina (Jetton et al. 2008a). In the northern states, heavy infestations of *A. tsugae* will result in the death of a hemlock tree within four or more years (McClure 1991a), or 2 to 3 years in the southern states (Trotter and Shields 2009). Other stress factors such as insect attack (elongate hemlock scale, hemlock borer and spittlebugs), fungal infection (*Fabrella* or *Korfia tsugae*) or prolonged drought could accelerate HWA infestation and lead to early tree mortality (Cheah and McClure 2000). Meanwhile, HWA is not a pest on western, *Tsuga heterophylla* (Raf.), or mountain hemlock, *Tsuga mertensiana* (Bong.) Carrière, although the insect occurs throughout much of the range of these two tree species (McClure 1991b, McClure 2001, Zilahi-Balogh 2003b). HWA were deliberately introduced into seed orchards of western hemlock to obtain quality seeds since they exert additional stress on hemlock (Zilahi-Balogh 2003b). However, unlike in eastern North America, the HWA observed in western North America are genetically distinct from HWA in others parts of the world (Havill and Montgomery 2008).

Hemlock is a gymnosperm that thrives in different forests of North America. These evergreen trees play a critical role within forest ecosystems supporting diverse flora and fauna; moreover, humans value them highly for their beauty. They are slow growing, shade tolerant trees with dense canopies that allow very little light to the forest floor. As an ornamental, hemlock trees are widely planted in a variety of landscapes (Cheah and McClure 2000). They are invaluable tree species in public areas such as parks, picnic areas, and trails on the east coast of North America and, therefore, have gained more attention in these sensitive sites in addition to forests.

Biology of Hemlock Woolly Adelgid

Hemlock woolly adelgid has two wingless generations per year, the sisten (summer) and the progredien (winter) (Annand 1928, McClure 1987a), and a winged generation (McClure 1987a, McClure 1989a). Wingless sistens occur from July to April while progrediens are found between April and June (McClure 1989a). Adelgids have six life stages in all three generations including the egg, four immature instars and the adult stages. The adult HWA are parthenogenetic, i.e. they reproduce without mating and all their progeny are females (McClure 1987b, McClure 1995). Adult *A. tsugae* are small, brownish-red in color and oval shaped aphid-like insects (Freeman 1987). Externally, *A. tsugae* ovisacs have a woolly or white mass covering their entire body and its eggs (Freeman 1987, McClure 1987b). An overwintering sisten adult oviposits approximately 50 brownish-orange eggs (McClure 1989a) in a cluster that darken when they mature (Freeman 1987). The first-instar nymphs are called ‘crawlers’ which are naked, flat and reddish-brown insects that are the only stage capable of actively moving around. Second-instar nymphs are black, immobile and produce white fringes around their edges when they settle down on hemlock branches at the needle cushion near the needle base (Freeman 1987). Nymphs then consume nutrients from xylem parenchyma cells (Shields et al. 1996). Mammals, wind or birds can play an active role in HWA dispersal with crawlers the most readily dispersed stage (McClure 1990, McClure 1991b).

Hemlock woolly adelgids undergo a polymorphic life cycle on hemlock (*Tsuga* spp.) and spruce (*Picea* spp.) (McClure 1989a). Annand (1928) reported that spruce is the primary host of *A. tsugae* and other species such as *Abies* spp., *Larix* spp., *Pinus* spp., *Pseudotsuga* spp., and *Tsuga* spp. are intermediate or secondary host species. The winged progredien females, otherwise called sexuparae, fly off in search of a suitable secondary host (spruce) and sometimes oviposit on available spruce that they come across (McClure 1989a), but emerged crawlers (sexuales) soon perish. The primary host of *A. tsugae* was recently identified as *Picea torano* (Koch) Koehne in Japan (Montgomery et al. 2009), but this exotic spruce species is not found in North America; resulting in an incomplete sexual life cycle (McClure 1987b, McClure 1989a). Winged progredien adults that choose to remain on hemlocks die without laying eggs (McClure 1989a). About 40-50% of progrediens develop as wingless morphs. In a study done in Essex forest (Connecticut) and Montgomery evergreen arboretum, McClure (1987a) reported that winged morphs colonized and oviposited on eleven species of spruce and on an exotic spruce, *P. polita* in the laboratory. These “settled” nymphs immediately died.

An apterous progredien female oviposits ≈25 eggs during June-July. Emerged sisten crawlers molt to second-instar nymphs once they settle on hemlock branches and aestivate from July to late October. After emerging from aestivation, sisten nymphs actively consume the stored reserves in parenchyma cells (McClure 1989a). Reports suggest that they inject toxins while feeding which causes needle loss by rapid desiccation, which leads to crown dieback and reduced new shoot growth (McClure 1991a, McClure 1991b). *Adelges tsugae* infest all hemlock trees irrespective of age or size (McClure 1992a).

McClure (1989a) observed that eggs and crawlers of the three generations were indistinguishable. However, older immature stages and adults could be differentiated from one another by presence or absence of thoracic sutures, wing bud notches, intensity of sclerotization, or body size and shape of the thorax, and the antennae. McClure (1989a) also suggested that progredien eggs produced by winged and wingless females are very difficult to distinguish.

Damage and Population Dynamics of HWA

New shoot growth is more vulnerable to HWA nymph infestation than mature regions of hemlocks (McClure 1991a). Studies have shown that feeding by nymphs on tender branches seriously affects production of new shoots from those branches the following year (McClure 1987b). Presence of even low densities of less than four individuals per 20 mm^2 of the branch has deleterious effects on new growth development the next year (McClure 1987b, McClure 1991a). Only 2% of new buds were found on infested hemlock branches relative to uninfested branches (McClure 1987b). Biomass of active buds was reduced from 71.2 ± 6.7 mg on uninfested to 4.3 ± 0.5 mg on infested previous-year branches. Heavy loss of foliar biomass results in tree mortality within four to ten years after infestation (McClure 1991b). Hemlock woolly adelgid population densities vary from time to time depending on the tree health and its physiology following the first infestation (McClure 1991a). *Adelges tsugae* exhibit a bimodal pattern with two consecutive peaks in their population (first and third years) and contributes to tree mortality in the fourth year. In the first year, adelgid infestations can occupy all ages of hemlock shoots (Lagalante et al. 2006, McClure 1991a). More adelgids were observed on the older hemlock branches in the second year because overall poor tree health reduced numbers of new shoots. Poor quality host material leads to high *A. tsugae* mortality (9-90% of the total live adelgids) or reduced fecundity. In second or fourth year of infestation, number of winged adults spiked by 100%, overall *A. tsugae* density was decreased to 2-40 individuals per 20 mm^2 branch and no new shoots were found (McClure 1991a). In the third year, 11-15% of growing shoot tips was attacked and their nutrients were completely exploited by adelgids resulting in severe stunting (McClure 1991a).

McClure (1991a) concluded that after a heavy initial adelgid infestation on younger shoots, their population naturally perishes because of severe reductions in current year shoots. Therefore, adeldgid population growth on eastern hemlock is often described as density dependent feedback (McClure 1989b, McClure 1991a). Randomized branch sampling of *T. canadensis* found that adelgid distribution on heavily infested trees tended to be greater on lower tree crown regions than upper crown regions, but in low infestations the pattern was reversed (Evans and Gregoire 2007).

Lamb et al. (2006) observed that the density dependent feedback of *A. tsugae* populations and tree health could indirectly influence the performance of the predator *L. nigrinus* in the forest. In another study, Broeckling and Salom (2003b) compared the volatile compounds emitted by shoots of various ages of eastern hemlocks. These researchers found higher levels of monoterpenes from previous year shoots than the current year's shoots. There was no clear difference in emitted volatile profiles between infested and uninfested branches except for a 57% reduction in α-pinene (monoterpene) in infested shoots compared to 66% in uninfested ones (Broeckling and Salom 2003b).

Additionally, progredien crawlers had better settlement rates relative to sisten crawlers on hemlock branches (Butin et al. 2007). Butin et al. (2007) artificially infested hemlock branches by attaching infested cuttings onto healthy hemlocks. They reported that settlement was greatest when adelgid-infested shoots were loosely placed, tied and covered with mesh sleeves on the uninfested hemlock foliage, and they did not find any effect of adelgid density on the cuttings on crawler settlement rates on healthy branches. In addition, they noted that settlement rate was greater with early-emerged crawlers than crawlers that emerge in late season (Butin et al. 2007).

Influence of Abiotic Factors

Hemlock woolly adelgids are highly adaptive and can tolerate low winter temperatures (Butin et al. 2005). Skinner et al. (2003) observed that survival of *A. tsugae* was adversely affected at -25°C or lower, < 3% of adelgids survived at -30°C and none survived at -35°C. This finding was consistent with Parker et al. (1999) as they observed no surviving adelgid at -35°C and -40°C. Parker et al. (1999) reported that relative mortality of HWA gradually increased as the temperature steadily declined from -20°C to -30°C. To determine this outcome, they sampled hemlock shoots between January and February. Furthermore, samples drawn from Holyoke, MA had heavy mortality. These particular samples were exposed to temperatures between -20°C and -40°C in the laboratory. The relative mortality of *A. tsugae* was significantly greater when the temperature dropped below -20°C in March compared to samples collected in the previous two months (Parker et al. 1999). Data showed that 14-22%, 8-12% and 0-4% of *A.*

tsugae survived in January, February and March, respectively, at -25^{o}C. This indicates that adelgids are less cold-hardy in the later part of the winter (Parker et al. 1999). In the laboratory, Parker et al. (1999) did not observe any influence of length of exposure (2, 4 and 8 hours) to lower temperatures on relative mortality of HWA. However, individual adelgids showed an ability to withstand short-term exposure of low temperatures. This indicates that *A. tsugae* has a high chance of acquiring cold-tolerance (Parker et al. 1999). Skinner et al. (2003) reported that an adelgid's tolerance to cold temperatures varies with the geographical location and time of the year. *Adelges tsugae* has been capable of adaptation to colder temperature regimes and could extend their geographical expansion to colder climatic regions (Butin et al. 2005). As *A. tsugae* has a high potential to build its population in a short time, negative evolutionary pressure acting on them is almost negligible. Since *A. tsugae* has exhibited strong genetic variation especially to cold-tolerance, it could rapidly evolve to within a short time of approximately 100 generations. Parthenogenetic reproduction lacks recombination among alleles between generations, so there is a very limited chance for *A. tsugae* to incorporate effects of possible mutations that acquire over time. Therefore, whatever existing diversity in alleles that are seen in *A. tsugae* could be dominant or epistatic effects passed on entirely to the offsprings (Butin et al. 2005).

Gouli et al. (2000) reported hemolymph of *A. tsugae* is composed of formative cells, plasmatocytes, adipohemocytes, vermiform cells, prohemocytes and oenocytoids. These researchers observed a variation in hemolymph cellular counts sampled from Mt. Tom State Reservation, Holyoke, MA between February and October. Significant reduction in adipohemocytes or prohemocytes and increases in oenocytoids or formative cells were noticed in February compared to October samples. They found all the hemocytes of HWA completely destroyed at -30^{o}C and could not recover live *A. tsugae*. Additionally, significant reductions in intact hemocytes were observed at -20^{o}C and -25^{o}C as compared to controls maintained at 0^{o}C. The only exception on *A. tsugae* survival was noted at -20^{o}C after 2 hours of exposure. Various cellular components responded differentially to cold tolerance. Plasmatocytes were resistant at -20^{o}C for 2, 4 and 8 hours of exposure but failed to resist cold at -25^{o}C. On the other hand, formative cells stayed intact only up to 2 hours at temperature below -20^{o}C (Gouli et al. 2000).

In contrast, at higher temperatures no progrediens survived temperatures of 27^{o}C and 32^{o}C (Salom et al. 2002). Salom et al. (2002) suggested a maximum temperature threshold falls between 22^{o}C and 27^{o}C in the laboratory. Most of the progrediens completed their development from the egg to adult between 4^{o}C (147 days) and 22^{o}C (52 days). The study noted a lesser influence of temperature on development time (53 days at 4^{o}C to 41 days at 22^{o}C) of first-instar nymphs. Meanwhile, the development time (56 days at 4^{o}C to 5 days 22^{o}C) for second-instar nymphs to adult was severely influenced by temperatures (Salom et al. 2002). Salom et al. (2001) also conducted experiments to determine the diapause-inducing cue of *A. tsugae* to possibly rear *A. tsugae* year-round by preventing aestivation between late June and October to provide food for predatory beetles in the laboratory without interruption. These researchers could prevent aestival-diapause by rearing the progrediens at 12^{o}C or 14.5^{o}C. However, they could not prevent aestival-diapause when progrediens were reared at17^{o}C. Prevention of diapause was also successful at a photoperiod of 12:12 (L: D) hours compared to either increased or decreased day length (Salom et al. 2001).

Management Measures for HWA

Various strategies have been used to control *A. tsugae* in the United States that included use of insecticides, enhanced host-plant resistance or vigor, and use of natural enemies (McClure 1989b). In the western United States, the natural populations of HWA are controlled in part by the natural resistance of hemlock species, but in the eastern United States, hemlock species are less resistant to hemlock woolly adelgid. No effective natural enemy was observed regulating *A. tsugae* population in forest of the eastern US (Cheah and McClure 2000, McClure 1991b). Chemical means to control adelgid infestations are usually not feasible because of the difficulty in moving spray equipment or personnel to interior regions of the forest (Cowles et al. 2006). Also, use of insecticides in the forest could create environment issues especially to aquatic fauna in nearby streams close to the insecticide treated hemlock trees (Cowles et al. 2006). Classical biological control by introduction of non-native natural enemies was identified as the only sustainable solution for this exotic pest in the forest (Butin et al. 2004, Cheah et al. 1998, Lamb et al. 2006, McClure 2001, Zilahi-Balogh et al. 2002)

Biological Control Agents

Various naturally occurring predatory arthropods were reported feeding on *A. tsugae* in the eastern (Wallace and Hain 2000) and Pacific northwestern United States (Kohler et al. 2008a). Although the non-native polyphagous coccinellid, *Harmonia axyridis* Pallas was released for aphid control in previous years, they actively consumed different stages of adelgid but failed to produce substantial control (Wallace and Hain 2000, Koch 2003). Other native predators including chrysopids (*Chrysoperla harrisii* Fitch), hemerobiids (*Hemerobius humulinus* L. and *Hemerobius* spp.), syrphids and cecidomyiids also failed to bring effective control of *A. tsugae* (McClure 1987a, Wallace and Hain 2000, Butin et al. 2004, McClure 2001). No parasitoid has been reported to date on *A. tsugae* (McClure 2001). *Laricobius nigrinus* Fender (Derodontidae) is a specialist predator native to the Pacific northwest that consumes *A. tsugae* (McClure 2001, Zilahi-Balogh et al. 2003b,) and is widely released to control *A. tsugae* in the forest. Two predators, a coccinellid beetle, *Sasajiscymnus tsugae* Sasaji & McClure (formerly called as *Pseudoscymnus tsugae*) (Sasaji and McClure 1997, Vandenberg 2004) and oribatid mite *Diapterobates humeralis* Hermann have been observed attacking HWA on *T. diversifolia*, *T. sieboldii* and *T. canadensis* (L.) Carrière in Japan (McClure 1995, McClure 2001).

Diapterobates humeralis feed on adelgid's woolly material that protects egg mass. Therefore, eggs fall from the tree and get destroyed or eaten by ground predators such as spiders or ants (McClure 1995, McClure 2001). Studies showed that 20 mites dislodged greater than 95% of *A. tsugae* eggs within seven days (McClure 1995). Although *D. humeralis* had good impact by destroying 99% of the *A. tsugae* ovisacs on susceptible *T. canadensis* at two ornamental sites in Japan (McClure 1995), it did not promise good HWA control because it had poor fecundity compared to HWA adults (McClure 2001).

Sasajiscymnus tsugae effectively suppressed *A. tsugae* population after a large scale release in the eastern US (McClure and Cheah 1999). They undergo a minimum of two generations per year and are well adapted to climatic conditions in eastern North America. In addition to their synchronous life cycle as compared to various stages of *A. tsugae,* they have superior searching behavior and dispersal ability.

Activity of the predators, *L. nigrinus*, *S. tsugae*, and *H. axyridis* varies temporally and spatially (Flowers et al. 2007). Behavioral studies on *L. nigrinus* showed that it was more active in feeding during spring than in other seasons. However, activity of *S. tsugae*, and *H. axyridis* was apparent during warmer seasons. In addition, diurnal behavior of *L. nigrinus*, *S. tsugae*, and *H. axyridis* showed that *L. nigrinus* was more active at night than during the day; the other two predators were more active during the daytime than at night (Flowers et al. 2007). In the Pacific Northwest, predaceous dipteran larvae, *Leucopis argenticollis* Zetterstedt (Chamaemyiidae) and *Leucopis atrifacies* (Aldrich) occur in association with *A. tsugae* settled on *T. heterophylla* (Kohler et al. 2008a). Kohler et al. (2008b) observed several parasitoids attacking the dipteran predators in the field.

Potential natural enemies of *A. tsugae* were rigorously investigated in China, and 54 species were collected feeding on them (Montgomery et al. 2000, Yu et al. 2000). Out of nine species screened to be imported to the United States, five were Coccinellidae in the tribe Scymini. The most important species included *Scymnus sinuanodulus* Yu and Yao, *S. camptodromus* Yu and Liu, and *S. ningshanensis* Yu and Yao. Although these beetles undergo only one generation per year, they prefer *A. tsugae* to other potential prey items (McClure 2001). A potential anthocorid predator, *Tetraphleps galchanoides* (Ghauri) collected from China was extensively tested for its compatibility with *A. tsugae,* found in the eastern United States (McAvoy et al. 2007). The no-choice tests showed that *T. galchanoides* consumed more *A. tsugae* eggs relative to adults of *A. tsugae*, eggs or adults of *P. strobe*. In addition, more hemlock woolly adelgid eggs were consumed when eggs of *A. tsugae* and *P. strobe* were provided in a choice test (McAvoy et al. 2007).

Important Biological Control Agents

a. *Laricobius nigrinus*

Laricobius nigrinus has a univoltine life cycle and undergoes pupal aestivation from April to September (Zilahi-Balogh et al. 2003c). Its summer aestivation is in synchrony with the *A. tsugae* aestivation. *Adelges tsugae* aestivates between late June and early October as second-instar nymph (Zilahi-Balogh et al. 2002). Adult beetles break their aestivation and become active (70% of males and 46.4% of females) by October. *Laricobius nigrinus* feed extensively on *A. tsugae* and excrete purple- pink colored liquid frass on hemlock needles (Zilahi-Balogh et al. 2003c). After multiple mating, a female beetle oviposits a single egg in *A. tsugae* ovisac

(Zilahi-Balogh et al. 2003b, Zilahi-Balogh et al. 2003c). The oval eggs turn bright yellow to pale greenish yellow after deposition and the sticky secretion on the egg enables these eggs to adhere to the woolly material on *A. tsugae* ovisacs. Oviposition starts by early December and continues for 10 to 32 weeks at 13°C (Zilahi-Balogh et al. 2003c). The average fecundity of *L. nigrinus* females was 108 eggs per female per season. Zilahi-Balogh et al. (2002) observed that time of *L. nigrinus* egg hatch was in synchrony with the oviposition of *A. tsugae*; therefore, the larva actively consumed *A. tsugae* eggs. It has been shown that larval density of *L. nigrinus* increased with increased availability of adelgid ovisacs in a field insectary (Mausel et al. 2008).

Zilahi-Balogh et al. (2003c) reported that developmental time of *L. nigrinus* from egg to adult was inversely proportional to temperature. They require 88.8 ± 2.2 (mean ± SE), 64.8 ± 2.6, and 46.6 ± 2 days to complete life cycle at 12, 15, 18°C, respectively. Larval development was completed in 14 days at 18°C. The pre-pupal stage of *L. nigrinus* did not tolerate cool soil temperature, especially <12°C (Zilahi-Balogh et al. 2003c). *Laricobius nigrinus* failed to develop at 21°C when their developmental time, at different ages, was examined at temperatures ranging from 6-21°C (Zilahi-Balogh et al. 2003a). These researchers also determined that the lower developmental threshold temperatures were 5.4, 3.2, 2.9 and 3.1°C for eggs, larvae, pre-pupae, and pupae, respectively. Zilahi-Balogh et al. (2003c) found that *L. nigrinus* larvae consumed on average 230 and 265 *A. tsugae* eggs at 12°C and 18°C, respectively. They also observed that adult *L. nigrinus* actively consume adults and nymphs of *A. tsugae* and beetle larvae prefer *A. tsugae* eggs for nutrition than other potential prey. Zilahi-Balogh et al. (2002) reported that *L. nigrinus* expressed strong preference for oviposition in *A. tsugae* ovisacs when they were offered a choice of other adelgid species including *Adelges piceae* Ratzeburg, *Pineus strobi* Hartig and *Adelges abietis* (L.); aphid species *Myzus persicae* Sulzer and *Cinara* *pilicornis* Hartig; and diaspidid species *Chionaspis pinifoliae* Fitch. *Laricobius nigrinus* consumed more HWA eggs than eggs of other prey species (Zilahi-Balogh et al. 2002).

Broeckling and Salom (2003a) studied the antennal morphology of *L. nigrinus* and *S. tsugae* to determine if its structures provide any hint towards their olfactory behavior in recognizing *A. tsugae*. Antennae of *L. nigrinus* were comparatively long, have nine annuli on flagellum segments and six types of sensillae. Similarly, *S. tsugae*'s antennae were relatively short, had only seven annuli and five types of sensillae. These researchers suggested that *L. nigrinus* may respond better to olfactory cues related to *A. tsugae* or hemlock species than the coccinellid, *S. tsugae* (Broeckling and Salom 2003a).

Lamb et al. (2005) showed that *L. nigrinus* preyed on all stages of *A. tsugae* throughout the year in field cages with HWA infested branches but *L. nigrinus* was most active during winter. Lamb et al. (2006) reported that female *L. nigrinus* survival was between 76% and 88% and their oviposition rate was higher in March than in April or May. Therefore, Lamb et al. (2006) recommended releasing *L. nigrinus* in the field by January to maximize production of predator eggs. They also found that predator density and prey abundance influence successful establishment of *L. nigrinus* in the field. In the laboratory, *L. nigrinus* oviposited fewer eggs in *A. tsugae* ovisacs when released as singles than as pairs and that *L. nigrinus* laid more eggs when *A. tsugae* density increased. However, in field cages, these researchers did not find significant suppression of *A. tsugae* density with increased predator numbers (Lamb et al. 2006). Lamb et al. (2006) speculated that multiple-location, low-density releases of *L. nigrinus* in trees that were fairly heavily infested with *A. tsugae* would aid successful establishment and persistence of this predator in the field (Lamb et al. 2006). However, predator recovery in the spring following a fall field release was inconsistent. This research suggested that successful predator establishment may be affected by poor tree health, tree physiology or adverse weather conditions (McClure and Cheah 1999). These conditions may not provide good oviposition sites for *L. nigrinus* in the forest (Lamb et al. 2006).

Three other species of *Laricobius* feed on adelgids; *Laricobius erichsonii* Rosenhauer, *Laricobius rubidus* LeConte and *Laricobius laticollis* Fall (Zilahi-Balogh et al. 2003b). *Laricobius erichsonii* is a native to Europe introduced to North America for biological control of the balsam woolly adelgid, *A. piceae*. Meanwhile, *L. rubidus*, the only native derodontid predator in the eastern United States, feeds primarily on pine bark adelgid in North America (Zilahi-Balogh 2003b, Zilahi-Balogh et al. 2005). Zilahi-Balogh et al. (2005) reported that although *L. rubidus* preferred to oviposit in ovisacs of *A. piceae* compared to *A. tsugae* in pair-choice tests, it oviposited similar numbers of eggs in ovisacs of *A. tsugae* and *A. piceae* during no-choice tests. *Laricobius rubidus* successfully completed its development on *A. tsugae*, and neither larval developmental time nor survivorship differed when it was fed *A. tsugae* alone. Since *L. nigrinus* requires *A. tsugae* to complete its development and *L. rubidus* can complete its development on either prey species (*A. tsugae* or *A. piceae*), the chance for competition between them is narrow. Conversely, *Laricobius laticollis* were collected from Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] and showed no preference for *A. tsugae* (Zilahi-Balogh et al. 2005).

Mausel et al. (2008) established a field insectary for *L. nigrinus* using 1.2- to 2.4 m tall *T. canadensis* that were then artificially infested with adelgids. This insectary program achieved a population of *L. nigrinus* synchronized with its adelgid host that also resulted in recovery of significant numbers of beetles for future release.

b. *Sasajiscymnus tsugae*

In the mid-1990s, *S. tsugae* produced remarkable success by reducing *A. tsugae* populations 47-88% following the release of 130000 adult beetles (McClure and Cheah 1999, McClure et al. 2000). Results indicated that the *S. tsugae* population overwintered, reproduced, and dispersed after release. The released predator had higher cold tolerance and a preference for hemlock woolly adelgids (McClure and Cheah 1999, McClure et al. 2000).

Cheah and McClure (2000) observed that the life cycle of *S. tsugae* was synchronous with the life cycle of *A. tsugae*. *Sasajiscymnus tsugae* adults were present in the forest throughout the year where they normally undergo two generations per year. However, a third generation was noted when some adults had survived two successive winters (Cheah and McClure 1998, McClure and Cheah 1999). Overwintering adults become active by March and start laying reddish-orange, oval (0.4-0.6 mm length) eggs covered with an opalescent sheath by early April, and by June F_1 adults begin oviposition.

The *S. tsugae* adults lay a single egg within concealed spots of bud-scales or empty cones or under the woolly material of the adelgid, which has four active larval instars. The first-instar larva has reddish-brown with darker brown head and the second-instar larva is more gray with a black head and other two larval instars that are dark-grayish to reddish brown with black heads and legs (Cheah and McClure 1998). The pre-pupal stage of the fourth-instar has a partial woolly covering and this larval skin which it sheds on pupation. Adults, 1.5-2.35 mm long have a jet-black exoskeleton with dorsal pubescence (Cheah and McClure 1998).

F_2 adults do not oviposit between October and March; rather they overwinter along with F_1 adults and they start oviposition when they both become active in March. Low temperatures in early spring can result in asynchrony of egg hatch and an overlap of the various stages of *S.*

tsugae. Multivoltinism is an attribute of *S. tsugae* which enables them to adapt to varied ecological conditions of the forest (Cheah and McClure 1998, Cheah and McClure 2000, McClure and Cheah 1999). Cheah and McClure (2000) have noted that *S. tsugae* survived on aphid honeydew or other extra-floral nectories when *A. tsugae* were in aestivation.

In an attempt to determine the prey preference and host range of *S. tsugae*, Butin et al. (2004) showed that they fed more on *A. tsugae* nymphs than on the other adelgid species when they were provided with various combinations including *Adelges cooleyi* Gillette, *P. strobi* and *Adelges laricis* Vallot. They also observed that by giving two adelgid species, *A. tsugae* and *A. laricis,* eggs in a combination, *S. tsugae* consumed a greater amount of *A. tsugae* eggs.

The polyphagous predatory beetles, *H. axyridis,* are common and occur in abundance on hemlock trees. High numbers of *H. axyridis* adults and eggs have been reported on heavily infested hemlock branches before woolly alder aphids appear on alder trees (Butin et al. 2004). Flower et al. (2005) studied the competitive interaction of three predators, *L. nigrinus*, *S. tsugae* or *H. axyridis* of hemlock woolly adelgid in the laboratory. According to these researchers, all predator species grouped into conspecifics (composed of same predator species) and heterospecifics (composed of dissimilar predator species), actively consumed *L. nigrinus* or *S. tsugae* eggs. However, predator groups, specifically heterospecific group, did not show any difference in *A. tsugae* predation and the predator survival was not affected.

c. *Scymnus ningshanensis*

Overwintering *S. ningshanensis* lay eggs in the spring, are univoltine (Butin et al. 2003) and consume all stages of *A. tsugae*. Butin et al. (2003) observed that their fecundity was higher when feeding on *A. tsugae* ovisacs. Laboratory and field performance of *S. ningshanensis* was compared to *S. tsugae* were compared. In laboratory, it was observed that *S. ningshanensis* performed better than *S. tsugae* as *S. ningshanensis* egg oviposition increased in response to an increase in HWA density. Population growth of *A. tsugae* declined when *S. ningshanensis* were released in field cages on infested hemlock branches. In contrast, *S. tsugae* displayed a density-independent response in suppressing *A. tsugae* density in the laboratory while it did not reduce adelgid population in the field cages (Butin et al. 2003). When given a choice of *A. laricis* or *A. cooleyi* prey*, S. ningshanensis* the predator did not show a preference (Butin et al. 2004).

Use of Insecticides

Application of chemical insecticides of any kind might impart environmental risks and their indirect impact on non-targets, especially mites in the forest, has been reported (Raupp et al. 2004, Cowles et al. 2006). Therefore, special attention has been given to the mode of application of insecticides to trees in forests to minimize non-target impacts. At the same time, these methods have demonstrated good protection of hemlock trees from *A. tsugae* infestation. McClure (1991b) tested efficacy of non-toxic horticultural oils (1% and 2%) during spring, summer and fall. He also sprayed insecticidal soap (18.72 g a.i /L of water) using a backpack sprayer on small trees and a high-pressure hydraulic sprayer on large trees (up to 30m) during summer and fall. Both these treatments were effective with 95-100% suppression of *A. tsugae* density (McClure 1991b). Others have found that foliar applications of insecticide were inefficient for reaching the highest tree branches (Gouger 1971, Cowles et al. 2006).

Gouger (1971) showed that foliar application of 70% Baygon (o-isopropoxyphenyl methylcarbamate) at the rate of 1.2 g/ L of water and 50% Thiodan (oxydemotonmethyl) at the rate of 2.4 g/ L of water were less effective on *A. tsugae* yielding ≈50% control. McClure (1992b) reported that 95-100% of adelgid mortality was observed when diazinon (7.1 g a.i /L water), fluvalinate (0.3 g a.i /L water), malathion (4.5 g a.i /L water), and oil (1%) + ethion (0.2

g a.i /L water) were used as compared to 6.7-9.3% of adelgid mortality on untreated trees after May or October treatment. McClure (1991b and 1992b) also observed that stem injection methods (Mauget system or CSI implanting) were effective in getting the active ingredients into the tree where it was moved to all shoots. In addition, to study the interaction effects of pesticide and fertilizer on *A. tsugae*, McClure (1992a) injected tree trunks with (J. J. Mauget Co., Arcadia, CA) or implanted (CSI system, Fremont. Nebr.) bidrin, oxydemetonmethyl and acephate in May 1990, and found 93-98% mortality in sistens and 60-88% mortality in progrediens as compared to their population on untreated control trees in all treatments. All the adelgids endured longer on trees that received fertilizer alone or in combination with insecticide. He also observed that fertilizer application reduced the effectiveness of the insecticide in suppressing *A. tsugae* population.

Cowles et al. (2006) reported that soil injection of imidacloprid was more effective than stem injection with Mauget System, Wedgle Direct-Inject Tree Treatment System (ArborSystems, Omaha, NE), or the Arborjet VIPER system (Arborjet, Winchester, MA) (Cowles et al. 2006). Higher numbers of live *A. tsugae* were noticed on Wedgle than Mauget or Arborjet treated trees. Since most of the absorbing roots were located at the subsurface beneath the tree canopy, the application of insecticide to this region would increase root interception and uptake. However, Cowles et al. (2006) found that soil-injection of imidacloprid throughout the area beneath the dripline of the canopy was no better than injections made with 25 cm of the trunk. Moreover, this study found similar levels of *A. tsugae* mortality whether imidacloprid was applied in fall or spring. In Connecticut, 80% and 98% reduction in *A. tsugae* population was noted within two consecutive years in the forest and that residues of imidacloprid were found in sap, needles and twigs for three years after soil-injection. However, Cowles et al.

(2006) did not find similar results from trunk injected trees where imidacloprid residues were recovered only from a few treated trees. Their results suggested that imidacloprid residues beyond 120 ppb (parts per billion) could suppress adelgid populations. Although residues of imidacloprid in the sap were evident in trunk or soil injected trees, the highest suppression of *A. tsugae* was noticed in soil-injected trees. Therefore, Cowles et al. (2006) suggested that residues of imidacloprid were more evenly distributed when roots absorbed the insecticide than when delivered through the trunk. These researchers observed that imidacloprid residues were stable and actively translocated to new shoots between years (Cowles et al. 2006).

Webb et al. (2003) observed that *A. tsugae* infested eastern hemlocks recovered following soil-drench of imidacloprid within 434 and 816 days of treatment. The trees that received soil-drench treatments had reduced adelgid populations and increased production of new shoot growth. Some severely damaged trees also showed signs of recovery relative to untreated trees even though they took at least 816 days after imidacloprid treatment. (Webb et al. 2003). Webb et al. (2003) noted that sap flow of infested hemlock trees was severely impaired; therefore, translocation of imidacloprid could have been delayed especially to the tree canopy regions. Daccola et al. (2005) observed that 85% *A. tsugae* population died in fall samples when imidacloprid was trunk-injected using arborjet viper in the summer as compared to 37.9% mortality on the untreated trees and suggested that the translocation of imidacloprid could be influenced by tree health during insecticide application (Daccola et al. 2005).

Studies conducted to determine possible non-target effects of imidacloprid on adelgid infested hemlocks in residential landscapes indicated high densities of spruce spider mites, *Oligonychus ununguis* Jacobi, and their damage was greater on imidacloprid treated hemlock trees as compared to untreated trees (Raupp et al. 2004). Raupp et al. (2004) suggested this increased in phytophagous mite populations could be the result of either imidacloprid-induced hormoligosis (higher reproductive rate of sucking arthropod pests), eradication of natural enemies due to imidacloprid toxicity, or favorable alteration in the tree physiology. Dilling et al. (2009) studied the non-target effects of imidacloprid applied as a soil drench, or soil or trunk injection, and foliar applications of horticultural oil on the community within the hemlock tree canopy. The overall abundance of the arthropod communities was reduced in all treatments especially in the imidacloprid-drench treatment. There was no reduction in the density of non-target arthropods among imidacloprid soil or trunk injections as well as horticultural oil applications. A single soil drench treatment of imidacloprid appears to have negatively affected lepidopteran and psocopteran densities, but their long-term effects were not studied (Dilling et al. 2009).

Eisenback et al. (2008) exposed *L. nigrinus* and *S. tsugae* to adelgid-infested branches obtained from trees that received 25% of the recommended dose (1.4g imidacloprid) and found that both predator species showed no evidence of indirect toxic effects and no detectable traces of imidacloprid residues was recovered from the beetle cadavers.

Use of Fertilizers

McClure (1991c) showed that fertilized *T. canadensis* had a two-fold increase in egg production of *A. tsugae* and their density increased five-fold compared to unfertilized trees. However, an increase in *A. tsugae* fecundity was not attained during the spring generation on older *T. canadensis* forest trees even though the N fertilizer had been delivered by trunk injection (McClure 1992a). In addition, fertilized trees showed higher levels of tree injury symptoms and severe reduction in the new growth production within three months of fertilization (McClure 1992a).

Performance and reproduction of *A. piceae* were also affected by N fertilizer-induced changes in the amino acid content within the tree (Carrow and Betts 1973). The highest *A. piceae* survival or reproductive rate was achieved with potassium ammonium nitrate and urea fertilizers instead of ammonium nitrate. Rapid assimilation and incorporation of N was observed in new flushes of fertilized, *A. piceae* infested *Abies amabilis* (Carrow and Graham 1968). These researchers found *A. piceae* nymphs settled on ammonium nitrate treated *A. amabilis* trees were significantly lower as compared to nymphs settled on untreated ones.

Host-Tree Resistance

Out of nine species of hemlock, eight species are reportedly infested by *A. tsugae* including two western North American hemlocks (western hemlock, *T. heterophylla* (Raf.) Sarg. and mountain hemlock, *T. mertensiana* (Bong.) Carrière); two Chinese hemlocks (*T. chinensis* (Franch.) E. Pritz and *Tsuga dumosa* (D. Don) Eichler); two Japanese hemlocks (northern Japanese hemlock, *T. diversifolia* (Maxim.) Mast., and southern Japanese hemlock, *T. sieboldii* Carrière); and two eastern North American hemlocks (Havill et al. 2008, Havill and Montgomery 2008). Havill et al. (2008) suggested that *T. mertensiana* had unique characters compared to other *Tsuga* spp. and therefore placed it in a monotypic group. Unlike other hemlock species, *T. mertensiana* had distinct needle, seed cone, and pollen morphology (Havill et al. 2008). Their needles were amphistomatic as in *Nothotsuga*, *Abies*, *Cedrus*, and *Keteleeria* genera; the morphology of their seed cone was erect as in *Abies*, *Cedrus*, *Keteleeria*, and *Pseudotsuga;* and their bisaccate pollen resembled the pollen morphology of *Larix* and *Pseudotsuga* (Havill et al. 2008). All other hemlock species had hypostomatic needles, pendulous seed cones, and monosaccate pollen. Moreover, studies based on volatile terpenoids also grouped *T. mertensiana* as a distant species relative to other *Tsuga* species (Lagalante and Montgomery 2003).

Several breeding projects involving inter-specific hybrids and intra-specific crosses have been conducted (Wang et al. 1997). The hybrids created were primarily between *T. chinensis* and *T. caroliniana* (Wang et al. 1997, Pooler et al. 2002). The idea of incorporating resistant genes by hybridizing Asiatic adelgid-resistant hemlocks (*T. diversifolia*, *T. sieboldii* or *T. chinensis*) and susceptible eastern hemlock has been unsuccessful (Bentz et al. 2002). Phylogenetic relationship using DNA sequence of worldwide specimens of *Tsuga* spp. indicate that the eastern American hemlocks (*T. canadensis* and *T. caroliniana*) are not closely related (Havill et al. 2008); instead, susceptible *T. caroliniana* is closely related to resistant *T. diversifolia* (Havill and Montgomery 2008). Montgomery et al. (2009) tested those hybrids by artificially infesting them with *A. tsugae* crawlers and observed intermediate adelgid population growth or resistance relative to their resistant-Asian and susceptible-eastern American hemlock parents. Interestingly, phylogenetic analysis of mitochondrial DNA suggests that HWA found in eastern North America has a closer resemblance to adelgids infesting *Tsuga sieboldii* Carriére in southern Japan than in mainland China, Taiwan or the Pacific Northwest (Havill et al. 2006).

McClure (1991b and 1992b) observed that *A. tsugae* survival or fecundity, and the numbers of dead tree twigs were significantly lower on resistant western hemlock as compared to susceptible eastern hemlock. Likewise, McClure (1992b) found that adelgids caused >85% bud injury on eastern hemlock and no bud mortality on *T. diversifolia* and western American hemlocks in Connecticut. Fewer adelgids survived on *T. diversifolia* and the western American hemlocks, *T. heterophylla* or *T. mertensiana* as compared to eastern American hemlocks *T. canadensis* or *T. caroliniana* (McClure 1992b). Jetton et al. (2008b) also found reduced *A.*

tsugae fecundity on *T. heterophylla* compared to *T. caroliniana* in North Carolina. However, Mausel (2005) observed greater adelgid fecundity on *T. heterophylla* (62% of the total) than on eastern hemlock in the Northwest. Thus, uncertainties exist in establishing a range of resistance among North American hemlock species. Furthermore, Havill et al. (2006) found that adelgids in the Pacific Northwest and East coast of the United States were not closely related and also noted that adelgids found in mainland China readily infested on *T. chinensis* but adelgids in North America do not. Genetic lineages of *A. tsugae* that occurred in the United States and China were non-sister groups and caused Havill et al. (2006) to speculate that they may be two different species. *Tsuga chinensis* as well as *T. diversifolia* are highly resistant to hemlock woolly adelgid infestation (Bentz et al. 2002, Del Tredici and Kitajima 2004), and in Japan native hemlock species tolerate the toxic saliva of *A. tsugae* (Paca 1993).

McClure (1992a and 1992b) reported that higher N content increased the palatability of hemlock thereby increasing the susceptibility to *A. tsugae* colonization. It was shown that if N content in trees were high, hemlock trees would support greater populations of *A. tsugae*. Pontius et al. (2006) showed the relationship between the foliar chemistry of *A. tsugae*-susceptible and *A. tsugae*-resistant hemlocks and correlated foliar chemistry of various hemlock species and tree health parameters. After analysis of various foliar concentrations after two successful sisten generations, a strong correlation was found between higher K or lower P and *A. tsugae* abundance. Resistant hemlocks had lower K and N but higher levels of P as compared to susceptible ones. Therefore, higher N and K content in foliage could have enhanced the palatability of hemlock for adelgids than if Ca or P were present in high concentration (Pontius et al. 2006).

Esham et al. (2005) collected open-pollinated seeds of eastern hemlock from Great Smoky Mountains National Park in Tennessee, New York and Massachusetts in the fall of 1995 and 1996 and raised seedlings in spring. This hemlock germplasm was maintained at four plantations in New York and Massachusetts, and they believe that hemlock germplasm collection might be important for future studies on *A. tsugae* infestations (Esham et al. 2005). Kessell (1979) classified eastern hemlock stands into two ecotypes called high response or low response, based on external morphology. In addition to higher growth rate, the trees in the high response ecotype were very sensitive to higher levels of moisture and had a greater tolerance to cold as compared to the low response ecotype. The low response ecotype hemlocks were more common on xeric slopes. However, Kessell (1979) also observed numerous intermediate types in the Allegheny and Appalachian Mountains as well.

Ecological Changes after Infestation

The eastern hemlock is evergreen, shade-tolerant, has dense foliage, and multi-layered crown branches that provide unique niches for wildlife (Jenkins et al. 1999, Tingley et al. 2002). Tingley et al. (2002) observed that the avian community, particularly black-throated green warbler, blackburnian warbler, and acadian flycatcher, would be especially vulnerable as a result of the destruction of hemlock trees in regions from Long Island Sound to southern border areas of Massachusetts and Connecticut River valley. Brooks (2001) observed that tree mortality caused by *A. tsugae* resulted in temporary reductions in the abundance of terrestrial, redback salamanders (*Plethodon cinereus* Green) in hemlock-dominated forests of Barkhamsted Reservoir areas in Connecticut and Massachusetts. However, salamander numbers increased to normal levels in later years after harvesting hemlocks infested with HWA (Brooks 2001). Ross et al. (2003) suggested that HWA infestation in hemlock-dominated forest could indirectly alter the diversity and abundance of the fresh water fish community, as these researchers were studying the aquatic composition of headwater streams flowing through hemlock or hardwood-dominated forests of Delaware. The study documented numerically higher functional diversity of fish in hemlock than in hardwood-dominated forest.

Hemlock woolly adelgids have caused a dramatic impact on the hemlock-dominated forest. The gaps resulting from tree mortality allow more light to the forest floor providing an opportunity for hardwoods seedlings such as red maple (*Acer rubrum* L.), various kinds of oaks (*Quercus* spp.) or black birch (*Betula lenta* L.) to flourish (Jenkins et al. 1999, Kizlinski et al. 2002, Stadler et al. 2005). Therefore, *A. tsugae* infestations accelerated the transition of hemlock-dominated coniferous forests to a climax forest dominated by deciduous trees (Jenkins et al. 1999). Brooks (2004) reported very slow regeneration of eastern hemlock (500 to 1100 stems per acre) in harvested areas, infested by adelgids while regeneration of the deciduous species was five times greater in northwest Connecticut (Brooks 2004). Small et al. (2005) reported that HWA caused a 70% reduction in *T. canadensis* dominated forest cover from 1982 to 2002 in New England. The gaps created in the forest were immediately filled by black oak, *Quercus velutina* Lam., scarlet oak, *Quercus coccinea* Münchh., and red oak, *Quercus rubra* L. (Small et al. 2005). These oaks increased in basal area from 28% of total basal area in 1982 to 41% in 2002. Small et al. (2005) also recorded an overall increase in sapling density from 80 stems/ha in 1982 to 5600 stems/ha in 2002. In addition, the highest increase was noticed in *Sassafras albidum* and *Acer rubrum* saplings following *A. tsugae* infestation.

Forest ecosystems experienced rapid variation in nutrient status (Stadler et al. 2006) especially in the rate of N cycling (Jenkins et al. 1999) and organic matter decomposition (Kizlinski et al. 2002). A gradual change in the microclimate of adelgid-damaged forest's floors was also observed (Kizlinski et al. 2002). High light infiltration into the forest floor might rapidly increase temperature or moisture levels, which ultimately favor germination and growth of shade-intolerant tree, shrub, or herb species, and accelerates decomposition of accumulated organic matter. The rate of organic matter decomposition in HWA damaged forest floors was greater than in undamaged forest floors. Adelgid-infested sites might also have higher soil pH and net nitrification, therefore being more prone to nitrate leaching in the soil. However, there was no appreciable change in the N pool in HWA damages vs. undamaged sites (Kizlinski et al. 2002).

Adelgid-infested hemlocks had lower canopy biomass and new shoot density relative to uninfested trees (Stadler et al. 2005). Stadler et al. (2006) reported that higher K and N fluxes as well as greater litter leachates were noticed in adelgid-infested hemlocks than uninfested ones. Bacterial count was higher in the infested hemlock, probably favored by high levels of N substrate in the soil. In addition, these researchers suggested that possibly high-energy, carbon-rich waxy wool eroded from infested hemlocks contributed to accelerate bacterial growth (Stadler et al. 2006). Yorks et al. (2003) reported higher concentrations of nitrates in water three months after girdling to simulate HWA infestation)compared to non-girdled control hemlock stands. Moreover, high levels of ions such as Ca^{2+}, K^{+} and Mg^{2+} were noted in the treated hemlocks.

Higher hemlock mortality or lower tree vigor was noted in hemlock stands located in the South compared to the stands in the North (Orwig et al. 2002). These researchers suggested that latitude may have some influence on tree mortality or vigor and degree of *A. tsugae* infestation. No relationship between effective crown damage and tree mortality was reported within under-storied or over-storied hemlock stands. Adelgid infestation was found irrespective of site location, altitude, overstory composition (with varied density of hardwood), structure and slope (hillside) in New England. Orwig et al. (2002) suggested that the trees in the hemlock-dominated stands and those located in northern regions should be given a greater priority for HWA management measures than isolated hemlocks within the forests. Orwig et al. (2002) also suggested that regular monitoring and management for HWA infestation might be important to prevent complete destruction of hemlock stands in the forest.

Research Objectives

Project 1: Effects of fertilizer and low rates of imidacloprid on *Adelges tsugae* (Hemiptera: Adelgidae)

The first objective of this project was to manipulate tree health and adelgid populations using imidacloprid insecticide and fertilizer to provide an adequate, uninterrupted supply of high quality adelgids to support long-term predator population growth. It was expected that fertilization would increase adelgid fecundity while low levels of insecticide would prevent adelgids from significantly weakening trees so that they would support sufficient populations of HWA for predator consumption. McClure (1991b) showed that adelgid fecundity was twice as high on fertilized hemlocks and suggested that fertilization may improve hemlock health when adelgid populations are controlled.

Project 2: Distribution and abundance of *Adelges tsugae* (Hemiptera: Adelgidae) within hemlock trees

Various sampling protocols including binomial sequential sampling (Fidgen et al. 2006) or randomized branch sampling (Evans and Gregoire 2007) were proposed and tested to assess *A. tsugae* population at the individual tree or forest scale (Costa 2005). The randomized branch sampling determined that sisten *A. tsugae* distribution within the tree crown depended on their population density suggesting that sampling from the lower regions of tree alone may not always yield accurate densities (Evans and Gregoire 2007). However, these studies did not evaluate sisten and progredien adelgid densities throughout the crown strata over multiple years. Moreover, studies have shown that feeding behavior of adelgid generations is influenced by terpenoid chemistry in the needles that varies with shoot development (Lagalante et al. 2006). Also, *A. tsugae* populations vary through time and these fluctuations are influenced by tree health and physiology following the initial infestation (McClure 1991a). This density dependent feedback of *A. tsugae* populations and tree health could indirectly influence the performance of predators like *L. nigrinus* (Lamb et al. 2006). Understanding how *A. tsugae* is distributed within different regions of hemlock trees over relatively longer periods will help to design monitoring strategies for evaluation of insecticide efficacy and to improve deployment of biological control agents.

Project 3: Effect of fertilizer on hemlock woolly adelgid (Hemiptera: Adelgidae) growth on various hemlock species and feeding preference of specialist predators

Fertilizer is commonly used on nursery trees and shrubs and in ornamental landscapes (Braman et al. 1998), where its effects on host plant resistance is widely debated (Herms 2002). McClure (1992c) showed that fertilized *T. canadensis* increased egg production of *A. tsugae* by two fold and its density by five times relative to unfertilized trees. However, effects of fertilization have not been examined on resistant hemlock species to date. Currently, *L. nigrinus* and *S. tsugae* are two specialist predators released against *A. tsugae* in the forest (Cheah and McClure 1998, Zilahi-Balogh et al. 2003c, Lamb et al. 2006). Quality adelgid eggs are vital for both the predators as food or ovipositional sites (Palmer and Sheppard 2002, Lamb et al. 2006). Our objectives for this project were: 1) to evaluate the host plant resistance among various hemlock species; 2) to determine if hemlock resistance to hemlock woolly adelgid is affected by chemical fertilization: and 3) to determine if tritrophic interactions are affected by the prey quality.

Project 4: The range and response of neonicotinoids on hemlock woolly adelgid (Hemiptera: Adelgidae)

Various insecticide materials have already been applied using different delivery mechanisms such as foliar spray, soil drench, trunk and soil injections to suppress hemlock woolly adelgid populations (Gouger 1971, McClure 1991b, Steward et al. 1998, Doccola et al. 2005, Cowles et al. 2006). Foliar sprays demonstrated greater than 90% mortality of HWA (McClure 1991b) but did not yield uniform insecticide exposure. Chemical applications may be challenging in a forest scenario as it is difficult to reach remote locations; however, landscape trees are relatively accessible. Neonicotinoids, especially, imidacloprid are widely accepted by the nursery and landscaping industries as they are effective against a variety of pests such as aphids (Mullin and Christie 1995, Pettis et al. 2005) and hemlock woolly adelgids (Cowles et al. 2006, McClure 1992a, Steward and Horner 1994, Webb et al. 2003). Their high oral toxicity and systemic activity make them especially useful for treating trees. Of late, more neonicotinoids are commercially available, but information about their efficacy against hemlock woolly adelgid is not known. The objectives of this project were: (1) to evaluate eight insecticides for suppression of hemlock woolly adelgid on eastern hemlock growing in various landscape sites; and (2) assessment of insecticide efficacy (a) using low or high volume methods, (b) varying sites of application (soil or tree trunk), (c) varying insecticide rates, (d) timing (spring versus fall), and (e) evaluation of speed and length of residual activity.

Project 5: Olfactory response of hemlock woolly adelgid (Hemiptera: Adelgidae) predators to hemlock species (*Tsuga* spp.)

Classical biological control programs involve introduction of exotic natural enemies from the native range to regions where their prey are invasive. In the native range, we know that the derodontid predator, *L. nigrinus* attack *A. tsugae* developing on *T. heterophylla* and the coccinellid predator, *S. tsugae*, consume *A. tsugae* on *T. sieboldii* in southern Japan. These predators have been widely released to control *A. tsugae* populations on *T. canadensis* in the eastern US. No research has been undertaken to understand how the predators find the host habitat or HWA infestations. Broeckling and Salom (2003a) suggested that *L. nigrinus* might have a greater inherent olfactory-mediated behavioral affinity to *A. tsugae* than the coccinellid *S. tsugae* but provided no proof. The objective of this project was to understand if odors of *T. canadensis* branches would affect the preferences of the specialist predators, *S. tsugae* and *L. nigrinus*.

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CHAPTER 2

EFFECTS OF FERTILIZER AND LOW RATES OF IMIDACLOPRID ON *ADELGES TSUGAE* (HEMIPTERA: ADELGIDAE)

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ABSTRACT Healthy hemlock trees, *Tsuga canadensis* (L.) Carrière, and adelgid, *Adelges tsugae* Annand (Adelgidae) populations should favor retention and population growth of adelgid predators like *Laricobius nigrinus* Fender (Derodontidae) and *Sasajiscymnus tsugae* (Sasaji & McClure) (Coccinellidae). Sixty eastern hemlock trees between 15.2 and 38.1 cm diameter at breast height (dbh) were selected in the Chattahoochee National Forest, Helen GA. Trees were treated with 0, 10 or 25% of 1.5g imidacloprid (Merit 75WP) per 2.5 cm of tree dbh and were either fertilized or not, in a 3 x 2 factorial design. After two years, more ovisacs and eggs were found on trees that received imidacloprid in the order 0% > 10% > 25%. Fertilized trees had greater adelgid fecundity which was positively correlated with total foliar N in both winter generations. In February 2009, N concentration and *A. tsugae* fecundity were higher on fertilized trees receiving 10% insecticide compared to unfertilized trees receiving the same amount of insecticide. In addition, N content was higher in insecticide treated trees than in untreated trees regardless of fertilizer in June 2009 samples. Tree growth parameters such as new shoots or needles and length of new shoots per unit length of branch exhibited a dose response (25% > 10% > 0%) to insecticide in June 2009 samples. Concentrations of N, P and K were higher in the foliage of trees treated with insecticide. Foliar aluminum concentrations were consistently lower in trees with higher insecticide dosages. Likewise, foliar calcium, zinc, manganese and boron were also higher in unhealthy trees that did not receive imidacloprid. Trees treated with low rates of imidacloprid were healthier than untreated trees, but only trees treated with 10% of the recommended rate of imidacloprid had sufficient adelgids to possibly sustain predators over extended periods of time.

KEYWORDS: Hemlock Woolly Adelgid, eastern hemlock, predators, imidacloprid, fertilizer

The hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), is a serious threat to two native hemlock species; eastern hemlock, *Tsuga canadensis* (L.) Carrière, and Carolina hemlock, *Tsuga caroliniana* Engelmann of eastern North America. *Adelges tsugae,* a native to Japan, was first reported in the eastern United States near Richmond, VA in 1951, and quickly expanded its range to include 17 states from New England in the north to Georgia in the southern Appalachians (Havill et al. 2006). It was first documented in Georgia in 2004 (Johnson 2005) where it now occurs in at least 11 northern counties and is spreading at 15 km/yr (Evans and Gregoire 2007). *Adelges tsugae* causes direct damage by feeding on the storage parenchyma cells containing the xylem rays at the base of the needle (Shields et al. 1996). Populations of *A. tsugae* increase to very high densities within a few years reducing new shoot production and causing branch dieback. Heavy infestations can lead to tree mortality in as little as 2 to 3 years in the southern states (Trotter and Shields 2009). Hemlock woolly adelgid has two parthenogenic generations per year called sistens and progrediens (McClure 1989). In addition, progrediens produce a winged, sexual generation that fails to colonize due to absence of a suitable primary host (*Picea torano* (Koch) Koehne) in the continental United States (Montgomery et al. 2009). Adelgid populations grow rapidly on eastern and Carolina hemlock because they lack resistance and there are no effective native natural enemies (McClure 1991).

Potential management strategies for hemlock woolly adelgid include insecticides (McClure 1991, Cowles et al. 2006), host-plant resistance (Lagalante et al. 2006, Montgomery et al. 2009, Jetton et al. 2009), and natural enemies (McClure 2001, Zilahi-Balogh et al. 2003, Cheah et al. 2004, Lamb et al. 2006). Although effective, use of insecticides to suppress adelgid infestations is limited in remote forest landscapes because of inaccessibility, expense, and post-treatment environmental risks to aquatic life in nearby rivers or streams (Cowles et al. 2006).

Classical biological control is a more feasible and sustainable alternative to insecticides for hemlock woolly adelgid control in forests (McClure 1991), although it has not been proven effective yet across the range of hemlock.

Two predators, *Laricobius nigrinus* Fender (Derodontidae) and *Sasajiscymnus tsugae* (Sasaji & McClure) (Coccinellidae), have been widely released to manage hemlock woolly adelgids (McClure 1999, Lamb et al. 2006). *Laricobius nigrinus* is a univoltine specialist predator native to the Pacific Northwest that has a life cycle synchronized with *A. tsugae* development (Zilahi-Balogh et al. 2003, Zilahi-Balogh et al. 2003a). Pupae of *L. nigrinis* reside in soil from April to September while second instar nymphs of *A. tsugae* also aestivate. Adult beetles and hemlock woolly adelgid break their aestivation and become active by late October and remain active until late June. Female *L. nigrinus* oviposition and larval eclosion is in synchrony with oviposition by *A. tsugae* so beetle larvae can prey on adelgid eggs (Zilahi-Balogh et al. 2003, Zilahi-Balogh et al. 2003a).

In the mid-1990s, *S. tsugae* was responsible for remarkable success of adelgid control in Connecticut, New Jersey and Virginia by reducing 47-88% of the *A. tsugae* population with the release of 130,000 adult beetles (McClure and Cheah 1999). Lamb et al. (2005) indicated that *S. tsugae* primarily feed on progrediens in late spring; however, their recovery following release has been inconsistent. *Sasajiscymnus tsugae* are multivoltine and the overwintering adults are generally active beginning in early summer. Egg laying begins in early April and continues through June (Cheah and McClure 1998, and 2000).

Both these predator species are being mass-reared for field release in various biocontrol facilities on the East Coast. *Laricobius nigrinus* is difficult to rear in the laboratory, owing to asynchrony with prey in adult emergence, after pupating in the soil, often resulting in high beetle mortality (Lamb et al. 2007). Mausel et al. (2008) established a field insectary in 1.2- to 2.4 m tall *T. canadensis* artificially infested with adelgids, before *L. nigrinus* was released on them. This insectary program resulted in a population of *L. nigrinus* synchronized with its adelgid host and also produced significant numbers of beetles for release.

As a hemlock woolly adelgid outbreak progresses in a forest, tree health deteriorates (McClure 1991a). Even low density infestations of less than four adelgids per 20 mm^2 of branch can cause negative physiological changes affecting new shoot growth the following year. This reduction in new growth and overall tree health results in a subsequent decline in adelgid populations. *Laricobius nigrinus* larvae consume an average of 265 adelgid eggs at 18^oC before pupating and they prefer adelgid eggs over other stages (Zilahi-Balogh et al. 2003a). Both *S. tsugae* and *L. nigrinus* often prefer high quality adelgid eggs in lab rearing facilities (Palmer and Sheppard 2002, Lamb et al. 2006). Therefore, declining hemlock health and *A. tsugae* densities could influence predator oviposition and survival in the forest (Lamb et al. 2006), since unhealthy trees may not sustain the quality and quantity of adelgids necessary for predator growth.

The objective of our study was to manipulate tree health and adelgid populations using imidacloprid insecticide and fertilizer to provide an adequate, uninterrupted supply of high quality adelgids to support long-term predator population growth. It was expected that fertilization would increase adelgid fecundity while low levels of insecticide would prevent adelgids from killing the trees but still make sufficient populations available for predator consumption. McClure (1991b) showed that adelgid fecundity was twice as high on fertilized hemlocks and suggested that fertilization may improve hemlock health when adelgid populations are controlled.

Materials and Methods

Study Site and Experiment Design. The study was initiated in November 2006 in White Co., ≈30 km North of Helen, GA in the Chattahoochee National Forest. Eastern hemlock trees in this area were naturally infested by adelgids beginning in 2004 (Johnson 2005). Sixty eastern hemlock trees between 15.2 and 38.1 cm diameter at breast height (dbh), 7.3-24.6 m tall (mean = 15.6 m), and 25-70 yrs old were selected based on their accessibility from the road for sample collection using a hydraulic lift. Trees were treated with either 0, 10 or 25% of 1.5g of imidacloprid insecticide (Merit 75WP Bayer Environmental Science, Research Triangle Park, NC) per 2.5 cm of tree dbh and one of two levels of fertilization; fertilized or not. Insecticide was applied in a circle around the tree approximately 30 cm from the tree root collar and 5 cm deep using a Kioritz soil injector (Kioritz Corp., Tokyo, Japan) on November 14, 2006. One injection point was made for each 2.5 cm of tree diameter by pressing the Kioritz dispensing knob six times to deliver 29.5 mL per 2.5 cm dbh of insecticide solution into the soil. On 9 and 19 April 2007, half of the trees received their initial fertilizer treatment. Fertilizer rates varied based on tree size so that trees < 19.1, 19.1-35.6 and > 35.6 cm dbh received 453.6, 907.2 and 1360.8g N, respectively. The initial fertilizer was applied using a combination of fertilizer spikes (12-6-12 NPK, Miracle-Gro, Marysville, OH) at a rate of one spike per 1.22 m of drip-line diameter and an additional broadcast application with polymer-coated urea fertilizer (29-2-5 NPK, Sta-Green broadcast, St. Louis, MO). In 2008, a total of 907.2, 1814.6 and 2721.5g of N (polymer-coated urea fertilizer, 29-0-5 NPK, Sta-Green broadcast, St. Louis, MO) were broadcast in two applications beneath trees in the respective diameter classes used in 2007. One half of the fertilizer was applied on March 4 and the remainder on June 11.

Predator Release. The predator *S. tsugae*, obtained from NCDA and CS Beneficial Insects Laboratory (Cary, NC) was maintained at 20°C and 45% relative humidity (RH) before being shipped to our laboratory. Mated *L. nigrinus* were obtained from the predator-rearing laboratory at the University of Georgia (Athens, GA). To facilitate release, 50 *S. tsugae* and 17 *L. nigrinus* adults were placed in separate, ventilated 1.9 liter plastic containers (Rubbermaid, Wooster, OH) and provided with 2 to 3 freshly cut 20-cm long *T. canadensis* terminals infested with hemlock woolly adelgid. Predators were then stored at 12°C and 50% RH for two days prior to release. On 19 February 2008, 50 *S. tsugae* and 17 *L. nigrinus* were released per tree on a total of thirty trees (one tree of each fertilizer/insecticide treatment per block). The hemlock terminals with predators were removed from the containers and fastened to tree branches using twist ties in the upper crown regions of the tree. In most cases, tree branches containing predators also were naturally infested with adelgids. The temperature at the study site was < 8°C and the wind was < 8 km/h during the releases. Predators were released between 1000 and 1600 EST.

Sample Collection and Evaluation. Hemlock terminals from the treated trees were sampled on 14 June 2007; 19 February and 26 June 2008; and 23 February and 8 June 2009 using a hydraulic lift truck to access all parts of the canopy. On June 14, 2007, four 30-cm long hemlock branch terminals were sampled from each of 30 trees representing one treatment from each block. Two branch terminals were cut from the lower, and two from the upper tree crown so that one sample at each crown location (lower and upper) was taken from the side of the crown facing the road (road-side) and the other on the opposite or forest-side of the crown. In February and June of 2008 and 2009, four 30-cm long terminal branches were sampled from each of the 60 trees. Samples were placed in polyethylene bags, labeled and transported to the laboratory where they were stored at -5°C.

The number of ovisacs, eggs, and nymphs (crawlers and second-instars) were counted on each 30-cm branch, and the number of new branch shoots, the length of new shoots and the number of needles on new growth were measured as an estimate of tree health.

In 2009, after one year of release, adult predatory beetles were sampled at 10 locations (five each in upper and lower crown regions) within each tree using an insect beat sheet (98 by 98 cm size, BioQuip Products Inc., Rancho Dominguez, CA). Adelgid infested branches, or uninfested branches if no adelgids could be found, were struck at least 10 times and carefully examined for adult beetles. Predaceous beetles were sampled between 1000 and 1600 h on relatively warm days to increase the probability of beetle activity and collection.

On September 10, 2009 we used the crown measurement and sampling procedures (Table 2.1) from the U.S. Forest Service FIA Core Field Guide (USDA, 2001) to assess the overall health of our study trees. Hemlock health was assessed on an individual tree basis for all sixty trees.

Insecticide Residue Analysis. Foliar imidacloprid residues were measured on 30 trees (1 for each treatment per block) to examine how insecticide residues correlated with adelgid population trends within treatments. Analyses were limited to 30 trees to minimize costs. Two to three 10-cm long terminals were taken from upper, middle and lower canopy locations of 30 trees (6 treatments, 5 replications) for analysis of imidacloprid residues in the foliage during each sampling date (February and June) in 2008 and 2009. Samples from each tree were combined into a single sample for analysis except in June 2008 when two samples of 10-cm long terminals were collected from both the lower and upper third of the crowns of 30 trees to examine for distribution of imidacloprid in the canopy. Samples were immediately placed in polyethylene bags and stored in a field cooler until they were brought to the laboratory and stored at -60^{o}C.

Samples for imidacloprid residue analyses were shipped overnight in insulated boxes containing 4.5 kg of dry ice to maintain the temperature below $0^{o}C$ to the University of California (Riverside, CA). Concentrations of imidacloprid within hemlock samples were measured using a competitive ELISA (enzyme linked immunosorbent assay) technique (Byrne et al. 2005). The imidacloprid results are expressed as ppb or ng of imidacloprid per gram of hemlock tissue (wet weight). The limit of detection for this assay was 50 ng/g hemlock tissue (wet weight) for all sample dates except the June 2008 samples in which the detection limit was 25 ng/g of hemlock tissue. Concentration below the 25 or 50 ng/g detection limit was recorded as zero.

Tree Nutrient Analysis. Foliar nutrient content was measured to assess N uptake within fertilized and unfertilized trees, and to examine for correlations of nutrient contents with adelgid densities and tree growth parameters. Samples consisted of 50g of hemlock terminals cut from branches throughout the canopy. The February 2008 sample included both needles and fine woody branch material up to 2 mm diameter. Subsequent samples (February and June 2009) contained only needles. All sixty trees were sampled and analyzed for each sample date. Samples were oven-dried (at $40^{o}C$ for 48 h) and taken to the Plant and Soil Testing Laboratory (University of Georgia, Athens, GA) for analysis. Nutrients (Mn, Fe, Al, B, Cu, Zn, Na, Pb, Cd, Ni, Cr, Mo, P, K, Ca and Mg) were analyzed using an inductively coupled plasma emission spectrograph (Isaac and Johnson 1985, AOAC, Official Method 985.01. 1995). Total percentage N was quantified by the combustion method of Colombo and Giazzi (1982).

Statistical Analyses. The experiment was arranged in a factorial design consisting of 10 replications in five blocks (two replications per block). Trees were grouped based on proximity to one another and blocks also represented changes in elevation. We selected 60 trees (2 trees/treatment/block) for treatment because the study area was scheduled for selective harvesting to remove hazard trees along the road. Since trees to be removed were scattered throughout our study area we were concerned some of our treated trees would be damaged or broken off during harvesting so we selected and treated twice as many trees as originally planned. Harvesting was delayed for a variety of reasons so we were able to sample all 60 trees throughout the study.

The effects of insecticide and fertilizer treatments on hemlock woolly adelgid populations and tree growth parameters were analyzed as a 3 x 2 factorial experiment with interaction using the general linear models procedure of SAS (Proc GLM, SAS Institute 2003). Adelgid count and tree growth parameter data from four canopy locations were combined into a single sample per tree. Each 30-cm long hemlock branch used for assessing adelgid densities and tree growth parameter varied in total length because they had differing numbers and lengths of side-branches. Therefore, all adelgid counts and growth parameters were standardized by dividing them with respective total hemlock branch lengths within a 30-cm long sample and expressed on a per cm of shoot length basis. These standardized independent variables, which included number of ovisacs, eggs, nymphs (crawlers and second instars), new shoots, total length of new shoots (in cm), needles on new shoots, and nymphs on new shoots, were transformed using the natural log (x + 1) after testing for homogeneity of variances using the PROC Univariate procedure of SAS (SAS Institute 2003). The variable eggs per ovisac and all percentage data were transformed using the natural log. Because variances were homogeneous for foliar nutrient, tissue imidacloprid concentrations and tree health survey data, these data were not transformed. The transformed and untransformed data for each sample date were examined using the PROC GLM procedure of SAS and means were separated using the least significant difference (LSD) method ($\alpha = 0.05$). The relationship between the numbers of *A. tsugae* or its fecundity and concentrations of N or imidacloprid were determined using regression analysis using PROC REG procedures of SAS. Untransformed means and standard errors are reported in tables and figures.

Results

Effects of Insecticide and Fertilizer. There were no interactions between fertilizer and imidacloprid for any adelgid life stage or tree growth parameter during any sample period so we present the insecticide and fertilizer effects separately. Imidacloprid applied in November 2006 did not affect *A. tsugae* progredien ovisac densities or eggs per cm of twig in June 2007 regardless of application rate (Table 2.2).

Likewise, sisten *A. tsugae* densities in February 2008 were unaffected by imidacloprid despite significantly higher imidacloprid residues in needles of 25% treated trees compared to untreated ones (Fig. 2.1a, Table 2.2). However, by June 2008 progredien ovisacs and eggs per cm of branch were higher on untreated trees than on trees treated at 10% or 25% of the recommended rate of imidacloprid (Fig. 2.1b, Table 2.2). In addition, sisten nymph densities were higher on untreated trees (0%) than on trees treated with 10% imidacloprid, which had higher numbers of nymphs than trees treated with 25% of the recommended rate of imidacloprid. Insecticide residues in foliage were higher in trees that received the highest rate of imidacloprid than in trees that received the lower rate (Figs. 2.1a and b). Trees treated at 10% of the recommended rate had detectable residues but they were not significantly higher than untreated trees.

The sisten generations of 2009, had fewer ovisacs and eggs per cm of branch on trees that received imidacloprid (Fig. 2.2a, Table 2.2). Trees treated at the highest rate had the fewest sisten life stages, followed by trees that received 10% of the recommended rate. Trees treated with both rates had fewer sistens than untreated trees. Despite having a significant impact on adelgid populations, imidacloprid residues in trees treated at the 10% rate were not significantly different from the controls.

In June 2009, the detected imidacloprid residues in the foliage were greater in the 25% insecticide treated tree followed by a significantly lower level in 10% treated trees (Fig. 2.2b). This was the first sample in which residues in foliage from trees treated at 10% of the recommended rate were significantly higher than the controls although adelgid populations were clearly being affected by both insecticide treatments (Fig. 2.2b). Despite higher imidacloprid residues in trees treated at 10% of the recommended rate, the progredien life stages and sisten nymphs exhibited the same trend as before, i.e., the highest populations were on untreated trees, followed by those treated at 10% of the recommended rate which were higher than adelgid populations on trees treated at the 25% rate. Alates were also reduced on imidacloprid treated trees.

Imidacloprid did not have an immediate effect on growth parameters. Length of new growth, numbers of new needles and numbers of new shoots were not significantly higher in the June 2007 sample (Tables 2.2 and Fig. 2.3). However, after 12 months (June 2008), trees treated with the highest rate of imidacloprid produced more shoots, more new needles and a greater length of new growth per cm twig relative to the 10% insecticide rate or untreated trees which were not significantly different. By June 2009 a dose response in the growth parameters was evident with the highest number of new shoots, new needles and length of new shoots per unit branch length on 25% treated trees followed by the 10% rate, and then by untreated trees. Fertilization had no effect on tree growth parameters throughout the study (Table 2.4).

Nitrogen fertilization did increase fecundity of sisten females in 2008 and 2009 (Fig. 2.4, Table 2.3). Although the initial fertilization did not result in significantly higher foliar N levels in winter 2008, fecundity exhibited a positive linear relationship with foliar N content of sample trees ($F = 6.6$; df = 1, 46; $P = 0.013$; Fig. 2.5a). By winter 2009, following two additional fertilizer applications in spring and summer 2008, foliar N was significantly higher in fertilized trees ($F = 18.2$; df = 1, 45; $P < 0.0001$) than in unfertilized trees. Likewise, sisten fecundity had a positive linear relationship with foliar N content of individual sample trees ($F = 5.7$; df = 1, 35; $P = 0.022$; Fig. 2.5b). Although foliar N content was significantly higher in fertilized trees ($F = 5.60$; df = 1, 44; $P = 0.0224$, Fig. 2.4), it did not contribute to more fecund sisten females in summer 2009 (Fig. 2.4).

In February 2009, foliar N content was higher in fertilized trees receiving 10% ($F = 7.54$; df = 1, 9; $P = 0.0226$) and 25% insecticide ($F = 21.30$; df = 1, 9; $P = 0.0013$) compared to unfertilized ones (Fig. 2.6). An increase in *A. tsugae* sisten fecundity was observed on fertilized trees within the 10% insecticide treatment ($F = 15.5$; df = 1, 9; $P = 0.0458$). The same trend was found in foliar N content ($F = 21.30$; df = 1, 9; $P = 0.0013$) on fertilized and 25% insecticide treated trees, but no effect on fecundity could be detected.

After a predator release (*S. tsugae* and *L. nigrinus*) in February of 2008, no adult predators were recovered from the beat-sheet samples of 2009. In addition, regular samples did not yield any predator stages between February and June samples of 2009.

Tree Health Survey. The effects of insecticide and fertilizer on tree growth parameters like amount of new growth and number of new needles presented in Fig. 2.3. Since fertilization did not affect these parameters, we grouped fertilized and unfertilized trees within insecticide treatments when analyzing tree health characteristics. Live crown ratio was similar within trees that received insecticide or not. Tree crown density of 25% insecticide treated trees was higher than untreated trees but not 10% treated trees (Table 2.5). In a related measurement, untreated and 10% insecticide treated tree canopies allowed more sunlight to pass through compared to trees that received the highest rate of insecticide. One of the tree decline symptoms, crown dieback, was greater on untreated than on insecticide treated trees. The percentage of living branches was greater in the 10% or 25% insecticide treatment groups than in the 0% treatment group. The percentage of newly growing branches was significantly greater in the following order: 25% > 10% > 0% of the recommended rate of imidacloprid.

Plant Nutrient Analyses. Our results suggest that foliar tissue nutrient levels changed as tree health improved. In summer 2009, N, P and K occurred at higher levels in foliage of insecticide treated trees than untreated ones. However, Ca and Zn levels were higher in untreated trees than in treated ones (Table 2.6). Likewise, Al concentrations were greater in control trees while tree treated with 10% of the recommended rate of imidacloprid had higher concentration of Al than those treated at the 25% rate. Boron (B) and Mn were also higher in untreated or 10% insecticide treated trees than in 25% insecticide treated trees. Besides the differences in mineral nutrients in insecticide treated trees, we also noted difference in P content in trees that received fertilizer or not. There was greater P content in unfertilized trees ($F = 5.65$; df = 1, 45; $P = 0.0217$; mean ± SE = 0.16 ± 0.01) than fertilized trees (mean ± SE = 0.14 ± 0.01) for the same sample date.

Discussion

Because *L. nigrinus* and *S. tsugae* require healthy adelgids which, in turn, require healthy hemlocks, we sought to determine the extent to which applications of insecticide and fertilizer are likely to promote predator establishment. It has been previously shown that soil injection of

71.4% rate of imidacloprid (of the recommended rate 1.4g [AI] per 2.5 cm dbh) caused 50-100% suppression of adelgid populations on eastern hemlocks (Cowles et. al 2006). However until now, the effect of yet lower rates of imidacloprid on *A. tsugae* population was unknown. After 14 months post-treatment, in summer of 2008, progredien adelgids were less dense on trees treated with all rates of insecticide compared to untreated trees. However, trees that received 10% rate had a moderate level of sisten nymphs, which was greater than those nymphs settled on trees treated with 25% rate, but lower than on untreated trees. In both winter and summer of 2009, sisten and progredien life stages exhibited a dose response in their abundance where the adelgid population followed this pattern: 0% > 10% > 25%. This suggests that a low rate of imidacloprid 10% or less of the recommended rate could allow trees to maintain a moderate density of *A. tsugae*. In addition, a tremendous improvement in the tree health was also found on the insecticide-treated trees proportional to the dose that they received. One year after application in summer 2008, trees treated with a quarter rate of imidacloprid produced more new shoots, than the 10% treated or untreated trees. Unlike the previous year, in the summer of 2009 new growth was denser on 25% imidacloprid treated trees followed by 10% treated trees, which had denser new growth than untreated trees. It seems that trees treated with a 10% rate of insecticide, not only maintained a moderate density of *A. tsugae* but also improve the tree health to possibly sustain predators for a longer time. However, those trees treated with a quarter rate of insecticide had a very low population of *A. tsugae*; thus, at least for a few years, they may not be suitable to maintain a sustainable population of specialist predators.

Imidacloprid residues in hemlock tissue were consistently higher in trees receiving the quarter rate of insecticide than in those trees that received the 10% rate in all sample dates. Indirect effects of insecticide-treated adelgid-infested branches on *L. nigrinus* and *S. tsugae* were not tested in this study. However, Eisenback et al. (2008) studied survival rate, feeding preference and level of toxicity of adelgid-infested branches treated at the quarter rate (of 1.4g) of imidacloprid on predators and did not find any evidence of indirect toxicity on either predator species, and no traces of insecticide residues were detected in beetle cadavers. Based on their study, it is unlikely that the low rates of imidacloprid in our study would have any direct or indirect toxic effects on survival and development of predators.

Our results showed that fertilized trees had greater adelgid fecundity in the winter generations. Nitrogen content was higher in fertilized trees than in unfertilized ones in 2009 samples. It is evident from several studies that a high level of N in host plants after application of mineral N would enhance the fecundity of piercing and sucking insects (Petitt et al. 1994, Kytö et al. 1996, van Emden 1996, Nevo and Coll 2001). In addition, McClure (1991b) showed an immediate increase in *A. tsugae* fecundity when they developed on young *T. canadensis* trees after a spring application of fertilizer. *Adelges tsugae* eggs are vital for *L. nigrinus* larval development (Zilahi-Balogh et al. 2003a). In the current study, adelgid females developed on fertilized trees produced 14-17 eggs/female compared to 7-13 eggs/female on unfertilized trees during winter generations. This higher adelgid fecundity would likely enhance predator survival and establishment. In addition, on trees within the 10% rate of insecticide, higher adelgid fecundity and foliar N content were seen on fertilized than on unfertilized trees in winter 2009. This result clearly demonstrated that it is possible to maintain healthy trees and high *A. tsugae* fecundity using low rates of insecticide and fertilizer applications so that the predators will have ample food as their populations grow.

As per our result, one could argue, why only sistens developing on fertilized trees were more fecund compared to those that developed on unfertilized trees. One explanation is that aestivating sisten nymphs possibly exert less stress on trees (Lagalante et al. 2006). As a result, trees could amass and store reserves in their xylem parenchyma cells. Once aestivating nymphs break diapause, they utilize these stored reserves and develop into healthy females. These healthier females developed on fertilized trees oviposit more progredien eggs than those developed on unfertilized trees. This might explain why sisten females had high fecundity in the winter. However, we did not find a consistently greater level of N on fertilized trees as compared to untreated in either winter 2008 or 2009. In 2008, we used both foliage and small branches in samples processed for nutrient content, while in 2009 samples we analyzed only foliage. The small branches in 2008 samples likely contained lower amounts of nutrients than the foliage and this might have masked the N content in the foliage where the adelgids feed.

Regardless of the foliar N content, late spring samples did not show a notable effect of fertilizer on progredien fecundity. This was consistent with a previous study where an increase in *A. tsugae* fecundity was not attained in the spring generation on older *T. canadensis* forest trees even though the fertilizer had been delivered by trunk injection (McClure 1992). Although the exact reason of this observation is unknown, there could be several possible reasons. First, progredien crawlers settle on the previous-year shoots beginning early-March to late April. Montgomery et al. (2009) observed that the bud-break of eastern hemlock usually started from the first-week of April; thus, crawlers have no new shoots immediately after their emergence. Moreover, terpenoid levels are more unstable in newly growing shoots than in older shoots, but sisten nymphs that have a choice between new expanding foliage and older foliage may not prefer to settle on new branches (Lagalante et al. 2006). Second, N is mobile within the tree and usually moves from the storage parenchyma cells in older shoots to newly developing tissues commencing in early spring which appears to occur in *T. canadensis* as well (Stadler et al. 2005, Lagalante et al. 2006). This N metabolism is rapid and in *A. piceae* (Ratzburg) infested Pacific silver fir, *Abies amabilis* (Dougl.) Forb. it is quickly incorporated into new flushes of fertilized trees (Carrow and Graham 1968). High N-remobilization also occurs from older leaves to new shoots of *Quercus glauca* Thunb. ex Murray (Miyazawa et al. 2004) and Norway spruce, *Picea abies* Karst. (Nommik 1966). Therefore, because the developing progredien adelgids settle on the older tissue where the N is moving from, they may not get access to N rich nutrients, newly deposited in the xylem parenchyma of the new tissues to enhance fecundity. In addition, nutrient analysis of summer 2009 samples detected more foliar N content in fertilized trees than in unfertilized ones, possibly because these samples were composed primarily of new shoots and foliage. Third, mature or older trees may not be as efficient as younger ones in nutrient uptake from the soil. For example, it has been shown that fertilizer uptake decreased with tree age in young Norway spruce stands (Nommik 1966).

We did not recover any of the life stages of *L. nigrinus* or *S. tsugae* in 2009 samples following their release in February 2008. Several factors may have affected the predators and our ability to recover them including low-densities of released predators, poor quality of adelgid population, or low available soil-moisture essential for *L. nigrinus* pupal development. McClure et al. (2000) reported that thousands of adult *S. tsugae* beetles were released per site and suppression of *A. tsugae* populations was observed but no predator recovery was reported. In 2004, Lamb et al. (2006) recovered only six adult *L. nigrinus* beetles after single release in 2003. So the low number of predators released may have resulted in our inability to recover their progeny the following year. Georgia also experienced a severe drought in 2007 and 2008 which may have both stressed the experimental trees and affected *L. nigrinus* pupal survival in the moisture-deficient soil. Furthermore, treated trees were relatively tall and widely spaced to facilitate treatment and sampling so it was less likely *L. nigrinus* adults would find a treated tree following pupation in the soil. A more effective method might be creation of clusters of treated trees so predators that disperse are likely to find suitable, healthy adelgid populations on nearby trees. This would be especially true for *L. nigrinus* because they pupate in the soil. Using clusters of treated trees should work since healthy populations of *L. nigrinus* were successfully reared on planted young hemlock trees artificially infested with hemlock woolly adelgid in a field insectary (Mausel et al. 2008). Multiple releases of predators on trees that receive fertilizer and low level of imidacloprid would likely improve establishment of predator populations. Flowers et al. (2005 and 2006) recommended combined release of both predators in the field since they found no competitive interaction between them so one cluster of treated trees could serve as a breeding site for both predators.

It seems that low rates of insecticide improved the foliage chemistry. Residual nutrient concentrations showed enhanced levels in essential nutrients especially N, P and K in the healthy foliage of insecticide treated trees. It is likely that high adelgid density depleted nutrient content of trees not treated with insecticides. Studies showed that *A. tsugae* susceptible *T. canadensis* had greater levels of N and K, and low P relative to resistant hemlock species, making it more attractive to colonization by adelgid (Pontius et al. 2006). Aluminum content in the needles was consistently reduced as the insecticide dosage increased. It has been previously reported that low levels of Ca in soil results in more Al in the soil solution and increased Al uptake by plants (Tisdale and Nelson 1975). Aluminum places additional stress on trees making them more vulnerable to insect attack (DeHayes et al. 1999, Schaberg et al. 2006). Other foliar minerals such as Ca, Zn, Mn and B tended to accumulate in unhealthy trees that did not receive imidacloprid. This suggests that *A. tsugae* colonization caused an imbalance in foliar chemistry and imposed indirect secondary stress to *T. canadensis*.

Trees treated with low rates of imidacloprid were healthier than untreated trees but only trees treated at the 10% rate and fertilized had sufficient adelgids to possibly sustain predators over extended periods of time. Higher populations of adelgids and increased fecundity on fertilized trees, especially in winter, may be beneficial for survival and development of specialist predators like *L. nigrinus* and *S. tsugae*. Prolonged tree health plus sustained, healthy adelgid populations should benefit other predators as well, allowing them to build up populations and spread to the surrounding forest. This will be one time application of insecticide to provide predator a window of opportunity to establish. More research should focus on multiple releases of predators and evaluation of their establishment on adelgid populations developed on healthy trees. Once the predator population grows in the forest, it should reduce *A. tsugae* populations, reducing the need for future insecticide treatments.

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Table 2.1. Criteria used in a survey of hemlock health on imidacloprid and fertilized trees using the assessment protocol in the U.S. Forest Service FIA field guide (2001)

Criteria	Description	Measurement
Tree class	Crown top (highest point of tree) relative to adjacent neighboring trees. Dominant (D): Crown extends above the general canopy. Co-Dominant (C): Crown held at the level of canopy. Intermediate (I): Crown shorter than adjacent trees. Suppressed (S): Adjacent trees surpass completely.	Indicated as D,C,I or S
Live crown Ratio	Crown's height relative to total height of the tree.	0-100%
Crown density	Accounts all structures within the crown including foliage and branches that interrupt light relative to healthy tree.	0-100%
Foliage Transparency	Sky-light permeability through the tree otherwise blocked by foliage.	0-100%
Crown dieback	Extent of dieback on the branch terminals.	1: <10%; 2: 10-50%; 3: 51-75%; 4: >75%[a]
Percent live branches	Relative presence of health branches to dead ones.	0-100%
Percent new shoots	Estimates amount of current-year shoots produced in the whole tree.	1: <10%; 2: 10-50%; 3: 51-75%; 4: >75%[a]

[a] The percentage range measurement is categorized into 1-4 scale system.

Table 2.2. Analysis of variance of insecticide effects on *A. tsugae* numbers per cm of branch, tree growth parameters per cm shoot and imidacloprid residues in the hemlock tissues from 2007 to 2009 for trees treated with low rates of imidacloprid insecticide in November 2006

Variable	2007 June			2008 Feb			2008 June			2009 Feb			2009 June		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*
Adelgid counts															
Ovisacs[a]	0.3	2, 20	0.7150	1.4	2, 45	0.2427	7.1	2, 45	0.0020	9.6	2, 45	0.0003	18.9	2, 45	<.0001
Eggs[b]	0.4	2, 20	0.6778	1.9	2, 45	0.1544	5.0	2, 45	0.0105	25.3	2, 45	<.0001	16.8	2, 45	<.0001
Eggs/ovisac	1.3	2, 20	0.2864	0.2	2, 33	0.8174	1.8	2, 35	0.1764	2.5	2, 23	0.0995	1.1	2, 32	0.3366
Nymphs	1.7	2, 20	0.1969	0.8	2, 45	0.4577	28.1	2, 45	<.0001	-	-	-	12.2	2, 45	<.0001
Tree growth															
New shoots[c]	0.5	2, 20	0.5653	-	-	-	6.9	2, 45	0.0024	-	-	-	22.4	2, 45	<.0001
Length of new growth	0.4	2, 20	0.6328	-	-	-	4.4	2, 45	0.0180	-	-	-	29.9	2, 45	<.0001
New needles	0.6	2, 20	0.5355	-	-	-	6.9	2, 45	0.0024	-	-	-	28.5	2, 45	<.0001
Imidacloprid															
Residue	-	-	-	3.8	2, 16	0.0432	3.7	2, 15	0.0468	7.3	2, 16	0.0054	11.9	2, 16	0.0007

Analysis performed on log-transformed adelgid population growth and tree growth parameters data. Imidacloprid residue data was untransformed. [a] Ovisacs represent progredien generation during June and sisten generation during February. [b] In contrast, eggs and nymphs represent progredien generation during February and sisten generation during June. [c] Growth parameters were only measured on hemlock branches during summer.

Table 2.3. Analysis of variance of effects of fertilizer applied in spring 2007 and spring and summer 2008 on *A. tsugae* numbers per cm of branch within insecticide treatments from 2007 to 2009

Adelgid counts	2007 June			2008 Feb			2008 June			2009 Feb			2009 June		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*
All Treatments															
Ovisacs[a]	0.61	1, 20	0.4425	3.70	1, 45	0.0608	0.91	1, 45	0.3454	2.40	1, 45	0.1277	0.20	1, 45	0.6109
Eggs[b]	0.00	1, 20	0.9494	2.20	1, 45	0.1449	1.08	1, 45	0.3053	2.50	1, 45	0.1179	0.00	1, 45	0.9847
Eggs/ovisac	0.77	1, 20	0.3903	8.26	1, 33	0.0070	0.90	1, 35	0.3490	6.10	1, 24	0.0207	0.00	1, 32	0.8025
Imidacloprid 0%[c]															
Ovisacs	5.31	1, 4	0.1029	1.30	1, 9	0.2878	0.16	1, 9	0.6961	1.50	1, 9	0.2424	0.71	1, 9	0.4182
Eggs	0.50	1, 4	0.5066	0.08	1, 9	0.7807	0.52	1, 9	0.4930	1.61	1, 9	0.2362	1.10	1, 9	0.4150
Eggs/ovisac	0.02	1, 4	0.8876	7.54	1, 9	0.0226	0.82	1, 9	0.3877	1.52	1, 9	0.2453	0.00	1, 9	0.9210
Imidacloprid 10%															
Ovisacs	0.01	1, 4	0.8744	2.11	1, 9	0.1843	0.61	1, 9	0.4562	1.60	1, 9	0.2262	0.20	1, 9	0.6139
Eggs	0.01	1, 4	0.8926	1.94	1, 9	0.2011	0.75	1, 9	0.4121	1.60	1, 9	0.2341	0.00	1, 9	0.8656
Eggs/ovisac	2.90	1, 4	0.1637	0.98	1, 5	0.3671	0.14	1, 8	0.7162	20.30	1, 2	0.0458	0.69	1, 5	0.4447
Imidacloprid 25%															
Ovisacs	15.10	1, 4	0.0301	5.34	1, 9	0.0496	3.17	1, 9	0.1127	0.10	1, 9	0.7332	0.40	1, 9	0.5066
Eggs	3.81	1, 4	0.1452	3.10	1, 9	0.1161	2.89	1, 9	0.1277	0.00	1, 9	0.8100	0.10	1, 9	0.7532
Eggs/ovisac	0.35	1, 4	0.5881	1.16	1, 3	0.3596	3.37	1, 4	0.1404	2.75	1, 1	0.3455	2.97	1, 1	0.3347

Analysis performed on log-transformed adelgid population growth. [a] Ovisacs represent progredien generation during June and sisten generation during February. [b] In contrast, eggs represent progredien generation during February and sisten generation during June. [c] Insecticide applied in November 2006.

Table 2.4. Analysis of variance of effects of fertilizer applied in spring 2007 and spring and summer 2008 on tree growth parameters per cm of branch within insecticide treatments from 2007 to 2009

Tree Parameters	2007 June			2008 June			2009 June		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*
All Treatments									
New shoots	0.13	1, 20	0.7266	0.01	1, 45	0.9095	0.02	1, 45	0.8790
Length of new growth	0.01	1, 20	0.9171	0.29	1, 45	0.5902	0.17	1, 45	0.6805
New needles	0.05	1, 20	0.8214	0.13	1, 45	0.7213	0.35	1, 45	0.5560
Imidacloprid 0%									
New shoots	0.88	1, 4	0.4011	0.34	1, 9	0.5755	0.10	1, 9	0.7596
Length of new growth	0.94	1, 4	0.3879	1.13	1, 9	0.3163	0.31	1, 9	0.5931
New needles	0.89	1, 4	0.3982	0.61	1, 9	0.4558	0.38	1, 9	0.5539
Imidacloprid 10%									
New shoots	0.75	1, 4	0.4361	0.34	1, 9	0.5734	0.13	1, 9	0.7228
Length of new growth	0.71	1, 4	0.4458	0.05	1, 9	0.8318	0.15	1, 9	0.7083
New needles	0.73	1, 4	0.4399	0.06	1, 9	0.8048	0.24	1, 9	0.6360
Imidacloprid 25%									
New shoots	0.78	1, 4	0.4278	0.05	1, 9	0.8343	0.48	1, 9	0.5062
Length of new growth	0.84	1, 4	0.4124	1.35	1, 9	0.2749	0.00	1, 9	0.9879
New needles	0.69	1, 4	0.4519	0.96	1, 9	0.3532	0.01	1, 9	0.9208

Table 2.5. Mean (± SE) tree crown health characteristics measured on September 10, 2009 on trees treated with different rates of imidacloprid insecticide in November 2006

	% of recommended rate			*F*	df	*P*
	0%	10%	25%			
Live crown ratio	65.7 ± 3.5b	67.0 ± 3.0a	73.2 ± 2.9a	2.17	2, 47	0.1251
Crown density	52.0 ± 3.7b	63.2 ± 4.4ab	73.2 ± 4.3a	5.93	2, 47	0.0051
Foliage transparency	66.7 ± 4.5a	50.9 ± 5.4a	33.7 ± 5.0b	13.19	2, 47	<.0001
Crown dieback	2.0 ± 0.1a	1.2 ± 0.1b	1.3 ± 0.1b	13.87	2, 46	<.0001
Percent live branches	49.5 ± 5.5b	68.0 ± 5.4a	72.1 ± 4.9a	10.76	2, 47	0.0001
Percent new shoots	1.8 ± 0.1c	2.8 ± 0.2b	3.5 ± 0.1a	26.51	2, 47	<.0001

Means in a row followed by different letters are significantly different ($P < 0.05$). Analyses of variance were performed on log-transformed live crown ratio, crown density, foliage transparency and percent live branches data, while untransformed data were used for crown dieback and percent new shoots.

Table 2.6. Mean (± SE) of the foliar nutrient content in June 2009 of hemlock trees treated with three rates of imidacloprid in November 2006

Foliar Nutrient	% of recommended rate					
	0%	10%	25%	*F*	df	*P*
N	1.320 ± 0.049b	1.466 ± 0.058a	1.446 ± 0.048a	4.5	2, 45	0.0159*
Ca	0.653 ± 0.033a	0.474 ± 0.031b	0.405 ± 0.030b	20.2	2, 45	<.0001*
K	0.431 ± 0.029b	0.599 ± 0.034a	0.669 ± 0.028a	5.2	2, 45	0.0088*
P	0.132 ± 0.009b	0.164 ± 0.007a	0.167 ± 0.007a	5.9	2, 45	0.0051*
Mg	0.127 ± 0.006a	0.119 ± 0.005a	0.113 ± 0.003a	1.9	2, 45	0.1537
S	0.161 ± 0.005b	0.176 ± 0.004a	0.152 ± 0.003b	6.5	2, 45	0.0031*
Al	584.265 ± 24.770a	506.375 ± 22.539b	406.310 ± 13.982c	20.1	2, 45	<.0001*
B	39.499 ± 2.166a	34.892 ± 2.223a	29.256 ± 1.817b	7.6	2, 45	0.0015*
Cu	1.837 ± 0.226a	2.617 ± 0.373a	2.451 ± 0.240a	2.5	2, 45	0.0902
Fe	79.482 ± 15.732a	70.558 ± 8.687a	53.928 ± 3.877a	1.3	2, 45	0.2619
Mn	1065.550 ± 66.139a	796.565 ± 92.755a	676.620 ± 58.445b	7.8	2, 45	0.0012*
Zn	24.634 ± 1.588a	20.861 ± 1.164b	19.600 ± 1.332b	4.0	2, 45	0.0235*

Means of Ca, K, Mg, N, P and S shown above are expressed in total % concentration (of dry wt.), while Al, B, Cu, Fe, Mn and Zn are in ppm in the hemlock foliage. Means in a row followed by different letters are significantly different (* $P < 0.05$). Analyses of variance were performed on the untransformed data.

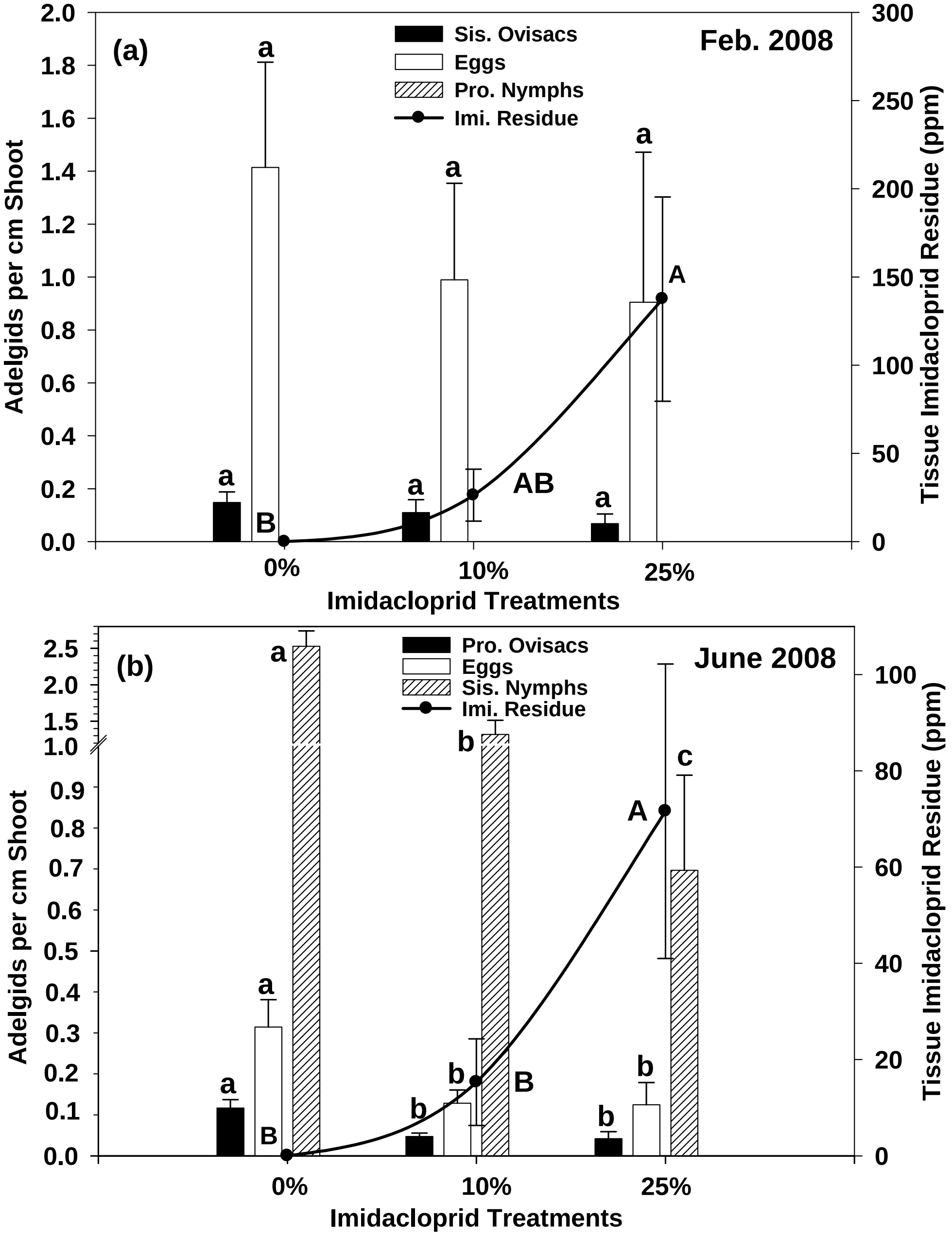

Fig. 2.1. Effects of different rates of imidacloprid (% of recommended rate of 1.5g imidacloprid/2.5cm dbh) on various life stages of hemlock woolly adelgid (left y-axis) and foliar imidacloprid residues (right y-axis) in *T. canadensis* (means ± SE, N = 20) in 2008. Insecticide was applied November 2006. The abbreviations Sis. = sisten and Pro = progredien. Bars of the same fill color with the same letters are not significantly different (LSD, $\alpha = 0.05$).

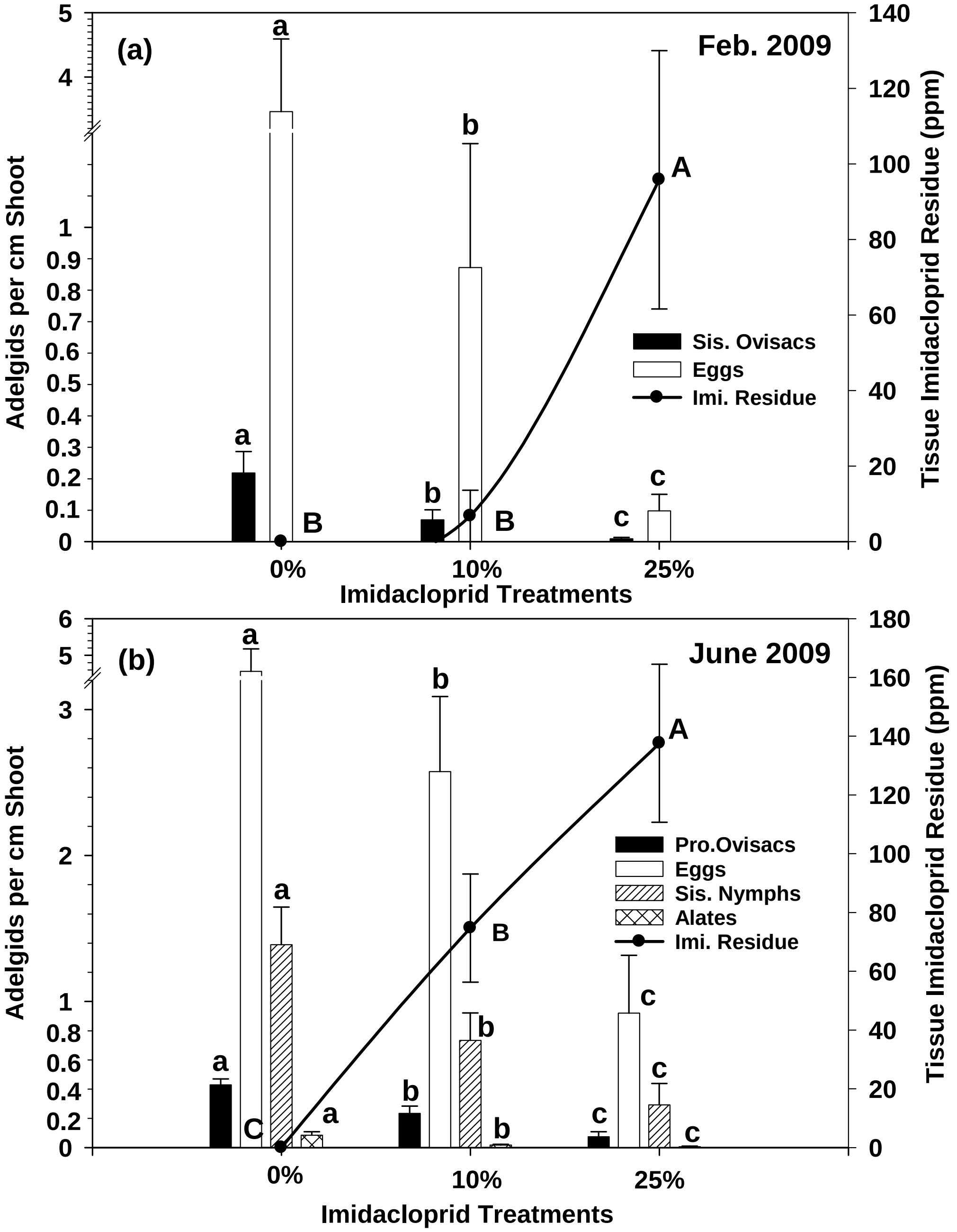

Fig. 2.2. Effects of different rates of imidacloprid (% of recommended rate of 1.5g imidacloprid/2.5cm dbh) on various life stages of hemlock woolly adelgid (left y-axis) and foliar imidacloprid residues (right y-axis) in *T. canadensis* (means ± SE, N = 20) in 2009. Insecticide was applied November 2006. The abbreviations Sis. = sisten and Pro = progredien. Bars of the same fill color with the same letters are not significantly different (LSD, α = 0.05).

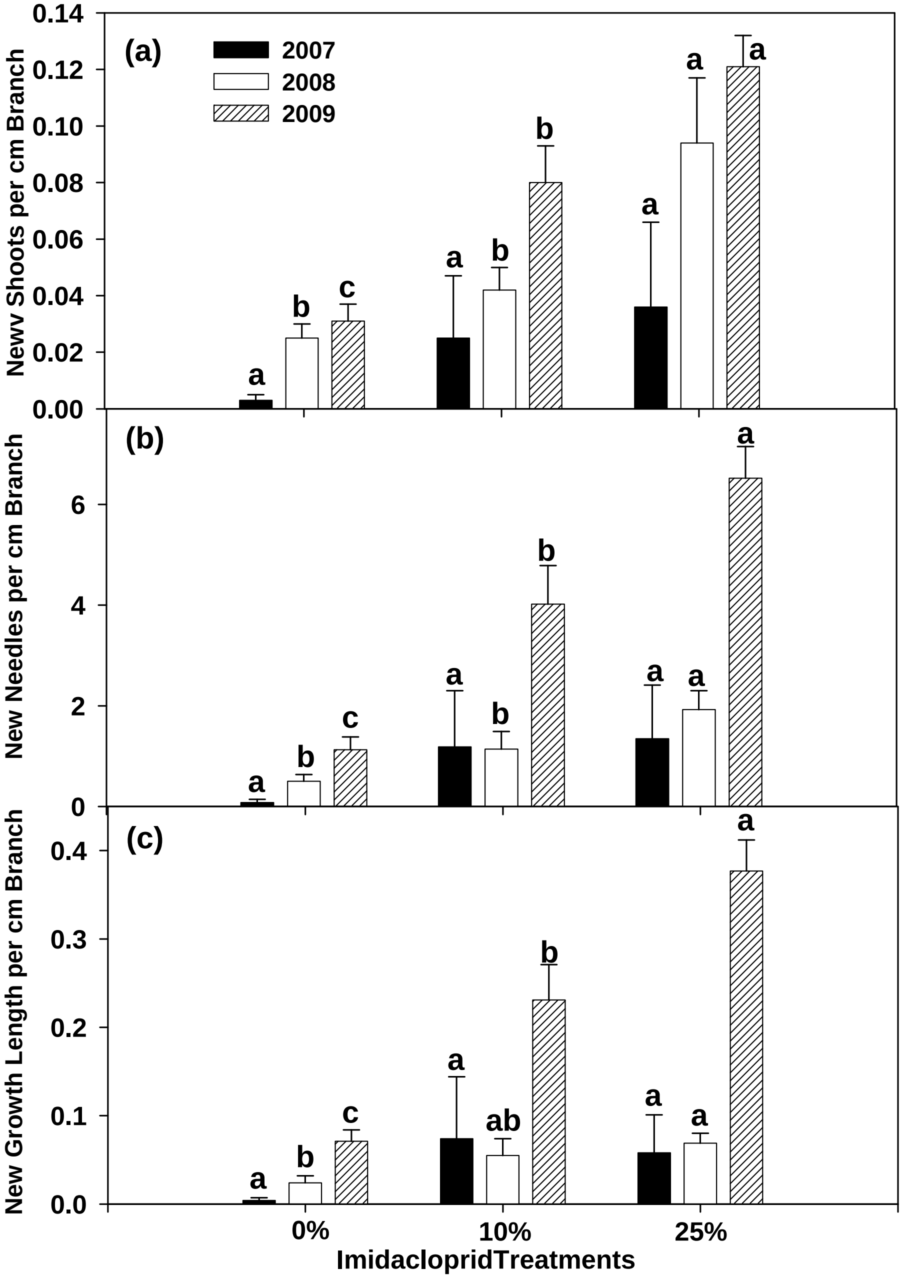

Fig. 2.3. Mean (± SE) growth response of *T. canadensis* trees treated with different rates of imidacloprid (% of recommended rate of 1.5g imidacloprid/2.5cm dbh) and expressed as: (a) new shoots, (b) needles on new shoots, and (c) length of new shoots per cm total branch length. Insecticide was applied November 2006. Bars with the same fill and the same letter are not significantly different (LSD, α = 0.05).

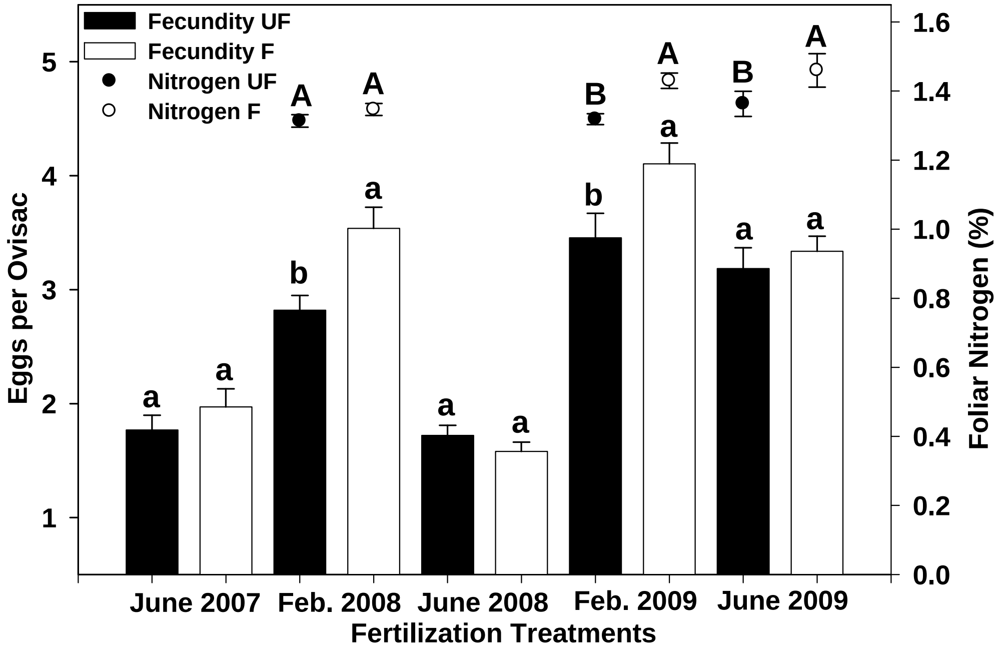

Fig. 2.4. Effects of fertilizer applied in spring 2007 and spring and summer 2008 on hemlock woolly adelgid fecundity (left y-axis) and total percentage N content (right y-axis) of *T. canadensis* foliage (means ± SE). Number of trees sampled per fertilizer treatment were N = 15 for the June 2007 sample and N = 30 for the rest of the sample dates. The abbreviations UF = unfertilized and F = fertilized. Bars or dots within the same sample with the same letter are not significantly different (LSD, α = 0.05).

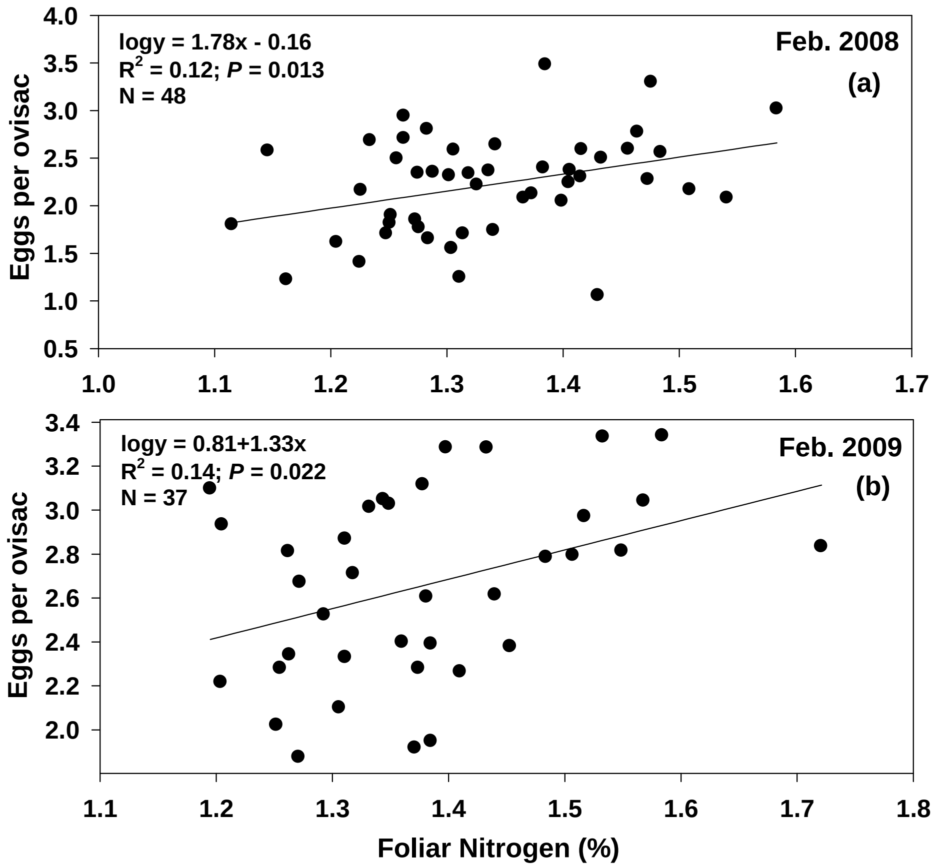

Fig. 2.5. Linear regression of total *A. tsugae* eggs laid per female and total foliar N content of *T. canadensis* trees on which they developed.

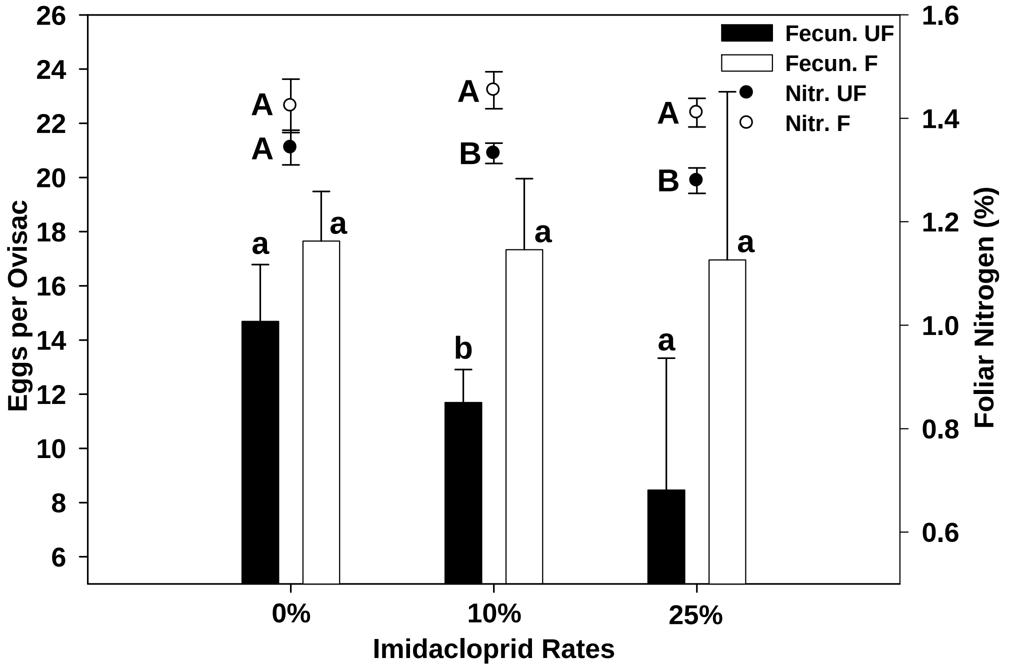

Fig. 2.6. Means (± SE) of *A. tsugae* eggs per female (right y-axis) and foliar N content of *T. canadensis* trees (left y-axis) in winter 2009 treated with different rates of imidacloprid (% of recommended rate of 1.5g imidacloprid/2.5cm dbh) in November 2006 (N = 10). The abbreviations Fecun. = fecundity, Nitr. = nitrogen, UF = unfertilized and F = fertilized. Bars and dots with the same fill and the same letter are not significantly different (LSD, α = 0.05).

CHAPTER 3

DISTRIBUTION AND ABUNDANCE OF *ADELGES TSUGAE* (HEMIPTERA: ADELGIDAE) WITHIN HEMLOCK TREES

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ABSTRACT We studied the distribution of hemlock woolly adelgid, *Adelges tsugae* Annand within hemlock trees for three summer (progredien) and two winter (sisten) generations in the Chattahoochee National Forest north of Helen, GA. In November 2006, sixty eastern hemlock, *Tsuga canadensis* (L.) Carrière trees were treated with either 0, 10 or 25% of 1.5g of imidacloprid insecticide per 2.5 cm of tree dbh and two levels of fertilization (fertilized or not) in the summer of 2007 and 2008. *Adelges tsugae* ovisacs per cm branch were significantly more abundant for consecutive generations from June 2007 to June 2008 in the upper tree crown of untreated trees and when all trees were combined, and that was the general trend for most comparisons. However, adelgid ovisacs were more abundant in the lower crown of insecticide treated trees in June 2008. More sisten nymphs were found settled on the upper crown branches than on the lower ones in the summers of 2007 and 2008. Higher adelgid fecundity was observed on the upper tree crown in February 2008 and in both the winter and summer of 2009. In contrast, *A. tsugae* were more fecund in the lower tree crown region regardless of insecticide rate they received in June 2008. On fertilized trees, *A. tsugae* fecundity was significantly higher in the upper crown region in February 2008. In the following summer in June 2008 fecundity was higher in the lower tree crown strata then recuperated to upper tree crown strata by summer 2009. New growth of branches also varied among sample dates. Greater density of sisten ovisacs was observed near branch tips than on the region closest to the tree trunk in summer 2007. These data demonstrate the variable distribution of hemlock woolly adelgid and hemlock growth in the tree crown over time and suggest that sampling only one crown area will not provide accurate estimates of adelgid densities or predator recovery.

KEY WORDS *Adelges tsugae*, eastern hemlock*, Tsuga canadensis*, distribution, tree height

Hemlock woolly adelgid, *Adelges tsugae* Annand, is a serious threat to eastern, *Tsuga canadensis* (L.) Carrière, and Carolina hemlocks, *Tsuga caroliniana* Engelmann, in the forests of eastern North America. Hemlocks are invaluable in sensitive public sites such as parks, picnic areas, and trails within their range (McClure 1991b) and they are also widely planted and valued in a variety of landscapes (Cheah and McClure 2000).

Hemlock woolly adelgid was first detected in the eastern United States near Richmond, VA in 1951, then in Pennsylvania in the 1960s and Connecticut by 1986 (McClure 1987a). Now, *A. tsugae* is distributed from north Georgia to New England and southern Canada, (USDA infestation map 2008). Mammals, wind and birds play active roles in hemlock woolly adelgid dispersal with the crawler stage being more amenable to movement (McClure 1990, McClure 1991b).

Adelges tsugae has two asexual generations, the sistens and the progrediens, on hemlocks which are its secondary host (McClure 1987a and Annand 1928). Both the overwintering sistens and progrediens undergo parthenogenic reproduction and oviposit ≈50 and 25 eggs per female, respectively (McClure 1989a).

Various sampling protocols including binomial sequential sampling (Fidgen et al. 2006) and randomized branch sampling (Evans and Gregoire 2007) were proposed and tested to assess *A. tsugae* populations at the individual tree or forest scale (Costa 2005). Randomized branch sampling determined that sisten *A. tsugae* distribution within the tree crown depended on their population density suggesting that sampling from the lower regions of tree alone may not always yield accurate densities (Evans and Gregoire 2007). However, these studies did not evaluate sisten and progredien adelgid densities throughout the crown over multiple years. Adelgid populations vary through time and these fluctuations are influenced by tree health and physiology following the initial infestation (McClure 1991a). This density dependent feedback of *A. tsugae* populations and tree health could indirectly influence the performance of predators like *L. nigrinus* (Lamb et al. 2006).

Joseph et al. (Chapter 2) studied the effect of low rates of imidacloprid insecticide and fertilizer on adelgid abundance and tree growth. The purpose of that study was to manipulate tree health and adelgid populations to provide an adequate, uninterrupted supply of high quality adelgids to support long-term predator population growth. As part of that project, we were also interested in how *A. tsugae* populations were distributed within hemlock trees over time. This information will help to design monitoring strategies for evaluating insecticide efficacy and to improve deployment of biological control agents.

Materials and Methods

Study Site and Experiment Design. The study was initiated in November 2006 in White Co., ≈30 km north of Helen, GA in the Chattahoochee National Forest. Eastern hemlock trees in this area were naturally infested by adelgids beginning in 2004 (Johnson 2005). Sixty eastern hemlock trees between 15.2 and 38.1 cm diameter at breast height (dbh), 7.3-24.6 m tall (mean = 15.6 m), and 25-70 yrs old were selected based on their accessibility from a forest road for sample collection using a hydraulic lift.

Trees were treated with either 0, 10 or 25% of 1.5g of imidacloprid insecticide (Merit 75WP Bayer Environmental Science, Research Triangle Park, NC) per 2.5 cm of tree dbh and one of two levels of fertilization; fertilized or not. Insecticide was injected into the soil in a circle around the tree approximately 30-cm from the tree root collar and 5 cm deep using a Kioritz soil injector (Kioritz Corp., Tokyo, Japan) on November 14, 2006. One injection point was made for each 2.5 cm of tree diameter by pressing the Kioritz dispensing knob six times to deliver 29.5 mL per 2.5 cm dbh of insecticide solution into the soil. On 9 and 19 April 2007, half of the trees received their initial fertilizer treatment. Fertilizer rates varied based on tree size so that trees < 19.1, 19.1-35.6 and > 35.6 cm dbh received 453.6, 907.2 and 1360.8g N, respectively. The initial fertilizer was applied using a combination of fertilizer spikes (12-6-12 NPK, Miracle-Gro, Marysville, OH) at a rate of one spike per 1.22 m of drip-line diameter and an additional broadcast application with polymer-coated urea fertilizer (29-2-5 NPK, Sta-Green broadcast, St. Louis, MO). In 2008, a total of 907.2, 1814.6 and 2721.5g of N (polymer-coated urea fertilizer, 29-0-5 NPK, Sta-Green broadcast, St. Louis, MO) were broadcast in two applications beneath trees in the respective diameter classes used in 2007. One half of the fertilizer was applied on March 4 and the remainder on June 11.

Sample Collection and Evaluation. Hemlock terminals from the treated trees were sampled on 14 June 2007; 19 February and 26 June 2008; and 23 February and 8 June 2009 using a hydraulic lift truck to access all parts of the canopy. On June 14, 2007, six 60-cm long hemlock branch terminals were sampled from 30 trees representing one treatment from each block. Two branch terminals each were cut from the lower, middle and upper tree crown so that one sample at each crown location (lower, middle and upper) was taken from the side of the crown facing the road (road-side) and the other on the opposite or forest-side of the crown. In February and June of 2008 and 2009, four 30-cm long terminal branches were sampled from each of the 60 trees. Two branch terminals were cut from the lower and two from the upper tree crown so that one sample at each crown location (lower and upper) was taken from the side of the crown facing the road (road-side) and the other on the opposite or forest-side of the crown. Samples were placed in polyethylene bags and transported to the laboratory where they were stored at -5^{o}C.

The 60-cm terminals were sub-divided into six 10-cm long sections. We examined both the distribution of adelgids and the distribution of new tree growth within the tree crown and along the length of the 30-cm long branches. The numbers of ovisacs, eggs, and nymphs (crawlers and second-instars) were counted on each 10-cm section of the 60-cm long branch samples and on 30-cm long branch samples. The number of new branch shoots, the length of new shoots and the number of needles on new growth were also measured.

Statistical Analyses. The experiment was arranged in a factorial design consisting of 10 replications in five blocks (two replications per block). Trees were grouped based on proximity to one another and blocks also represented changes in elevation. We selected 60 trees (2 trees/treatment/block) for treatment because the study area was scheduled for selective harvesting to remove hazardous trees along the road. Since trees to be removed were scattered throughout our study area we were concerned some of our treated trees would be damaged or broken off during harvesting so we selected and treated twice as many trees as originally planned. Harvesting was delayed for a variety of reasons so we were able to sample all 60 trees after the initial sample period.

The various rates of insecticide had significant effects on numbers of adelgid ovisacs, eggs or nymphs and tree growth parameters from the June 2008 sample onwards (Chapter 2). Fertilizer treatments increased adelgid fecundity (Chapter 2), but had no demonstrable effects on other life stages. Likewise, tree growth parameters were unaffected by fertilization. Therefore, we included data from trees treated with insecticide in our analyses for the June 2007 and February 2008 sample dates where insecticide treatment was not significant. On fertilized trees we only examined variation in adelgid fecundity associated with crown position.

The 10- or 30-cm-long sections of each branch were measured along the main branch stem so they also had varying lengths of side branches. Therefore, all adelgid counts and growth parameters were standardized by dividing them with the respective total hemlock branch lengths (main stem plus side branches) within a 10- or 30-cm-long sample and expressed on a per cm of branch length basis. The data obtained from 10-cm long subsampling of 60 cm branches in June 2007 was used to shape the sampling procedure in the later dates. Standardized independent variables which included number of ovisacs, eggs and nymphs (crawlers and second instars), number of new shoots, total length of new growth (cm), needles on new growth, and nymphs on new growth, were transformed using the natural log (x + 1) transformation after testing for homogeneity of variances using the PROC Univariate procedure of SAS (SAS Institute 2003). The variables eggs per ovisac were transformed using the natural log. The transformed data for each sample date were examined using the PROC GLM procedure of SAS and means were separated using the least significant difference (LSD) method ($\alpha = 0.05$). We looked for correlations in ovisac densities between the upper and lower tree crown using PROC Reg of SAS and a linear model of log transformed data. Untransformed means and standard errors are reported in tables and figures.

Results

Effects of Tree Crown Position on Adelgid Counts. When all the trees were included in the analysis, *A. tsugae* ovisacs per cm branch were more abundant in the upper tree crown than in the lower crown between the summers of 2007 and 2008 (Table 3.1, Fig. 3.1a). However, trees that did not receive insecticide had similar densities of adelgid ovisacs in both crown positions for all the sample dates except in June 2007 where we observed a marginally higher number of ovisacs ($P < 0.1$) in the upper crown (Fig. 3.1b). In June 2008, significantly greater numbers of females were recorded in the upper crown of 10% insecticide treated trees (Fig. 3.1c). This is the only sample date where we found a difference in the adelgid distribution within 10% insecticide treated trees. Whereas on 25% insecticide treated trees, significantly higher numbers of ovisacs were found in the upper tree crown in the June 2007 sample while later samples had similar female densities at both crown positions (Fig. 3.1d). Since the insecticide did not reduce adelgid numbers until the June 2008 sample (Joseph et al. Chapter 2), this difference is not a result if differential toxicity but is consistent with the overall trend shown in Figure 3.1a.

We found more eggs per cm branch in the upper crown than in the lower crown in both June 2007 and February 2008 when all trees were combined (Table 3.1; Fig. 3.1e). Likewise, samples from untreated trees in June 2007, February 2008 and June 2009, had higher egg densities in the upper crown (Fig. 3.1f). There were very few significant differences in egg densities on insecticide-treated trees (Figs. 3.1g and h). However, generally eggs densities were higher in the upper canopy of those trees but only in the June 2008 sample from 10% insecticide treated trees were they significantly higher.

Our winter sampling was targeted to sample females and eggs so very few nymphs were recorded in February samples. However, summer samples had sufficient densities of adelgid nymphs for analysis (Table 3.1, Fig. 3.2). In 2007 and 2008, sisten nymphs that settled per cm branch were significantly greater in the upper tree crown than in the lower crown region (Fig. 3.2a-d) in all cases except in the 25% insecticide treated trees in June 2008. In June 2009, the upper and lower crown had similar numbers of nymphs in all treatments. By that date insecticide treated trees had low densities due to insecticide and the untreated trees were in poor health (Joseph et al. Chapter 2).

In June 2007 we also sampled branches from the mid-crown region. Sistens ovisac and progredien egg densities in the middle and upper crown were not different but there were fewer of both life stages in the lower crown (Table 3.2). Crown position did not affect adelgid fecundity, but greater numbers of nymphs were observed in the upper third of the crown, followed by middle third, and the fewest in the lower third of the crown (Table 3.2).

Adelgid fecundity tended to be higher in the upper crown but not always (Table 3.3). Fecundity was higher in the upper tree crown than on the lower crown in February 2008 when all the trees were pooled or within untreated trees. In February and June 2009 fecundity was higher in the upper crown when all trees were combined and in the untreated trees alone. In contrast, *A. tsugae* were more fecund in the June 2008 samples in the lower crown regardless of insecticide rate they received or whether all trees were pooled. We did not see any pattern in fecundity between tree crown strata within 25% insecticide treated trees except during the June 2008 sample period when fecundity was higher in the lower crown. *Adelges tsugae* fecundity was significantly higher in the upper crown of fertilized trees in February 2008 and June 2009 (Table 3.3), while fertilized trees sampled during June 2008 had higher fecundity in the lower tree crown than in the upper crown. Fertilization had no effect on fecundity of adelgids from the two crown positions in February 2009. However, in summer 2009 adelgids had higher fecundity in the upper crown regardless of fertilizer.

In June 2008 we counted the numbers of sisten nymphs found on new growth to see if crown position affected settling position. The densities of nymphs on new growth were not affected by crown position (all trees $F = 0.73$; df = 1, 89; $P = 0.3938$: untreated trees $F = 2.7$; df = 1, 19; $P = 0.1117$: 10% insecticide $F = 0.01$; df = 1, 23; $P = 0.9107$: 25% insecticide $F = 1.6$; df = 1, 25; $P = 0.2173$).

Moreover in February 2009, sisten ovisacs (*F* = 0.06; df = 1, 83; *P* = 0.8124), progredien eggs (*F* = 0.19; df = 1, 83; *P* = 0.6626), and fecundity (*F* = 1.11; df = 1, 30; *P* = 0.3006) of females that developed on the previous-year's shoots (in 2008) were not significantly affect by crown position.

Ovisacs densities exhibited a positive correlation with the upper and lower tree crown in June 2008 (r^2=0.0916; *t* = 2.42; df = 1, 58; *P* = 0.0188), February 2009 (r^2=0.3118; *t* = 5.13; df = 1, 58; *P* < 0.0001), and June 2009 (r^2=0.3709; *t* = 5.85; df = 1, 58; *P* < 0.0001). In June 2007 (r^2=0.0082; *t* = 0.48; df = 1, 28; *P* = 0.6340) and February 2008 (r^2=0.0558; *t* = 1.85; df = 1, 58; *P* = 0.0693) the results were not significant.

Effects of Tree Crown Position on Tree Growth. Crown position had relatively little effect on the number of new shoots produced on branches and, where differences occurred, there was no consistent trend (*P* < 0.1; Table 3.4, Fig. 3.3). The largest differences were noted on untreated trees which had significantly more new shoots in the lower crown in 2008 and 2009 (Fig. 3.3b). Trees treated with 10% insecticide had similar density of new growth for all summer sample dates (Fig. 3.3c) while the 25% insecticide treated trees had slightly more new shoots in the upper crown in 2009 (Fig. 3.3d). Other growth measurements (new needles and new shoot length) were also variable or unaffected by crown position (Table 3.4, Fig. 3.4)

Effects of Distance from Branch Tip. Numbers of progredien ovisacs per cm branch were greater on the branch tips as compared to the regions of the branch near the tree trunk when all tree sample positions were combined and for the lower and middle crown positions (*P* < 0.1; Table 3.6, Fig. 3.5a,b,c) but not for the upper crown position (Fig. 3.5d). Furthermore, sisten eggs were slightly more abundant on the first 10 cm than on the portion of the branch located closest to tree trunk on branches collected from the lower and middle one-third of the crown

(Figs. 3.6b, and 3.6c). No difference was noticed in egg density in relation to position on the branches for branches collected from the upper tree crown (Figs. 3.5d and 3.6d). Density of settled nymphs and adelgid fecundity were unaffected by distance from the branch tip regardless of crown position. Similarly, number of new shoots, new needles and length of new growth parameters were similar regardless location along the branches (Table 3.6).

Discussion

Our results show that adelgid ovisacs, eggs, and nymphs tend to be higher in the upper crown than in the lower crown so sampling only the lower crown may be insufficient to obtain accurate estimates of adelgid densities. We did observe significant relationships of adelgid ovisac densities between upper and lower crown positions for 3 of the sample dates. However, on the other 2 dates the relationships were not significant. In addition, the R^2 values were low, so prediction of upper crown densities from lower crown samples would not be precise. Gray et al. (1998) sampled both upper and lower crown positions but did not report differences in adelgid densities between them. Past research using randomized branch sampling has shown that *A. tsugae* infestation levels may influence its distribution within the tree crown, at least for sisten populations (Evans and Gregoire 2007). Evans and Gregoire (2007) observed that sisten densities were higher in the upper crown when trees had low level infestations but this pattern was reversed for high level of infestations when sisten densities were higher in the lower tree crown. Although Evans and Gregoire (2007) suggested that adelgid densities were likely to be greater in the upper strata of the crown on those trees that have low or new infestation, we believe that the distribution of adelgids in newly infested trees would depend on the crown position at which they initially colonize a tree. However in a hemlock stand with an established infestation, the density of *A. tsugae* might vary depending on tree health (McClure 1991a, Pontius et al. 2006), minimum winter temperature, seasonal temperatures (Trotter and Shields 2009), latitude (Orwig et al. 2002) and their interactions. Therefore, the precise infestation level of *A. tsugae* can only be determined by quantifying their density throughout the crown. For an accurate assessment of adelgid densities in a given tree, we recommend sampling upper and lower crown regions and pooling the subsamples into an estimate for the tree.

Sampling procedures vary in their purpose. Costa and Onken (2006) and Fitzpatrick et al. (2009) provide methods for early detection and monitoring of infested stands while Fidgen et al. (2006) estimated relative levels of infestation for individual trees. In their non-destructive binomial sequential sampling only branches from the lower third of the crown were examined (Fidgen et al. 2006). An *in situ* visual observation of adelgid woolly ovisacs on a certain number of trees can provide an estimate of the general infestation level in an area (Costa and Onken 2006, Faulkenberry et al. 2009). However, Fitzpatrick et al. (2009) reported a high incidence of biased misidentifications between trained observers and volunteers in the monitoring program developed by Costa and Onken (2006), especially when *A. tsugae* infestation on a given hemlock tree was low. Based on our observations, we suggest that sisten woolly ovisacs might be adequately visible to record infestation, but it might be difficult to distinguish progredien woolly ovisacs because they occur along with the dead sistens or even with the previous-year progredien woolly mass in a cluster on hemlock branches leading to population overestimates.

We found that adelgids tend to be more abundant in the upper crown. If adelgid predators seek higher prey densities for oviposition or establish better at higher densities, then this study suggests that predator releases and subsequent recovery efforts should focus on the upper crown, if possible, where higher prey densities are more likely. Most predator releases have been made on the lower third of the hemlock tree crown (McClure and Cheah 1998 and

1999, McClure et al. 2000, Flower et al. 2006, Lamb et al. 2006). Although adult predators can fly, their ability to locate and colonize branches based on adelgid density is not well understood. Because the mobility of larvae is limited it is likely that predator larvae might have a greater chance of survival if placed on shoots having a patch of high adelgid density. Future research should examine the pattern of predator establishment within the tree crown to streamline open-release of adult predators in the forest.

Hemlock branch terminals are often sampled to determine the insecticide efficacy using a pole pruner to reach branches that are otherwise inaccessible from the ground and probably this is a feasible procedure for sampling trees in the forest interior (Cowles 2009, Cowles et al. 2006, Doccola et al. 2005, Doccola et al. 2007, Gouger 1971, Webb et al. 2003). Even though most studies report the sampling height, actual tree height is rarely specified; therefore, it is difficult to determine the crown position where samples were collected. However, based on the diameter at breast height (13-135 cm) of the experimental trees, we believe sampling was limited to the lower half of the tree crowns in most cases (Cowles 2009, Cowles et al. 2006, Doccola et al. 2005, Doccola et al. 2007, Webb et al. 2003). We suggest that including samples from the upper tree crown would be ideal for a better appraisal of the adelgid density but recognize the current limitations of sampling the upper crowns in forests.

Interestingly, the new growth, which presumably adelgid nymphs prefer and settle on more frequently (Lagalante et al. 2006, McClure 1991a), was not more abundant in the upper crown. We did not find any difference in new growth within the tree crown of the control trees initially in 2007 summer; however, new shoots were more abundant in the lower crown as compared to the upper crown in the successive summers of 2008 and 2009. One potential explanation may be the stress induced by the adelgids on the trees, particularly in the upper crown where adelgid densities were higher in 2007 and winter 2008. These higher populations may have impacted the upper crown more resulting in an inability of the tree to produce new growth in that region. Throughout the study, trees that received insecticide had a uniform density of new shoots between tree crown positions. Previously, enhanced plant growth has been reported in hybrid poplar, *Populus nigra* L. (Chiriboga 2009) and cotton (Gonias et al. 2007, Hundley 2004) mediated by imidacloprid treatment. Thus, it is also possible that imidacloprid insecticide improved the tree health and facilitated a uniform distribution of the new growth within the crown.

Nymphs were abundant in the upper crown of trees regardless of insecticide treatments on all sample dates. Warmer conditions in the upper tree crown might have caused early progredien egg laying and egg hatch. Therefore, if nymphal stages are included in assessments of adelgid densities, samples should be taken from the same crown position. Because new growth is not evenly distributed and the nymphs are distributed throughout the branches, not just new growth, it may not be reliable to estimate sisten nymphal density by merely counting them on the new growth.

McClure (1991) found a two-fold increase in adelgid fecundity on fertilized *T. canadensis*. Likewise, fertilization had a small but significant effect on sisten fecundity on our trees in 2008 and 2009 (Joseph et al. Chapter 2). However, fecundity was not consistently higher in a particular crown region. For example, adelgids were more fecund in the upper tree crown in February 2008 and June 2009 but adelgids in that region were less fecund in June 2008. In June 2009 fecundity was higher in the upper crown regardless of fertilizer treatment.

Since most adelgid stages remain stationary and consume stored reserves from hemlock branches, quality of these branches might be important for their survival or reproduction. Active allocation, re-allocation, or metabolism of nutrients occurs within the tree to generate new shoot growth during the summer (Miyazawa et al. 2004, Nommik 1966). Progredien density and sisten eggs tended to be denser on the terminal tip region of previous-year branches than on the region furthest away from the branch tip on insecticide-untreated or 10% insecticide treated trees. However, branches sampled from trees treated with 25% insecticide had a similar distribution of progredien ovisacs and sisten eggs over the entire branch. These results suggest that sampling terminal tips of hemlock branches may be adequate for estimating adelgid density.

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Table 3.1. Analysis of variance of effect of tree crown position (lower or upper) on adelgid life stages per cm branch on 30 cm long *T. canadensis* branches (from 30 trees in 2007 and 60 trees in 2008-09) sampled from trees treated with 0-25% of 1.5g imidacloprid/cm tree diameter in 2006

Adelgid Counts	2007 June			2008 Feb			2008 June			2009 Feb			2009 June		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*
All Treatments															
Ovisacs[a]	9.3	1, 54	0.0034**	6.7	1, 109	0.0108*	5.9	1, 109	0.0163*	0.0	1, 109	0.9313	0.9	1, 109	0.3356
Eggs[b]	3.3	1, 54	0.0166*	8.4	1, 109	0.0044**	2.1	1, 109	0.1478	0.1	1, 109	0.7415	1.9	1, 109	0.1685
Nymphs	28.8	1, 54	<0.0001***	-	-	-	21.9	1, 109	<0.0001***	-	-	-	0.0	1, 109	0.9235
Imidacloprid 0%															
Ovisacs	4.2	1, 14	0.0597[a]	2.7	1, 29	0.1113	1.3	1, 29	0.2629	0.3	1, 29	0.5429	2.5	1, 29	0.1183
Eggs	8.3	1, 14	0.0118*	6.9	1, 29	0.0131*	0.2	1, 29	0.6528	0.0	1, 29	0.8012	9.2	1, 29	0.0049**
Nymphs	14.9	1, 14	0.0017**	-	-	-	27.4	1, 29	<0.0001***	-	-	-	0.5	1, 29	0.4601
Imidacloprid 10%															
Ovisacs	1.2	1, 14	0.2766	2.0	1, 29	0.1645	19.9	1, 29	0.0001***	0.6	1, 29	0.4155	1.4	1, 29	0.2467
Eggs	1.8	1, 14	0.1909	2.1	1, 29	0.1557	6.9	1, 29	0.0137*	0.3	1, 29	0.5590	1.4	1, 29	0.2344
Nymphs	7.1	1, 14	0.0183*	-	-	-	28.0	1, 29	<0.0001***	-	-	-	0.6	1, 29	0.4222
Imidacloprid 25%															
Ovisacs	5.6	1, 14	0.0326*	2.3	1, 29	0.1394	0.0	1, 29	0.7619	0.0	1, 29	0.9284	1.2	1, 29	0.2815
Eggs	2.8	1, 14	0.1119	2.5	1, 29	0.1210	0.0	1, 29	0.9851	0.2	1, 29	0.6374	0.5	1, 29	0.4624
Nymphs	15.6	1, 14	0.0014**	-	-	-	2.1	1, 29	0.1560	-	-	-	0.0	1, 29	0.7812

Analysis performed on log-transformed data. [a] Ovisacs represent progredien generation during June and sisten generation during February. [b]In contrast, eggs represent progredien generation during February and sisten generation during June. The notations indicate the significant difference (*P*: [a] < 0.1; *< 0.05; **<0.01 and ***<0.001) of log-transformed data.

Table 3.2. Analysis of variance and means (± SE) of effect of tree crown positions (lower or upper) on *A. tsugae* and tree growth parameters per cm of branch length within 60 cm long branches from 30 *T. canadensis* trees in 2007

Variables	*F*	df	*P*	Tree Crown Positions		
				Lower	Middle	Upper
Adelgid Counts						
Ovisacs	6.67	2, 82	0.0021**	0.09 ± 0.01b	0.20 ± 0.03a	0.20 ± 0.02a
Eggs	6.65	2, 82	0.0021**	0.29 ± 0.06b	0.82 ± 0.20a	1.05 ± 0.26a
Nymphs	18.51	2, 82	<0.0001***	1.24 ± 0.18c	2.50 ± 0.28b	4.44 ± 0.81a
Fecundity	0.32	2, 82	0.7252	2.83 ± 0.31a	3.23 ± 0.30a	3.96 ± 0.52a
Growth Parameters						
New shoots	2.34	2, 82	0.1027	0.003 ± 0.002a	0.009 ± 0.005a	0.021 ± 0.008a
New needles	2.95	2, 82	0.0582^{a}	0.092 ± 0.082a^{b}	0.321 ± 0.204a^{ab}	0.909 ± 0.388a^{a}
New shoot length	3.10	2, 82	0.0505^{a}	0.004 ± 0.003a^{b}	0.014 ± 0.007a^{b}	0.048 ± 0.021a^{a}

The notations indicate the significant difference (*P*: ^{a} < 0.1; *< 0.05; **<0.01 and ***<0.001) of log-transformed data. In the means (± SE), same letters among each row are not significantly different (LSD, α = 0.05) and different superscripted letters are significantly different at α = 0.1 within each row.

Table 3.3. Analysis of variance and means (± SE) of effect of *T. canadensis* tree crown position (lower or upper) on number of eggs per *A. tsugae* ovisac on 30 cm long branches (from 30 trees in 2007 and 60 trees in 2008-09) for trees treated with low rates of imidacloprid insecticide in November 2006, and fertilizer in 2007 and 2008

Sample Date	Tree Crown Positions	All Treatments	Eggs per ovisac				
			% of Recommended Rate of Imidacloprid			Fertilizer Regime	
			0%	10%	25%	Unfertilized	Fertilized
2007 June							
	Lower	3.0 ± 0.3	2.8 ± 0.4	3.6 ± 0.7	2.6 ± 0.3	2.7 ± 0.5	3.3 ± 0.3
	Upper	4.1 ± 0.5	4.2 ± 0.8	4.3 ± 0.9	3.7 ± 1.1	3.5 ± 0.6	4.7 ± 0.8
	F (df)	0.07 (1, 53)	1.29 (1, 14)	0.00 (1, 14)	0.23 (1, 14)	0.40 (1, 23)	0.01 (1, 24)
	P	0.7905	0.2751	0.9966	0.6370	0.5319	0.9046
2008 Feb.							
	Lower	8.1 ± 0.9	6.9 ± 0.9	9.2 ± 2.2	8.7 ± 1.9	7.9 ± 1.1	8.2 ± 1.4
	Upper	11.4 ± 0.9	12.2 ± 1.5	11.2 ± 1.4	10.2 ± 2.2	9.1 ± 1.0	14.4 ± 1.5
	F (df)	5.39 (1, 71)	7.31 (1, 25)	1.62 (1, 15)	0.22 (1, 11)	0.37 (1, 37)	7.03 (1, 23)
	P	0.0231*	0.0122*	0.2227	0.6471	0.5478	0.0142*
2008 June							
	Lower	4.3 ± 0.4	3.7 ± 0.4	4.3 ± 0.7	5.9 ± 1.9	5.1 ± 0.7	3.5 ± 0.5
	Upper	2.6 ± 0.2	2.6 ± 0.4	2.1 ± 0.3	3.3 ± 0.4	2.5 ± 0.3	2.7 ± 0.3
	F (df)	13.4 (1, 75)	6.1 (1, 28)	5.4 (1, 20)	6.70 (1, 9)	1.18 (1, 31)	17.61 (1, 33)
	P	0.0004**	0.0192*	0.0299*	0.0293*	0.2866	0.0002**
2009 Feb.							
	Lower	13.6 ± 1.3	13.3 ± 1.5	14.3 ± 2.2	13.5 ± 4.9	10.5 ± 1.6	16.5 ± 1.8
	Upper	16.0 ± 1.0	17.5 ± 1.6	14.4 ± 1.1	11.0 ± 1.9	14.2 ± 1.5	17.9 ± 1.4
	F (df)	3.71 (1, 58)	5.95 (1, 29)	0.12 (1, 10)	0.02 (1, 4)	0.52 (1, 49)	0.03 (1, 49)
	P	0.0591[a]	0.0211*	0.7412	0.9072	0.4723	0.8633
2009 June							
	Lower	9.2 ± 0.6	8.6 ± 0.5	10.6 ± 1.3	8.4 ± 1.6	9.1 ± 0.9	9.2 ± 0.8
	Upper	12.9 ± 0.8	12.2 ± 1.1	13.3 ± 1.4	14.9 ± 2.1	12.7 ± 0.9	13.0 ± 1.3
	F (df)	12.0 (1, 69)	4.65 (1, 29)	3.45 (1, 16)	2.39 (1, 3)	4.62 (1, 29)	9.35 (1,29)
	P	0.0009**	0.0395*	0.0149*	0.2199	0.0401*	0.0048**

The notations indicate the significant difference (*P*: [a] < 0.1; *< 0.05 and **<0.01) of log-transformed data.

Table 3.4. Analysis of variance of effects of crown positions (lower or upper) on *T. canadensis* tree growth parameters per cm of branch from 2007 to 2009 for trees treated with 0, 10 or 25% of the recommended rate of imidacloprid insecticide in November 2006, and fertilizer in 2007 and 2008

Tree Growth Parameters	2007 June			2008 June			2009 June		
	F	df	*P*	*F*	Df	*P*	*F*	df	*P*
All Treatments									
New Shoots	3.1	1, 54	0.0797^{a}	3.6	1, 109	0.0601^{a}	0.3	1, 109	0.5678
New Needles	4.3	1, 54	0.0413*	0.0	1, 109	0.7886	0.6	1, 109	0.4337
New Shoot length	3.7	1, 54	0.0593^{a}	1.6	1, 109	0.1988	0.0	1, 109	0.9292
Imidacloprid 0%									
New Shoots	1.6	1, 14	0.2253	5.3	1, 29	0.0277*	12.1	1, 29	0.0016**
New Needles	1.8	1, 14	0.1941	2.9	1, 29	0.0960^{a}	11.8	1, 29	0.0018**
New Shoot length	1.2	1, 14	0.2816	0.4	1, 29	0.5302	11.5	1, 29	0.0020**
Imidacloprid 10%									
New Shoots	1.0	1, 14	0.3349	2.5	1, 29	0.1188	0.7	1, 29	0.4015
New Needles	1.6	1, 14	0.2154	0.7	1, 29	0.3919	0.7	1, 29	0.4012
New Shoot length	1.9	1, 14	0.1821	0.0	1, 29	0.7766	0.1	1, 29	0.6666
Imidacloprid 25%									
New Shoots	1.2	1, 14	0.2819	0.1	1, 29	0.7567	4.0	1, 29	0.0544^{a}
New Needles	1.4	1, 14	0.2559	2.7	1, 29	0.1106	6.6	1, 29	0.0154*
New Shoot length	1.1	1, 14	0.2957	4.1	1, 29	0.0501^{a}	8.4	1, 29	0.0069**

Analysis performed on log-transformed tree growth parameters data. Growth parameters were only measured on hemlock branches during summer. The notations indicate the significant difference (*P*: $^{a} < 0.1$, *< 0.05 and **<0.01).

Table 3.6. Analysis of variance of effects of proximity to *T. canadensis* branch tip or tree trunk within tree crown positions (lower or upper) on *A. tsugae* life stages and tree growth parameters per cm of branch in June 2007

	Whole Tree Crown			Tree Crown Positions								
				Lower			Middle			Upper		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*
Adelgid Counts												
Ovisacs	2.00	5, 170	0.0815[a]	1.93	5, 164	0.0912[a]	2.20	5, 170	0.0570[a]	1.23	5, 170	0.2987
Eggs	1.02	5, 170	0.4089	2.23	5, 164	0.0541[a]	2.07	5, 170	0.0709[a]	0.71	5, 170	0.6161
Nymphs	0.70	5, 170	0.6253	0.34	5, 164	0.8867	0.63	5, 170	0.6785	0.81	5, 170	0.5461
Eggs/Ovisac	0.23	5, 170	0.9501	1.04	5, 153	0.3954	1.56	5, 162	0.1753	0.17	5, 158	0.9742
Growth Parameters												
New Shoots	0.47	5, 170	0.7998	0.83	5, 164	0.5320	0.24	5, 170	0.9453	0.18	5, 170	0.9696
New Needles	0.41	5, 170	0.8392	0.60	5, 164	0.6986	0.35	5, 170	0.8811	0.18	5, 170	0.9685
Shoot length	0.63	5, 170	0.6740	0.86	5, 164	0.5071	0.32	5, 170	0.9018	0.35	5, 170	0.8794

The notations indicate the significant difference (*P*: [a] < 0.1).

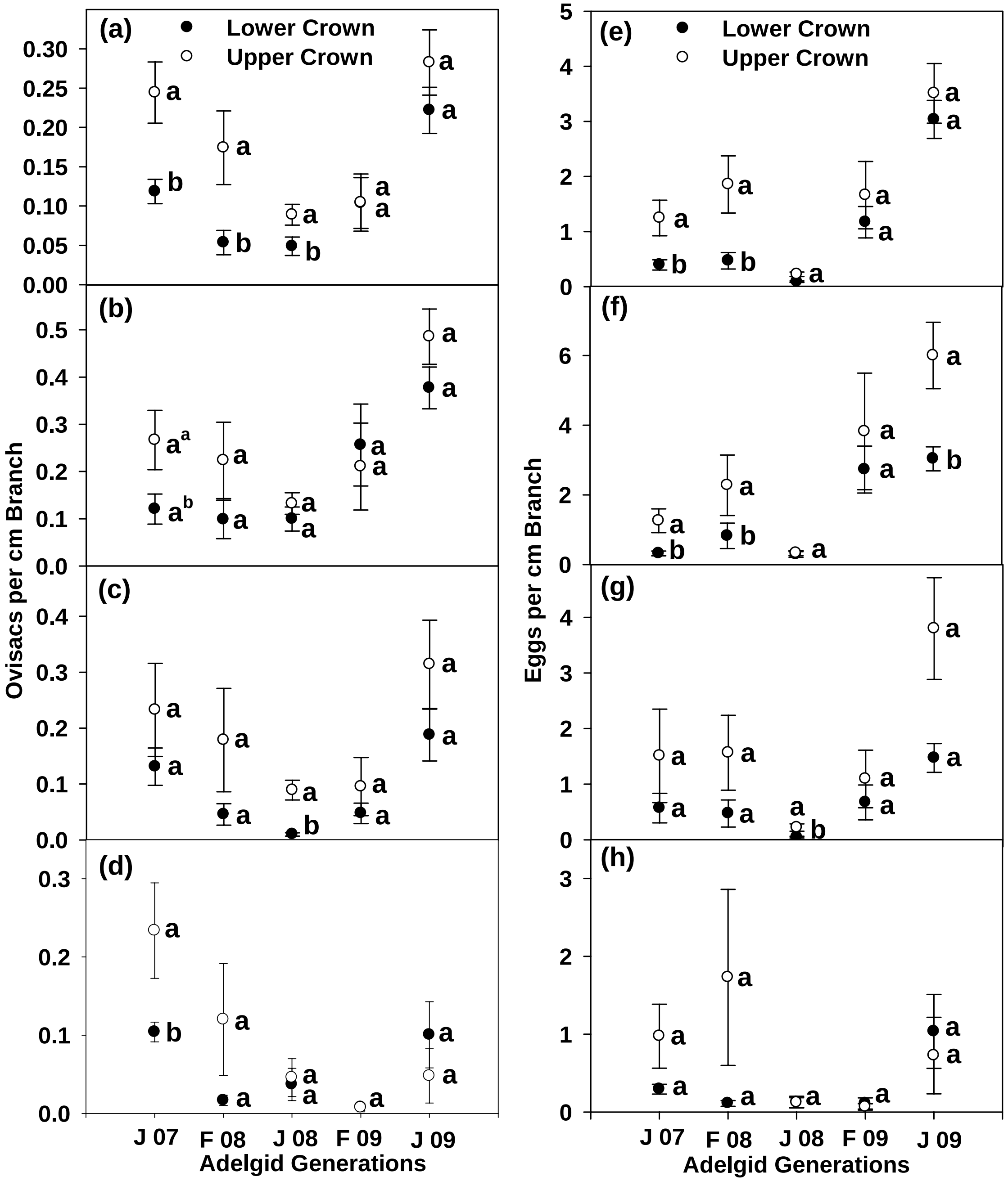

Fig. 3.1. Effects (means ± SE) of tree crown position on abundance of ovisacs and eggs per cm branch from June 2007 to 2009. Graphs are (a and e) all treatments combined, (b and f) 0% imidacloprid, (c and g) 10% imidacloprid and (d and h) 25% imidacloprid. . In figs. (a) and (e) N = 30 for 2007 data and N = 60 for 2008-09 data, and in figs. (b), (c), (d), (f), (g) and (h) N = 10 for 2007 data and N = 20 for 2008-09 data. The abbreviation J = June and F = February. Within the same date, circles with the same case letters are not significantly different (LSD, α = 0.05) and different superscripted letters are significantly different at α = 0.1.

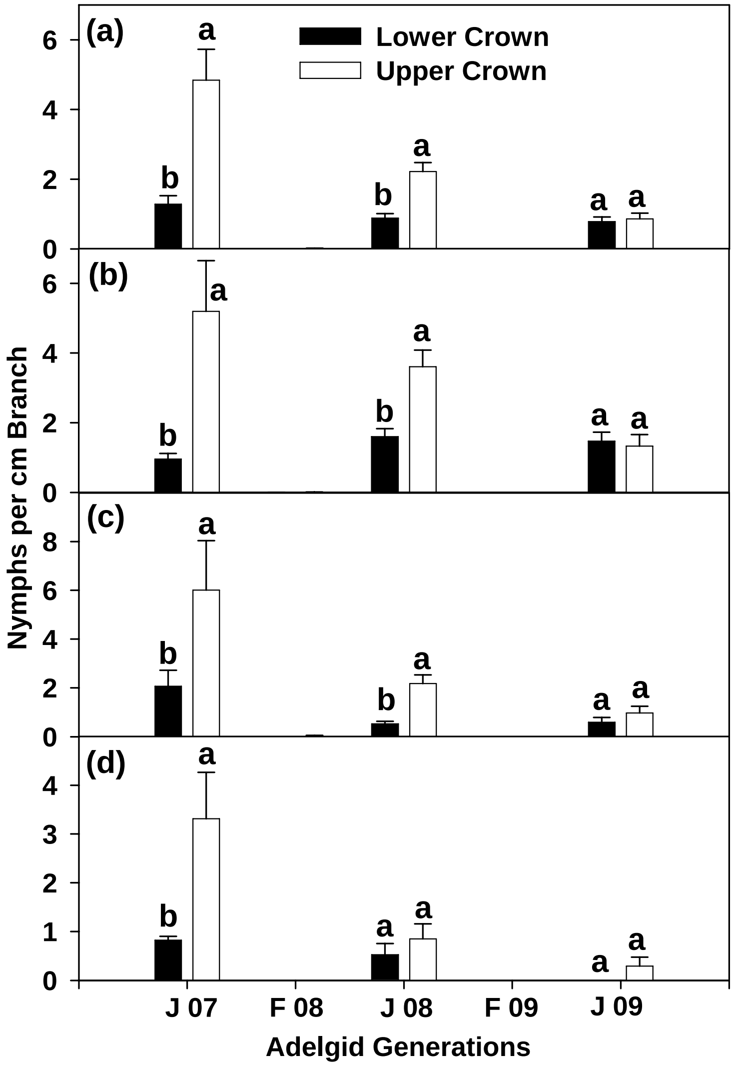

Fig. 3.2. Effects (means ± SE) of tree crown position (lower and upper) on abundance of adelgid nymphs per cm branch from June 2007 to 2009. Graphs are (a) all treatments combined, (b) 0% imidacloprid, (c) 10% imidacloprid and (d) 25% imidacloprid. The abbreviation J = June and F = February. Same case letters are not significantly different (LSD, $\alpha = 0.05$).

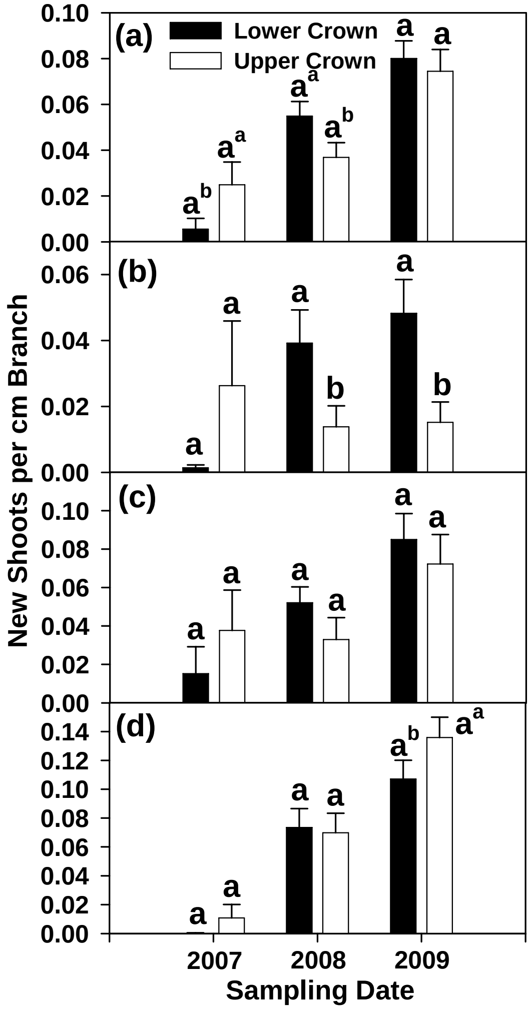

Fig. 3.3. Effects (means ± SE) of tree crown position (lower and upper) on abundance of new shoot growths per cm branch in *T. canadensis* from June 2007 to 2009. Graphs are (a) all treatments combined, (b) 0% imidacloprid, (c) 10% imidacloprid and (d) 25% imidacloprid. In fig. (a) N = 60 for 2007 data and N = 120 for 2008-09 data, and in figs. (b), (c) and (d) N = 20 for 2007 data and N = 40 for 2008-09 data. Same case letters are not significantly different at α = 0.05 (LSD) and different superscripted letters are significantly different at α = 0.1.

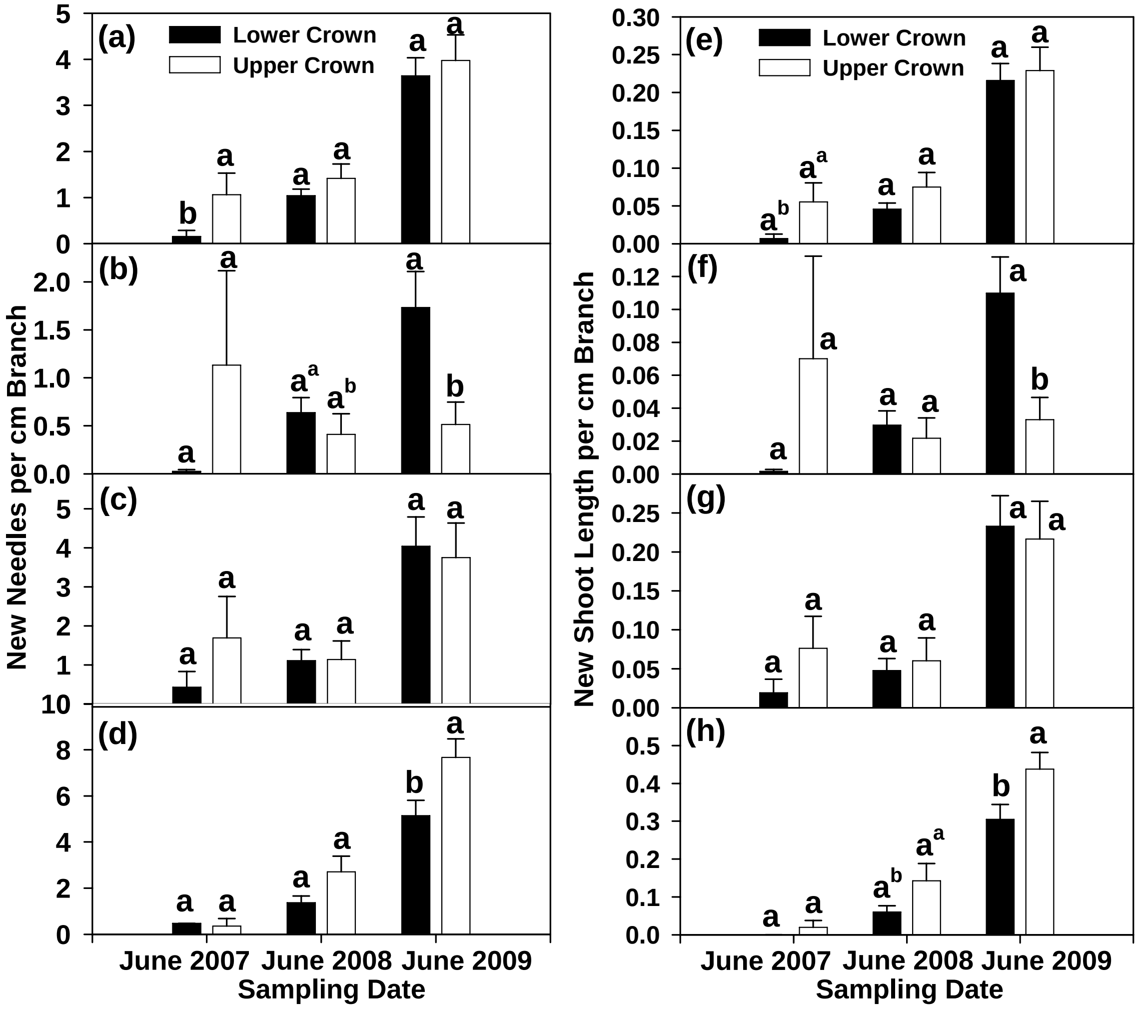

Fig. 3.4. Effects (means ± SE) of tree crown position on new needles and shoot length per cm branch from June 2007 to 2009. Graphs are (a and e) all treatments combined, (b and f) 0% imidacloprid, (c and g) 10% imidacloprid and (d and h) 25% imidacloprid. In figs. (a) and (e) N = 60 for 2007 data and N = 120 for 2008-09 data, and in figs. (b), (c), (d), (f), (g) and (h) N = 20 for 2007 data and N = 40 for 2008-09 data. Within the same date and graph, bars with the same case letters are not significantly different at α = 0.05 (LSD) and different superscripted letters are significantly different at α = 0.1.

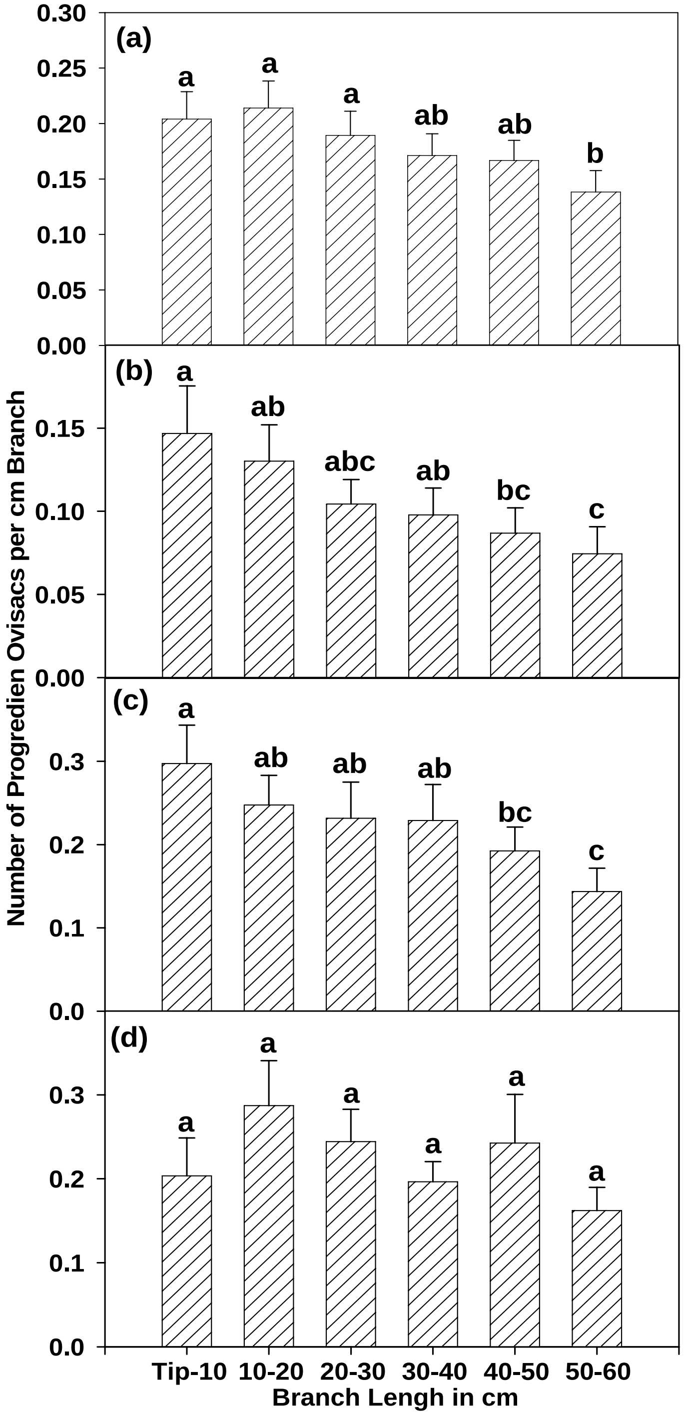

Fig. 3.5. Effects (means ± SE) of distance from branch tip of 60 cm long *T. canadensis* branches on ovisacs abundance of hemlock woolly adelgid (N = 180) in June 2007. The figures represent tree crown position, (a) whole tree (b) lower (c) middle, and (d) upper. Bars of the same fill color with the same letters are not significantly different (LSD, $\alpha = 0.1$).

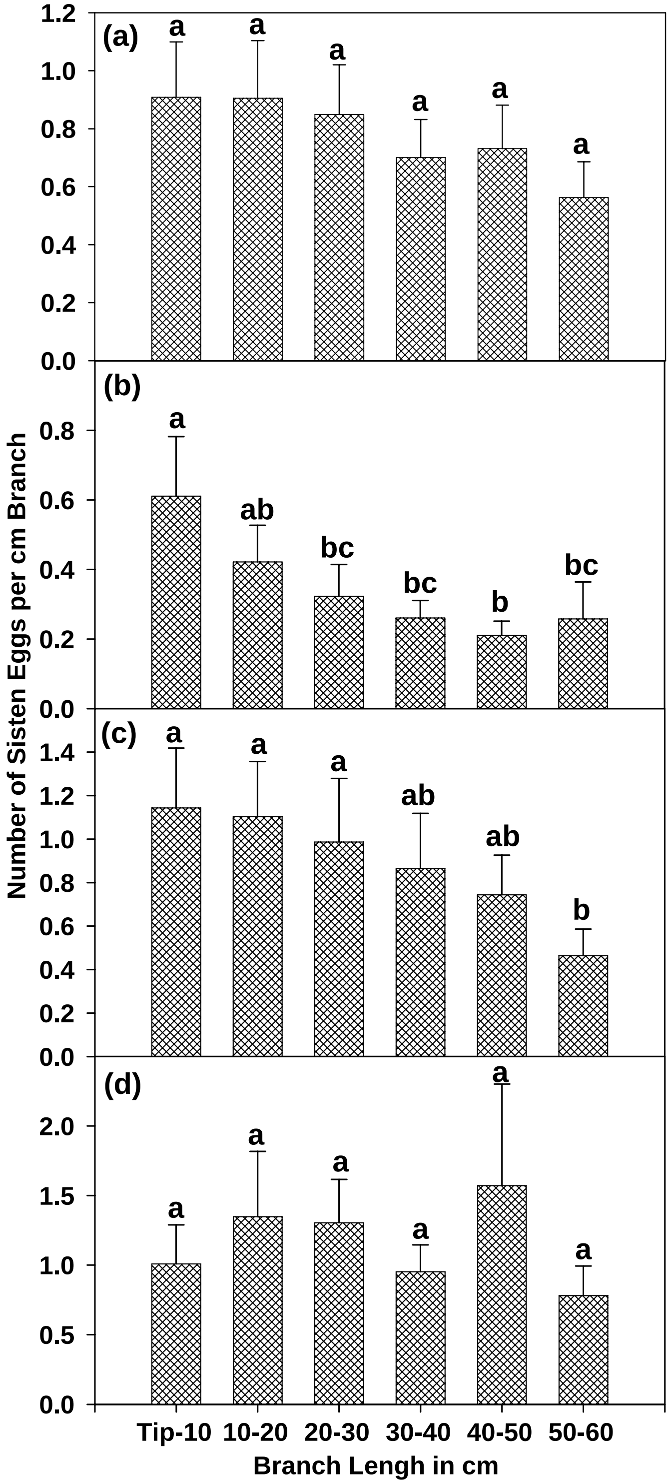

Fig. 3.6. Effects (means ± SE) of distance from branch tip of 60 cm long *T. canadensis* branches on eggs of hemlock woolly adelgid (N = 180) in June 2007. The figures represent tree crown position, (a) whole tree (b) lower (c) middle, and (d) upper. Bars of the same fill color with the same letters are not significantly different (LSD, α = 0.1).

CHAPTER 4

EFFECT OF FERTILIZER ON HEMLOCK WOOLLY ADELGID (HEMIPTERA: ADELGIDAE) GROWTH ON VARIOUS HEMLOCK SPECIES AND FEEDING PREFERENCE OF SPECIALIST PREDATORS

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S. V. Joseph, S. K. Braman, and J. L. Hanula

ABSTRACT We evaluated host plant resistance to hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae) among five hemlock species under three different fertilizer regimes to assess whether or not fertilizer affected difference in susceptibility of various hemlock species, and to examine if fertilizer and host tree species affected prey quality. The three treatments included a long-term fertilizer application (June 2008 to June 2009) a short-term fertilizer application of same rate (March to June 2009), and an unfertilized group. On 31 March 2009, all plants were artificially infested. Among unfertilized hemlocks, foliar N content was highest in *Tsuga mertensiana* (Bong.) Carrière, lowest in *T. chinensis* (Franch.) E. Pritz and intermediate in *T. canadensis* (L.) Carrière and *T. heterophylla* (Raf.) Sarg. A greater number of progredien ovisacs or sisten eggs per cm shoot were noted on *T. mertensiana* than on other hemlock species, an intermediate density was noted on *T. canadensis* and *T. heterophylla,* and none developed on unfertilized *T. chinensis*. Compared to unfertilized *T. canadensis*, recently-fertilized trees had more ovisacs, eggs and adelgid fecundity. Fertilizing *T. heterophylla* had no effect on *A. tsugae* feeding on them. However, densities of developing *A. tsugae,* especially older-instar nymphs, were greater on unfertilized *T. heterophylla* than on fertilized *T. heterophylla* regardless of fertilizer. In the spring, long-term fertilized *T. canadensis* or *T. heterophylla* plants produced abundant new growth and had a higher dry weight biomass relative to unfertilized plants. Feeding preference of adelgid predators was tested by providing equal numbers of adelgid eggs developed on various hemlock species that received fertilizer or not, in a small choice arena. More eggs from fertilized *T. canadensis* were consumed by the predators *Laricobius nigrinus* Fender and *Sasajiscymnus tsugae* (Sasaji & McClure) than from unfertilized ones. No predator interaction-effects were noted on any other choices.

KEYWORDS: hemlock woolly adelgid, resistance, fertilizer, *T. canadensis*, *T. heterophylla*, *T. mertensiana*, *T. chinensis*

The non-native pest hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), has caused widespread mortality to eastern hemlock, *Tsuga canadensis* (L.) Carrière, and Carolina hemlock, *Tsuga caroliniana* Engelmann, in the Eastern United States. Currently, hemlock woolly adelgid occurs in 17 states, which include >50% of the native range of eastern hemlock and the entire range of Carolina hemlock (USDA-Forest Service 2008). Carolina hemlock is endemic to the southern Appalachian Mountains within the boundaries of Virginia, Tennessee, Georgia and North and South Carolina (Jetton et al. 2008a). Upon adelgid infestation, needles gradually begin to yellow, new shoot production slows, and eventually dieback occurs (McClure 1991a) as adelgids consume stored nutrients in the xylem parenchyma cells using their long stylets. In general, tree mortality occurs approximately 10 yrs after initial infestation. However, mortality may occur 2 to 3 yrs in the southeastern United States (Trotter and Shields 2009).

Hemlock woolly adelgid has a polymorphic (both winged and wingless), bivoltine life cycle, which include sisten (from June to March) and progredien (from March to June) generations on hemlocks of eastern North America. Both *A. tsugae* generations develop parthenogenetically on the secondary hemlock host; however, other winged morphs of the progrediens generation undergo sexual reproduction. The winged females, called sexuparae have an incomplete generation owing to the absence of a primary host (spruce) in the eastern United States. The primary host of *A. tsugae* was identified as *Picea torano* (Koch) Koehne in Japan (Montgomery et al. 2009). The severity of impact in the eastern US is attributed to a high replacement rate (≈50 nymphs per sisten female), lack of effective native natural enemies, relatively easy dispersal by mammals, birds, or wind (McClure 1990), and weak hemlock resistance (Trotter and Shields 2009). Although use of chemical insecticides is effective in managing this pest, it is only feasible in recreational areas or various ornamental landscapes having valuable hemlocks, or on a limited basis in forests. Widespread use in forests is too expensive, impractical and has unacceptable environmental risks (Cowles et al. 2006). However, biological control might provide a feasible and sustainable solution to this pest problem in the forest (Cheah and McClure 1998).

Eight of nine hemlock species are reportedly infested by *A. tsugae*, including two western North American hemlocks, western hemlock, *Tsuga heterophylla* (Raf.) Sarg. and mountain hemlock, *Tsuga mertensiana* (Bong.) Carrière; two Chinese hemlocks, *Tsuga chinensis* (Franch.) E. Pritz and *Tsuga dumosa* (D. Don) Eichler; two Japanese hemlocks, northern Japanese hemlock, *Tsuga diversifolia* (Maxim.) Mast., and southern Japanese hemlock, *Tsuga sieboldii* Carrière (Havill et al. 2008, Havill and Montgomery 2008); and the two eastern American hemlocks. Although hemlock woolly adelgid is common on western hemlock, *T. heterophylla* and mountain hemlock, *T. mertensiana* in the Pacific Northwest, it is not injurious to hemlock health in this region (Furniss and Carolin 1977) unless the tree is seriously stressed (McClure 1992a). Lower *A. tsugae* survival and fecundity on 1.8 m tall plants of *T. heterophylla*, *T. mertensiana* and *T. sieboldii* relative to *T. canadensis* or *T. caroliniana* was reported in Connecticut (McClure 1992a). Jetton et al. (2008b) also found lower *A. tsugae* fecundity on *T. heterophylla* than on *T. caroliniana* in North Carolina. However, Mausel (2005) observed greater adelgid fecundity on western hemlock (62% of the total) than on eastern hemlock in the Northwest. These studies indicate a lingering uncertainty regarding the level of resistance among Pacific Northwest hemlock species.

The Asian hemlocks, *T. chinensis* as well as *T. diversifolia* are highly resistant to hemlock woolly adelgid infestation (Bentz et al. 2002, Del Tredici and Kitajima 2004). However, dense populations of *A. tsugae* were reported on *T. chinensis* in China. Upon investigation, using mitochondrial and nuclear DNA sequences, phylogenetic analysis among the *A. tsugae* populations around the world showed that hemlock woolly adelgid infesting the eastern hemlock is closely related to those infesting *T. sieboldii* in Japan. Adelgids found on *T. chinensis* in China are not related to *A. tsugae* on hemlocks in the eastern United States (Havill et al. 2006). In addition, Havill et al. (2006) could not establish a strong evolutionary relationship between the *A. tsugae* populations observed on western and eastern North American species. Therefore, performance of the adelgid may differ on resistant western and Asian hemlock species planted in the eastern United States.

Several breeding projects involving inter-specific hybrids, intra-specific crosses and vegetative propagation are being conducted (Del Tredici 1985, Wang et al. 1997, Jetton et al. 2005, Bentz et al. 2002, Pooler et al. 2002). The hybrids created were mostly a cross between *T. chinensis* and *T. caroliniana* (Wang et al. 1997, Pooler et al. 2002). Attempts to incorporate resistant genes into species of the eastern US by hybridizing Asiatic adelgid-resistant hemlocks (*T. diversifolia*, *T. sieboldii* or *T. chinensis*) and susceptible eastern hemlock has been unsuccessful (Bentz et al. 2002). Phylogenetic relationship using DNA sequences of worldwide specimens of *Tsuga* spp. indicate that the eastern American hemlocks (*T. canadensis* and *T. caroliniana*) are not closely related; instead, susceptible *T. caroliniana* is closed related to resistant *T. diversifolia* (Havill and Montgomery 2008). Montgomery et al. (2009) tested those hybrids by artificially infesting them with *A. tsugae* crawlers and observed intermediate adelgid population growth relative to their resistant-Asian and susceptible-Eastern North American hemlock parents.

Fertilizer is commonly used on nursery trees and shrubs and in ornamental landscapes (Braman et al. 1998), where its effects on host plant resistance is widely debated (Herms 2002). In most of the breeding programs, plants have been grown under a scheduled fertilizer regime and tested for adelgid resistance (Caswell et al. 2008, Montgomery et al. 2009). McClure (1992) showed that fertilizing *T. canadensis* increased egg production of *A. tsugae* by two-fold and its density by five-fold relative to unfertilized trees. However, effects of fertilization have not been examined on resistant hemlock species to date. *Laricobius nigrinus* Fender (Derodontidae) and *Sasajiscymnus tsugae* (Sasaji & McClure) (Coccinellidae) are two specialist predators released against *A. tsugae* in the forest (Cheah and McClure 1998, Zilahi-Balogh et al. 2003, Lamb et al. 2006). Quality adelgid eggs are vital for both the predators as food or oviposition sites (Palmer and Sheppard 2002, Lamb et al. 2006). Our objectives for this project were to: 1) evaluate the host plant resistance among various hemlock species; 2) determine if hemlock resistance to hemlock woolly adelgid is affected by chemical fertilization: and 3) determine if tritrophic interactions are affected by prey quality.

Materials and Methods

Plant Material and Treatment. The experiment was conducted at the Mountain Research Station in Blairsville, Union Co., GA from June 2008 to June 2009. One-year-old hemlocks-*T. canadensis*, *T. heterophylla*, *T. mertensiana* and *T. chinensis* - were purchased from Porcupine Hollow Tree Nursery (Central Lake, MI), Brooks Tree Farm (Brooks, OR), Mineral Springs Ornamental Plant Nursery (OR) and Parry Tree Farm Nursery (Forest Grove, OR), respectively, during May-June 2008. Eastern and western American hemlocks were received as bare-root seedlings and the *T. chinensis* as potted plants. The bare-root plants were planted into 3.8 L pots with unfertile soil media (Sun-Gro Horticulture, Metro-Mix 300 series, Bellevue, WA) and the *T. chinensis* were re-planted to same sized-pots and soil media. Plants were maintained in a screen house with screen mesh allowing 50% sunlight. Plants were irrigated on a 2-day schedule. During the winter (November to February), the average minimum temperature was -1.4^{o}C with a low of -16.1^{o}C on 16 January 2009. Spaces between the plants were mulched with hay up to the pot height to prevent root injury from frost.

The experiment was a 4 X 3 factorial design with 10 single plant replications per treatment per hemlock species. The fertilizer treatments were long-term fertilized (between June 4, 2008 and June 2, 2009), short-term fertilized (between 31 March 2009 to 2 June 2009) and unfertilized. Fertilized plants received 240.1 ppm N through water-soluble fertilizer (Scott Peter Professional 20-20-20 General purpose, Marysville, OH) at biweekly intervals starting from June 4, 2008 to June 2, 2009, except during winter months (from November to February) when they were fertilized once per month. The long- and short-term fertilizer treatments received fertilizer 20 and 6 times, respectively. Fertilizer was applied by soil drench using 200 ml of an aqueous solution of 240 mg of fertilizer per plant. The fertilizer was composed of 3.94% ammonia, 6.05% nitrate and 10.01% urea N. Although all the plants were established after planting, *T. heterophylla*, and *T. mertensiana* suffered mortality regardless of fertilizer treatment (Table 4.1). This was consistent with a previous finding that suggested a poor acclimation of *T. heterophylla* and *T. mertensiana* to eastern North America (Bentz et al. 2002). Unfertilized dead plants were replaced by extra healthy plants that were maintained under the same condition throughout the course of the experiment.

Artificial Infestation and Measurements. On 31 March 2009, hemlock plants were artificially infested by loosely attaching woolly sisten ovisacs along with a hemlock shoot collected from nearby infested trees using paper clips. Ovisacs on each hemlock branch were counted and branch bundles of 25 ovisacs were prepared. Four such bundles (100 ovisacs) were attached to each plant. Ovisacs average 142 progredien eggs/sac. Branches with ovisacs remained on the plants for 3 weeks to facilitate settling of emerged crawlers. We decided to infest plants during early spring because progredien crawlers have a better settlement rate relative to sisten crawlers on hemlock branches (Butin et al. 2007). Butin et al. (2007) also observed that crawler settlement was greatest when adelgid-infested shoots were loosely tied and settlement rate was not affected by increasing density of adelgid inoculation.

On May 1, plants were evaluated for sedentary second-instar progredien nymphs settled on the hemlock twigs. Four branches were non-destructively examined under a compound microscope (10X), and second-instar nymphs were counted.

On June 3 and 11, 2009, two 15-cm-long branches were cut from each plant and stored in a cold chamber at 12^{o}C. Total length of the branch (including length of side-branches), winged exuviae, live apterous and pterous nymphs, ovisacs, and eggs were recorded. Additionally, number of growing hemlock tips and their length were measured from a 10 cm long branch.

On June 11 2009, the entire plant was cut ≈1 cm above the soil and retained 12^{o}C. Later, the plants were oven-dried at 40^{o}C for 48 h, and total plant biomass was recorded. Those four 15-cm-long hemlock branches destructively sampled per plant in June were also included with the respective entire-plant sample prior to oven drying. Dried needles were removed and analyzed for total N (Colombo and Giazzi 1982) and other mineral nutrients (Isaac and Johnson, 1985, AOAC, Official Method 985.01. 1995) at a plant and soil testing lab (University of Georgia, Athens, GA).

Predator Choice Tests. Adult *S. tsugae* and *L. nigrinus* were obtained from the predator-rearing laboratory, Young Harris College (Young Harris, GA) and the University of Georgia (Athens, GA), respectively. During the summer, adult *S. tsugae* beetles were maintained at 18-20^{o}C, 55-65% RH; 12:12 (L: D) and provided with a 15.4-25.4 cm long *A. tsugae* infested hemlock twig in a storage cage. Adult *L. nigrinus* beetles were maintained at 8-10^{o}C (day and night), 65% RH; 12:12 (L: D). Adult beetles, 50 *S. tsugae* and 40 *L. nigrinus,* were brought to the USDA-Forest Service laboratory (Athens, GA) for a beetle preference study and were maintained under the same abiotic conditions.

Beetle response was tested in a 9-cm diameter petri dish with moistened tissue paper on the bottom (Butin et al. 2004). Uninfested shoots (1 cm long) were collected from each hemlock species except *T. chinensis* because almost no *A. tsugae* developed on it. Twenty *A. tsugae* eggs deposited by adults reared on fertilized or unfertilized hemlock of differing species were placed on the shoots. One shoot with eggs from fertilized trees and one with eggs from unfertilized trees was placed on opposite sides of the arena, and an adult beetle (starved 24 h) was introduced in the center. Intact or damaged eggs were quantified after 24 h. All the tests were conducted at 26-27^{o}C and 45-55% RH. In another trial predators were given a choice of 1-cm long branches of *T. canadensis* with 10 *A. tsugae* eggs developed on unfertilized *T. canadensis* and 10 *A. tsugae* eggs deposited by adults reared on either unfertilized *T. heterophylla* or *T. mertensiana*. All the choice tests were replicated 10 times.

Statistical Analyses. A generalized linear model (GLM) was used to analyze the effects of hemlock species and fertilizer treatments on adelgid densities. Because of their sedentary habit, their density is proportional to the branch length. Thus, adelgid counts were standardized by dividing the total length of hemlock branch. Data, including the growth parameters, were transformed by natural log (x + 1) to attain homogeneity of variance. However, the dry weight data were not transformed and eggs per ovisac were transformed using the natural log. Too few adelgids or none developed on *T. chinensis* plants; therefore, to prevent unnecessary inflation of experimentwise error rates, the adelgid counts were not included in the multiple comparisons of hemlock species for each fertilizer treatment (Montgomery et al. 2009, Reeve and Strom 2004). It is noteworthy that only two *T. mertensiana* plants survived in the long-term fertilization group. Thus, the adelgid count data obtained from the two *T. mertensiana* plants were not included in the analysis to determine differences within hemlock species that received long-term fertilizer treatment. The data were examined using the PROC GLM procedure of SAS (SAS Institute 2003), and means were separated using a least significant difference (LSD) test at $\alpha = 0.05$. The correlations between adelgid density and foliar nutrient content were examined using PROC CORR procedure of SAS. The multicolinearity, if it existed among the nutrient elements, was removed by considering the partial correlations and adding a PARTIAL statement to the CORR procedure. Regression analysis using PROC REG procedure of SAS examined the relationship between fecundity and percent foliar N concentration. The predator feeding choice tests were analyzed by paired t-tests using PROC TTEST of SAS. The data presented in the tables and figures are untransformed data.

Results

Effect of Hemlock Species. In May 2009, one month after being infested, second-instars were more abundant on unfertilized *T. mertensiana* than on *T. canadensis* or *T. heterophylla*, and lowest on *T. chinensis* ($F = 99.6$; df = 3, 51; $P < 0.0001$; Fig. 4.1). For this analysis, the data were combined from two fertilizer treatments (long-term fertilized and unfertilized) per hemlock species.

After two months of infestation in June 2009, unfertilized plants had significantly more ovisacs per cm branch on *T. mertensiana* than on *T. canadensis* or *T. heterophylla* and none developed on *T. chinensis* (Table 4.2; Fig. 4.2). Eggs laid by apterous females per cm branch were greater on *T. mertensiana* compared to *T. canadensis* or *T. heterophylla* on unfertilized trees (Table 4.2; Fig. 4.3). No eggs were found on *T. chinensis*. Adelgid fecundity was similar among various hemlock species (Table 4.2; Fig. 4.4a). The progredien second- and third-instar nymphs were greater on *T. heterophylla* than on *T. mertensiana* or *T. canadensis* (Table 4.2; Fig. 4.5a). Similarly, progredien older-apterous nymphs were also higher on *T. heterophylla* compared to *T. mertensiana* plants (Table 4.2; Fig. 4.5b). A higher density of sisten second-instar nymphs settled on *T. mertensiana* (mean ± SE = 4.42 ± 1.04) than on both *T. canadensis* (mean ± SE = 1.66 ± 0.48) and *T. heterophylla* (mean ± SE = 1.70 ± 0.59; Table 4.2).

Effect of Fertilizer Treatments. The total foliar N content was significantly greater in both the short-term and long-term fertilized *T. canadensis* ($F = 21.15$; df = 2, 11; $P < 0.0001$) or *T. heterophylla* ($F = 17.88$; df = 2, 13; $P < 0.0002$) plants than their respective unfertilized counterparts (Fig. 4.6). A dose response of total foliar N was observed in *T. chinensis* with higher N concentration recorded on long-term fertilized plants followed by the short-term fertilizer treatment and by the non-fertilizer treatment ($F = 38.38$; df = 2, 18; $P < 0.0001$). Again, only two *T. mertensiana* plants survived in the long-term fertilization group, and they were not included in the analysis.

The short-term fertilizer treatment had a significant effect on fecundity (Table 4.3), illustrated by a higher number of eggs produced per ovisac on short-term fertilized *T. canadensis* than on the non-fertilizer treatment (Fig. 4.4a). Adelgids on long-term fertilized *T. canadensis* were not significantly more fecund than those on the other two treatments. Fecundity on *T. heterophylla* was not affected by fertilizer treatment. Progredien adult and egg densities were also greater on short-term fertilized *T. canadensis* than on the unfertilized ones (Table 4.3; Figs. 4.2 and 4.3). We did not see this pattern in *T. heterophylla*. Regression analysis also showed a significant correlation between adelgid fecundity and N concentrations in *T. canadensis* (F = 5.04; df = 1, 28; $P < 0.033$; Fig. 4.4b).

Progredien second and third-instars were more abundant on unfertilized *T. heterophylla* than on plants that received short-term fertilization but not those that received long-term fertilization (Table 4.3; Fig. 4.5a). Similarly, more fourth-instar apterous females were found on the unfertilized *T. heterophylla* compared to either the short-term or long-term fertilizer treatments (Fig. 4.5b). This trend was not noted on the other hemlock species studied.

In both short-term and long-term fertilizer treatments, ovisacs per cm branch were not significantly different among hemlock species (Table 4.2; Fig. 4.2). Few ovisacs were seen on *T. chinensis*. A slightly greater number of eggs per cm branch and significantly higher fecundity were noticed on *T. canadensis* than on *T. heterophylla* in the short-term fertilizer treatment (Table 4.2; Figs. 4.3 and 4.4a). On short-term fertilized treatment, winged females (alates) per cm branch were higher on *T. canadensis* (mean ± SE = 0.02 ± 0.00) than on *T. heterophylla* (mean ± SE = 0.01 ± 0.00; Table 4.2).

Fertilizer Effects on Tree Growth. Long-term fertilized *T. canadensis* plants had abundant new shoots and needles, greater shoot length, and higher total dry weight than untreated or short-term treated plants (Table 4.4). Similarly, *T. heterophylla* plants treated with fertilizer for a year had longer new shoots and greater biomass compared to unfertilized or plants fertilized for a short duration. The number of new shoots produced by *T. heterophylla* did not differ between fertilizer treatments but greater shoot growth was found on short-term treated plants compared to unfertilized ones. No clear indication of fertilizer effect was noticed on the long-term fertilized *T. mertensiana* or both long- and short-term fertilized *T. chinensis* plants.

Plant Nutrient Analyses. Most of the nutrients varied significantly with species in the unfertilized treatment. Percent total N and Al content were highest in *T. mertensiana* foliage and lowest in *T. chinensis* within the unfertilized category (Table 4.5). The P content was significantly higher in *T. chinensis* compared to all other hemlock species. *Tsuga heterophylla* had the most K and the smallest quantity was detected in both *T. canadensis* and *T. mertensiana*. Concentrations of Mg did not differ among the American hemlocks but all three had significantly more Mg than *T. chinensis*. Both *T. canadensis* and *T. mertensiana* had higher levels of S relative to the *T. heterophylla* and *T. chinensis*. The highest and lowest Mn and Zn content were found in the *T. mertensiana* and *T. heterophylla* plant needles, respectively. Foliage B levels were highest in *T. heterophylla* while *T. Canadensis* had the lowest. A significantly higher concentration of Ca was recovered from *T. mertensiana* and *T. chinensis* plant foliage compared to *T. canadensis* followed by the *T. heterophylla*.

The partial correlation analysis revealed a significant relationship between N, P, Mg, S, Al, B and Mn and live adelgid stages settled or the secondary population derived from the settled crawlers (Table 4.6). Developing immature stages of adelgid settled as of June 2009 were positively correlated with the Mg or Al while maintaining a negative association with the P or S content. Increasing levels of the Mn were related to a high number of ovisacs and their eggs. Conversely, a negative association between the P content and ovisac and its egg densities was noted. Results show a slight positive relationship between the Al or B contents and density of ovisacs ($P < 0.05$). Number of eggs produced by the progredien females was positively related with the quantity of S in the foliage. Interestingly, N, P, S and B concentrations were positively correlated with adelgid fecundity in our study. Fecundity showed a slight increase association with a amount of needle Fe ($P < 0.1$). There was a significant positive correlation between the Mn quantities and secondary nymphs. In addition, sisten nymphs had a negative association with the P or B concentrations ($P < 0.1$).

Choice Arena Tests. After 24 h, significantly more *A. tsugae* eggs derived from fertilized *T. canadensis* were consumed by *L. nigrinus* adults than eggs from unfertilized trees (Table 4.7). *Sasajiscymnus tsugae* also consumed more eggs from females that developed on fertilized *T. canadensis* than on unfertilized ones ($P < 0.1$). However, neither predator showed any preference for *A. tsugae* eggs developed on fertilized versus unfertilized *T. heterophylla* or *T. mertensiana*. When given a choice of eggs derived from 3 unfertilized hemlock species neither predator exhibited a preference for eggs of a given species. When second-instar nymphs developed on *T. canadensis* were provided as prey in the test arena, neither predator consumed many of them.

Discussion

Initially, more *A. tsugae* crawlers settled on *T. mertensiana* than on *T. canadensis* or *T. heterophylla*. Usually, adelgid crawlers settle at the needle base (McClure 1987) but we found them all over the plants including the bark on the stems and branchlet regions of *T. mertensiana*. A greater number of adult females were observed on *T. mertensiana* than other hemlock species, but they had the fewest developing nymphs compared to other American hemlock species tested. Our findings suggest that *T. mertensiana* is more susceptible to *A. tsugae* than *T. heterophylla* or *T. canadensis* when they are young plants. Whether this would continue to be the pattern for older trees is unclear. However, these results are consistent with our nutrient content analyses which showed that unfertilized *T. mertensiana* had higher foliar nitrogen content than unfertilized *T. canadensis* or *T. heterophylla* (Fig. 4.6). *Tsuga chinensis*, which had almost no adelgids settling or surviving on them, had the lowest foliar nitrogen content among unfertilized plants.

We found no apparent difference in numbers of ovisacs or eggs on *T. canadensis* and *T. heterophylla*. However, progredien nymphs developed more slowly on the *T. heterophylla* plants relative to other hemlock species. This was indicated by the higher number of second and third instar nymphs found on unfertilized *T. heterophylla* plants in June (Fig. 4.5a) while almost all adelgids on *T. mertensiana* had developed into adults (Figs. 4.5a and 4.2). Previous reports have shown that *A. tsugae* performed poorly on *T. heterophylla* and *T. mertensiana* suggesting these species were relatively resistant compared to *T. canadensis* or *T. caroliniana* (McClure 1992a, Pontius et al. 2006, Jetton et al. 2008b). In addition, Havill et al. (2006, 2007) have identified separate genetic lineages for the *A. tsugae* that occur in western and eastern North America. Others suggested that *A. tsugae* development on *T. heterophylla* might be inhibited by the levels of α-pinene, β-caryophyllene or α-humulene while high levels of isobornyl acetate in the *T. canadensis* encourage feeding (Lagalante and Montgomery 2003). Therefore, Lagalante and Montgomery (2003) categorized *T. heterophylla* and *T. canadensis* as resistant and susceptible, respectively, to hemlock woolly adelgid infestation. Several other reports also concluded that natural adelgid populations had little impact on *T. heterophylla* or *T. mertensiana* in their native range because of the combined effects of various limiting factors including effective natural enemies, host-plant resistance and low temperatures that keep *A. tsugae* populations under control (McClure 1992a, McClure and Cheah 1999, Kohler et al. 2008).

As in previous studies (Bentz et al. 2002, Del Tredici and Kitajima 2004), we found very few second instars settled and none developed on the unfertilized *T. chinensis* plants. These plants also had much lower foliar nitrogen content compared to the other unfertilized hemlock species tested (Fig. 4.6). Clearly, *T. chinensis* is highly resistant predominantly due to antibiosis resistance. Whether this is linked to their low nitrogen content is unclear. Phylogenetic analysis of mitochondrial DNA suggests that hemlock woolly adelgid found in eastern North America more closely resembles adelgids infesting *T. sieboldii* in southern Japan than those in mainland China or Taiwan (Havill et al. 2006, Havill et al. 2007). Therefore, adelgid populations adapted to Chinese hemlock in China are not of the same lineage as those found on eastern hemlock and which we used in our study. This suggested genetics difference in *A. tsugae* further complicates their interaction of the adelgid with various hemlock species. However based on our study in North Georgia, we observed a gradient in hemlock resistance to *A. tsugae* derived from Japanese adelgids in the following order: *T. chinensis* > *T. heterophylla* ≥ *T. canadensis* > *T. mertensiana*.

Nitrogen is an important nutrient for all insects, especially piercing and sucking insects which often respond to higher nitrogen levels in their host plants (Petitt et al. 1994, Kytö et al. 1996, van Emden 1996, Nevo and Coll 2001). Our results on unfertilized plants showed that foliar nitrogen was more abundant in *T. mertensiana* than any other hemlock studied. Moreover, the nitrogen concentration in *T. canadensis* or *T. heterophylla* was similar, while the smallest amount was found in the *T. chinensis*. Interestingly, a dose response was noticed in the adelgid populations that developed on these plants. As the nitrogen content in foliage increased, higher densities of *A. tsugae* were noted. These data and those of Pontius et al. (2006) suggest that nitrogen content of host plants plays a role in hemlock resistance or susceptibility. It also suggests that the role that foliar N may play in *A. tsugae* survival or establishment on hemlock.

In our study, little or no *A. tsugae* was found on resistant, low nitrogen *T. chinensis* while higher populations were seen on *T. mertensiana* which had the highest nitrogen content. These data may be useful to researchers interested in detecting and screening for host tree resistance within eastern hemlock species.

Tsuga canadensis under both short- and long-term fertilization had higher levels of foliar nitrogen compared to unfertilized trees (Fig. 4.6) but only the long-term fertilized trees had more plant biomass (Table 4.4). Interestingly, short-term fertilized *T. canadensis* had more mature ovisacs and eggs per cm branch than both unfertilized and long-term fertilized trees (Fig. 4.2 and 4.3). Fecundity exhibited a similar pattern although females on long-term fertilized plants did not produce more eggs than those on the other two treatments (Fig. 4.4). McClure (1991) noted an immediate increase in *A. tsugae* fecundity when they developed on young *T. canadensis* trees after a spring application of fertilizer. However, it is unclear why there was no difference in adelgid density between long-term fertilized *T. canadensis* and unfertilized ones.

Application of fertilizer may reduce the level of secondary metabolites thereby decreasing tree-resistance to sap-feeding insects (Herms 2002). Herms and Mattson (1992) proposed that under the growth/differentiation balance hypothesis, plants growing in nutrient-limited conditions would produce more secondary metabolites. Production of secondary metabolites would decrease as nutrient availability increased and favored growth. Our data showed that *A. tsugae* developed on reportedly resistant *T. heterophylla* plants but did not show significant difference in the density of developed adults regardless of the length of fertilization or high N concentration in the foliage of fertilized plants. Interestingly, densities of developing *A. tsugae,* especially older-instar nymphs, were greater on the unfertilized plants than on both the fertilized treatments of *T. heterophylla* plants. Again, second/third-instar nymphs were most abundant on plants not treated with fertilizer than those provided with fertilizer for a short period. It is likely that antibiosis resistance affected growth of nymphs on unfertilized *T. heterophylla*. Perhaps to a certain extent, available nitrogen increased palatability of fertilized *T. heterophylla* plants to settled *A. tsugae* and aided development. Unlike fertilized *T. canadensis* or *T. heterophylla*, fertilized *T. chinensis* did not show a change in susceptibility to *A. tsugae*. However, we recovered three ovisacs with 13 eggs and 23 developing nymphs from 2 fertilized *T. chinensis* plants.

In the spring, long-term fertilized *T. canadensis* or *T. heterophylla* plants produced abundant new growth. However, in spite of comparatively high levels of foliar nitrogen in fertilized plants, new shoot production in the *T. chinensis* was substantially less than in the *T. canadensis* or *T. heterophylla* plants. *Tsuga chinensis,* however, had earlier bud-break, when bud-break phenology among American and Asian hemlocks was compared (Montgomery et al. 2009). Because we inoculated the hemlock plants with high egg densities (avg. 14, 200 eggs per plant), a dilution effect from increased surface area for settling crawlers resulting from increased plant growth on plants that received fertilizer should not have been a problem. Moreover, the dry weight data also mirrored the response of new shoot growth as fertilized *T. canadensis* or *T. heterophylla* plants had greater biomass than plants that were never fertilized. The short-term fertilized plants may not have had sufficient time to allocate nitrogen so that overall plant growth and biomass were comparable to unfertilized plants.

Our data showed that increased Al content was associated with high numbers of developing nymphs. This is consistent with our data on unfertilized *T. mertensiana* which had the highest foliar Al levels as well as higher numbers of adelgid ovisacs and eggs. Similarly, our data showed a positive correlation between Mg levels and developing nymphal density, and Mg was uniformly higher among relatively susceptible North American hemlocks than in the *T. chinensis* plants with no adelgids. Retnakaran and Beck (1967) showed that pea aphid, *Acyrthosiphon pisum* (Harris), requires low quantities of Mg in its diet for normal performance and reproduction. This means that even a small variation in the Mg content, in our case as a low content recorded in *T. chinensis* hemlock, may be contributing to hemlock resistance to *A. tsugae*. Interestingly, the Mn content was correlated with adelgid abundance and again was high in *T. mertensiana*, but how Mn levels might affect tree resistance is not known. Although we detected little difference in the P levels among American hemlocks, our data suggest that even a minute increase in the P concentration has a deleterious impact on *A. tsugae* abundance. Moreover, the P content was highest in *T. chinensis*, and adelgids failed to complete development on this species. This suggests that the P levels may play an important role in hemlock resistance. *Adelges tsugae* infestations appear to decline when P is present in high concentrations in hemlocks (Pontius et al. 2006). Combined N, P and S in fertilizer applied to *Pinus radiata* (D. Don) improved tree growth and reduced colonization of Monterey pine aphid, *Essigella californica* (Essig.) (Hopmans et al. 2008). Hopmans et al. (2008) concluded that availability of the added P and S helped the tree to rectify its deficiencies and produce abundant new shoot growth. A greater S or B content was also found to be associated with an increase in adelgid fecundity. In our *A. tsugae* infestation experiment on young hemlocks, concentration of N, Al, and S were associated with susceptibility while P and K were related to resistance.

Tritrophic studies involving interaction of biological control agents and prey when mediated by host plant quality may help to better understand bottom-up effects in the population. Host plant quality could be influenced by plant chemistry including both available nutrient and secondary metabolite levels. For *T. canadensis*, our choice arena results showed that *L. nigrinus* consumed more eggs that developed on fertilized plants than those on the unfertilized plants. We also know that fertilized *T. canadensis* had high levels of N, which increased *A. tsugae* survival and reproduction. Apparently, the predator *L. nigrinus* preferred eggs produced by *A. tsugae* on a more nutritional host. *Laricobius nigrinus* larvae and adults prefer *A. tsugae* eggs to other stages and eggs are vital for their development (Zilahi-Balogh et al. 2003a). Zilahi-Balogh et al. (2003a) reported that approximately 300 *A. tsugae* eggs are required for *L. nigrinus* larvae to complete development. In our feeding studies, *S. tsugae* adults also prefer eating *A. tsugae* eggs produced by females on the fertilized *T. canadensis* plants. It has been shown that consumption of at least one egg is vital for *S. tsugae* adults to begin their oviposition (Butin et al. 2003). Previous tritrophic studies showed that performance of a hymenopteran larval-endoparasitoid, *Diadegma insulare* (Cresson), doubled on larvae of diamondback moth, *Plutella xylostella* (L.) (Plutellidae), reared on N-fertilized canola, *Brassica napus* L., in the greenhouse (Sarfraz et al. 2009). Similarly, a surge in populations of a specialist egg predator, *Tytthus vagus* Knight (Miridae), on delphacids, *Prokelisia dolus* Wilson and *Prokelisia marginata* (Van Duzee), occurred on fertilized perennial cordgrass, *Spartina alterniflora* (Lois.), demonstrating a strong bottom-up effect in the field (Claudio and Denno 2003). However, *L. nigrinus* and *S. tsugae* adults in our small arena trials did not prey more heavily on eggs from fertilized plants when the host was *T. heterophylla*. It is possible that the levels of secondary compounds in the *T. heterophylla* plants have a cascading effect on the nutritional quality of *A. tsugae* eggs, indirectly affecting predation regardless of fertilizer. There was considerable variation in terpenoids and their levels between the *T. heterophylla* and *T. canadensis*, and this might affect predator feeding (Lagalante and Montgomery 2003).

This study suggests that interactions between hemlock woolly adelgid and hemlock species are difficult to predict when hemlocks are grown outside of their native range. A wide variety of ornamental cultivars representing various hemlock species are being grown in alien ornamental landscapes (Bentz et al. 2002). Additional studies are required to determine hemlock species susceptibility/resistance to *A. tsugae*, specifically on popular cultivars, even though their resistance levels are determined in the native range. Performance of hemlock woolly adelgid may be affected by nutritive status of the hemlock species as per our experiment. Although it is not possible to firmly conclude that fertilization disrupts resistance of relatively resistant hemlock species to *A. tsugae*, it is important to note that antibiosis resistance may be partly affecting their nymphal development. In addition, levels of nutrients and endogenous compounds vary remarkably upon fertilization; in particular, this variation is more intense in young plants than in mature trees (Kytö et al. 1996). Thus, fertilized plants involved in breeding programs should be evaluated for resistance more carefully. Further research might elucidate the effect of fertilizer on mature hemlock tree species in ornamental landscapes. Our study determined that the nutritive value of adelgid eggs could influence predatory behavior of *L. nigrinus* or *S. tsugae* adults and depends on the hemlock species examined. Therefore, a nutritionally weak site or stand of eastern hemlock may have limiting effects on predator fitness. Future studies should investigate, more specifically, the effect that changes in the nutritive value of host trees in the field has on biological control.

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Table 4.1. Number of hemlocks planted, infested and surviving June 2008- June 2009

Hemlock spp.	No. of plants fertilized through 2008-09		No. of plants only fertilized in 2009		No. of plants not fertilized 2008-09		
	N	Infested (% Survival)	N	Infested (% Survival)	N	Infested (% Survival)	Replaced[a]
T. canadensis	10	10 (100)	10	10 (100)	10	10 (100)	0
T. heterophylla	6	6 (60)	9	9 (90)	10	10 (60)	4
T. mertensiana	2	2 (20)	-	-	10	10 (10)	9
T. chinensis	10	10 (100)	10	10 (100)	10	10 (100)	0

[a] dead hemlock plants were replaced by healthy ones from the repository maintained under the same conditions as experimental plants throughout the study and none of the plants were replaced after March 31st, 2009.

Table 4.2. Analysis of variance of the adelgid populations settled on various hemlock species per fertilizer treatment in June 2009

		Progrediens										Sistens						
		Nymphs																
Species effects		2nd/3rd instars		Older[a] Apterous		Older Pterous		Alated adults		Ovisacs		Eggs		Nymphs[b]		Fecundity[c]		
	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	df	*P*
Unfertilized	2, 18	6.2	0.0085	3.9	0.0384	1.1	0.3357	1.46	0.2581	5.6	0.0124	7.3	0.0045	3.6	0.0470	2.2	2,16	0.1389
Short-term fertilized	1, 8	0.0	0.8250	0.6	0.4488	0.0	0.9018	6.81	0.0312	0.5	0.4907	3.6	0.0932	0.0	0.8614	9.6	1, 6	0.0208
Long-term fertilized	1, 6	0.8	0.4731	0.4	0.6830	0.2	0.7679	0.4	0.6624	0.4	0.6794	1.17	0.3716	0.5	0.5862	2.4	2, 6	0.1629

[a] fouth-instar nymphs settled on the hemlock species. [b] comprised of both sisten crawlers and second-instar nymphs. [c] Eggs per apterous female.

Table 4.3. Analysis of variance on effect of fertilizer treatment on hemlock woolly adelgid settled on various hemlock species in June 2009

Fertilizer effects	Progrediens													Sistens						
		Nymphs						Alated adults		Ovisacs		Live progrediens settled		Eggs		Nymphs[b]		Fecundity[c]		
		2nd/3rd instars		Older[a] Apterous		Older Pterous														
	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	df	*P*
T. canadensis	2, 18	0.6	0.5507	2.5	0.1065	0.9	0.4142	1.7	0.2072	5.8	0.0108	1.8	0.1870	7.0	0.0054	2.6	0.0963	5.0	2, 18	0.0186
T. heterophylla	2, 13	6.3	0.0121	4.6	0.0297	0.2	0.8170	1.5	0.2436	1.4	0.2774	0.5	0.6167	1.2	0.3114	3.0	0.0817	1.2	2, 9	0.3259
T. mertensiana	1, 1	1.5	0.4289	2.3	0.3680	74.1	0.0736	8.2	0.2133	0.8	0.5312	3.4	0.3134	6.1	0.2444	0.2	0.6900	7.4	1, 1	0.2242
T. chinensis	-	-	-	-	-		-	-	-	-	-	0.8	0.4587	-	-	-	-	-	-	-

[a]fouth-instar nymphs settled on the hemlock species. [b]comprised of both sisten crawlers and second-instar nymphs. [c]Eggs per apterous female.

Table 4.4. Effect of fertilizer treatments (mean ± SE) on growth parameters of various hemlock species infested with hemlock woolly adelgid in June 2009

Hemlock species	Growth parameters	Unfertilized	Short-term fertilized	Long-term fertilized	*F*	df	*P*
T. canadensis							
	New shoots	0.17 ± 0.02b	0.10 ± 0.03b	0.37 ± 0.06a	7.58	2, 18	0.0041
	Length of new shoots	0.30 ± 0.06b	0.23 ± 0.07b	0.88 ± 0.20a	6.36	2, 18	0.0081
	Needle dry weight	12.11 ± 1.33b	13.83 ± 1.52b	26.42 ± 2.26a	22.45	2, 18	<.0001
	Total dry weight	26.79 ± 3.09b	36.45 ± 6.77b	50.93 ± 4.70a	6.85	2, 18	0.0061
T. heterophylla							
	New shoots	0.26 ± 0.03b	0.91 ± 0.41a	0.42 ± 0.04ab	3.72	2, 13	0.0528
	Length of new shoots	0.41 ± 0.07b	0.90 ± 0.17a	1.03 ± 0.11a	6.25	2, 13	0.0125
	Needle dry weight	4.83 ± 0.70b	3.80 ± 0.31b	8.52 ± 2.00a	8.06	2, 13	0.0053
	Total dry weight	12.60 ± 1.85b	8.77 ± 0.76b	19.72 ± 3.68a	10.86	2, 13	0.0017
T. mertensiana							
	New shoots	0.16 ± 0.04a	-	0.09 ± 0.09a	32.25	1, 1	0.1110
	Length of new shoots	0.07 ± 0.02a	-	0.04 ± 0.04a	3.27	1, 1	0.3215
	Needle dry weight	2.52 ± 0.24a	-	4.14 ± 2.57a	1.10	1, 1	0.4848
	Total dry weight	7.08 ± 0.49a	-	12.84 ± 0.96a	16.60	1, 1	0.1532
T. chinensis							
	New shoots	0.54 ± 0.04a	0.40 ± 0.09a	0.36 ± 0.05a	2.07	2, 18	0.1554
	Length of new shoots	1.12 ± 0.08a	1.11 ± 0.26a	1.07 ± 0.16a	0.16	2, 18	0.8571
	Needle dry weight	22.65 ± 2.84a	13.58 ± 1.41b	18.91 ± 2.47ab	3.63	2, 18	0.0474
	Total dry weight	50.88 ± 6.48a	33.45 ± 3.72a	41.19 ± 4.84a	2.73	2, 18	0.0925

Means in a row followed by different letters are significantly different ($P < 0.05$) on the log-transformed data.

Table 4.5. Mean (± SE) foliar nutrient concentrations among four unfertilized hemlock species in June 2009

Nutrients	Foliage nutrient concentrations				*F*	df	*P*
	T. canadensis	*T. heterophylla*	*T. mertensiana*	*T. chinensis*			
N	1.05 ± 0.04b	1.03 ± 0.04b	1.22 ± 0.04a	0.86 ± 0.06c	7.27	3, 27	0.0010
Ca	0.52 ± 0.03b	0.39 ± 0.02c	0.65 ± 0.02a	0.68 ± 0.06a	11.26	3, 27	<0.0001
K	0.44 ± 0.03c	0.74 ± 0.06a	0.43 ± 0.03c	0.56 ± 0.02b	14.44	3, 27	<0.0001
P	0.26 ± 0.02b	0.26 ± 0.01b	0.28 ± 0.03b	0.36 ± 0.01a	5.13	3, 27	0.0062
Mg	0.31 ± 0.02a	0.33 ± 0.01a	0.31 ± 0.02a	0.23 ± 0.01b	5.44	3, 27	0.0047
S	0.21 ± 0.02a	0.11 ± 0.01b	0.26 ± 0.04a	0.12 ± 0.01b	7.45	3, 27	0.0009
Al	166.32 ± 12.83b	190.01 ± 39.25b	282.99 ± 27.21a	79.31 ± 7.19c	10.62	3, 27	<0.0001
B	29.97 ± 2.02c	49.91 ± 2.47a	40.16 ± 2.96b	41.88 ± 4.30ab	7.24	3, 27	0.0010
Cu	3.78 ± 0.54a	2.60 ± 0.56a	2.90 ± 0.32a	2.65 ± 0.39a	1.17	3, 26	0.3386
Fe	148.91 ± 10.78ab	385.94 ± 190.02a	220.88 ± 21.85ab	102.57 ± 8.89b	1.68	3, 27	0.1950
Mn	86.26 ± 12.34bc	223.16 ± 21.67b	659.44 ± 94.05a	61.99 ± 9.21c	32.97	3, 27	<0.0001
Zn	27.45 ± 12.34bc	36.67 ± 6.92ab	49.43 ± 5.04a	22.90 ± 1.31c	6.19	3, 27	0.0024

Means of Ca, K, Mg, N, P and S shown above are expressed in total % concentration (of dry wt.), while Al, B, Cu, Fe, Mn and Zn are in ppm in the hemlock foliage. Means in a row followed by different letters are significantly different (* $P < 0.05$). Analyses of variance were performed on the untransformed data.

Table 4.6. Pearson's correlation coefficients between adelgid stages or growth parameters and foliar nutrient concentration of hemlock species

A. Pairwise correlation variables	Foliar nutrient concentration											
	N	Ca	K	P	Mg	S	Al	B	Cu	Fe	Mn	Zn
2nd or 3rd instars	-0.01	-0.33*	0.45**	-0.16	0.29	-0.17	-0.01	0.20	-0.14	0.09	-0.11	0.03
Older apterous instars	0.12	-0.40*	0.23	-0.16	0.49**	-0.14	0.33*	0.21	0.13	0.47**	0.02	0.25
Older alated instars	0.35*	-0.05	-0.14	-0.01	0.49**	0.11	0.55**	0.13	0.34*	0.36	0.38	0.48**
Total immatures	0.21	-0.39*	0.25	-0.19	0.53**	-0.09	0.37*	0.22	0.10	0.37*	0.13	0.31
Alated adults	0.32*	0.00	-0.15	0.01	0.27	0.03	0.11	0.02	-0.11	0.03	0.19	0.18
Ovisacs	0.61***	-0.09	-0.12	-0.23	0.39*	0.36*	0.57	0.05	0.25	0.23	0.54**	0.53**
Eggs	0.63***	-0.01	-0.11	-0.19	0.41**	0.44**	0.56	0.02	0.19	0.19	0.58**	0.56**
Eggs / ovisac	0.47*	0.63**	0.03	0.23	0.11	0.40*	0.17	0.04	-0.02	-0.06	0.35a	0.39*
Sis. nymphs	0.64***	-0.08	-0.11	-0.08	0.31	0.27	0.51**	0.04	0.17	0.23	0.54**	0.54**
B. Partial correlations variable	N	Ca	K	P	Mg	S	Al	B	Cu	Fe	Mn	Zn
2nd or 3rd instars	-0.11	-0.08	0.09	-0.13	0.24*	-0.16	-0.03	-0.12	-0.03	0.03	-0.07	0.05
Older apterous instars	-0.02	-0.11	0.01	-0.17	0.35**	-0.26*	0.37**	-0.13	-0.05	0.14	-0.20^a	-0.14
Older alated instars	0.03	0.10	0.02	-0.29**	0.25	-0.11	0.36**	0.09	0.09	-0.13	0.15	-0.08
Total immatures	-0.06	-0.08	0.03	-0.28**	0.35**	-0.23*	0.25*	-0.09	-0.02	0.04	-0.05	-0.05
Alated adults	-0.01	-0.01	-0.18	-0.19^a	0.18^a	-0.07	0.08	0.05	-0.11	-0.02	0.09	-0.03
Ovisacs	0.11	0.11	0.05	-0.32**	0.08	0.17	0.21^a	0.17^a	-0.02	-0.11	0.39**	-0.13
Eggs	0.14	0.10	0.05	-0.26*	0.06	0.26*	0.12	0.19^a	-0.05	-0.12	0.40***	-0.08
Eggs / ovisac	0.28*	0.12	0.01	0.29*	-0.17	0.42**	-0.23	0.28*	0.04	-0.26^a	0.18	0.18
Sis. nymphs	0.18^a	-0.02	0.01	-0.19^a	0.06	0.14	0.10	-0.14^a	0.03	-0.07	0.26*	0.06

The notations indicate the significant correlation (*P*: $^a < 0.1$; *< 0.05; **<0.01 and ***<0.0001) between variables and foliar element concentrations. A. Pairwise correlations between elements and response variables, while B. partial correlations accounts for the multiple correlation effects among the elements.

Table 4.7. Mean (± SE) of eggs consumed after 24 h by two predators when given a choice of eggs developed on hemlock spp. that received long-term fertilizer (between June of 2008 and 2009) or unfertilized

Predator spp.	Hemlock spp.	Trials (no.)	*A. tsugae* eggs provided	No. of *A. tsugae* eggs consumed		Paired *t*-test	df	*P*
				Unfertilized	Long-term fertilized			
S. tsugae	*T. canadensis*	10	20	2.6 ± 0.7	5.5 ± 1.3	-2.25	9	0.051[a]
	T. heterophylla	10	20	5.2 ± 1.6	7.2 ± 2.2	-1.08	9	0.306
	T. mertensiana	10	20	6.4 ± 1.9	5.4 ± 1.4	0.61	9	0.557
L. nigrinus	*T. canadensis*	10	20	6.9 ± 2.2	10.8 ± 2.2	-2.56	9	0.031*
	T. heterophylla	10	20	5.9 ± 1.9	5.8 ± 2.0	0.09	9	0.929
	T. mertensiana	10	20	8.7 ± 1.6	8.4 ± 1.5	0.17	9	0.865

The notations indicate the significant differences (*P*: [a] < 0.1 and *< 0.05)

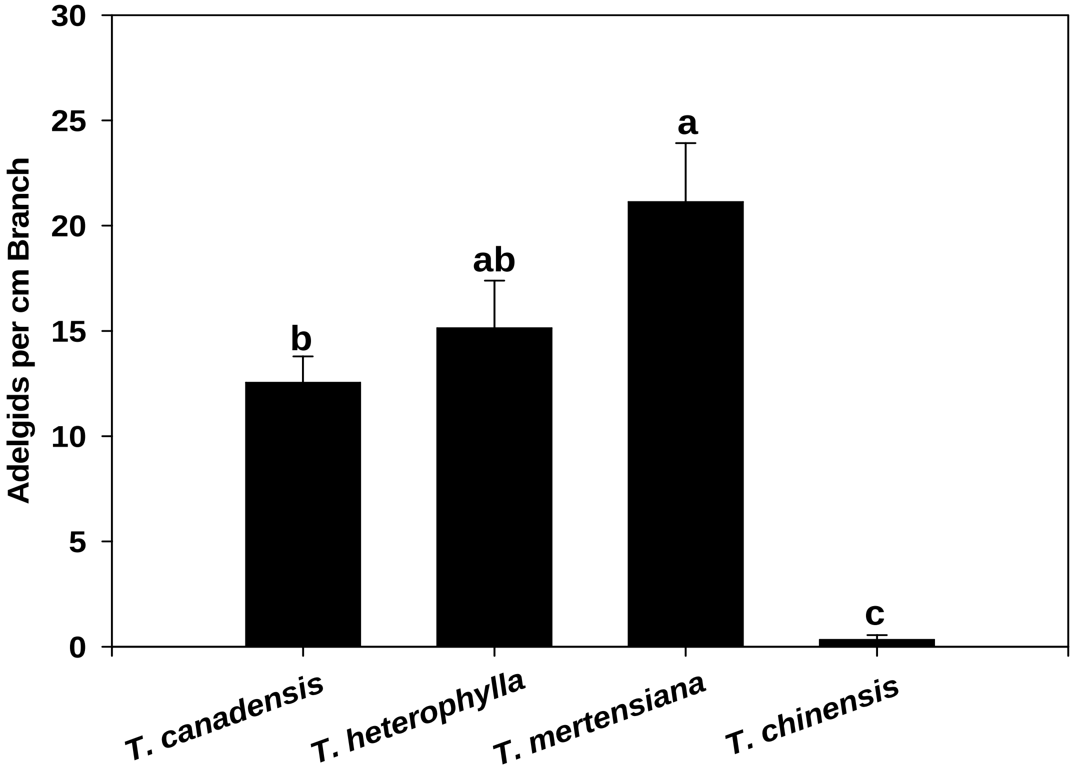

Fig. 4.1. Mean (± SE) number of second-instars observed on various hemlock species (N=20) (both unfertilized and long-term fertilized treatments) May 2009. Same letters are not significantly different (LSD, $\alpha = 0.05$).

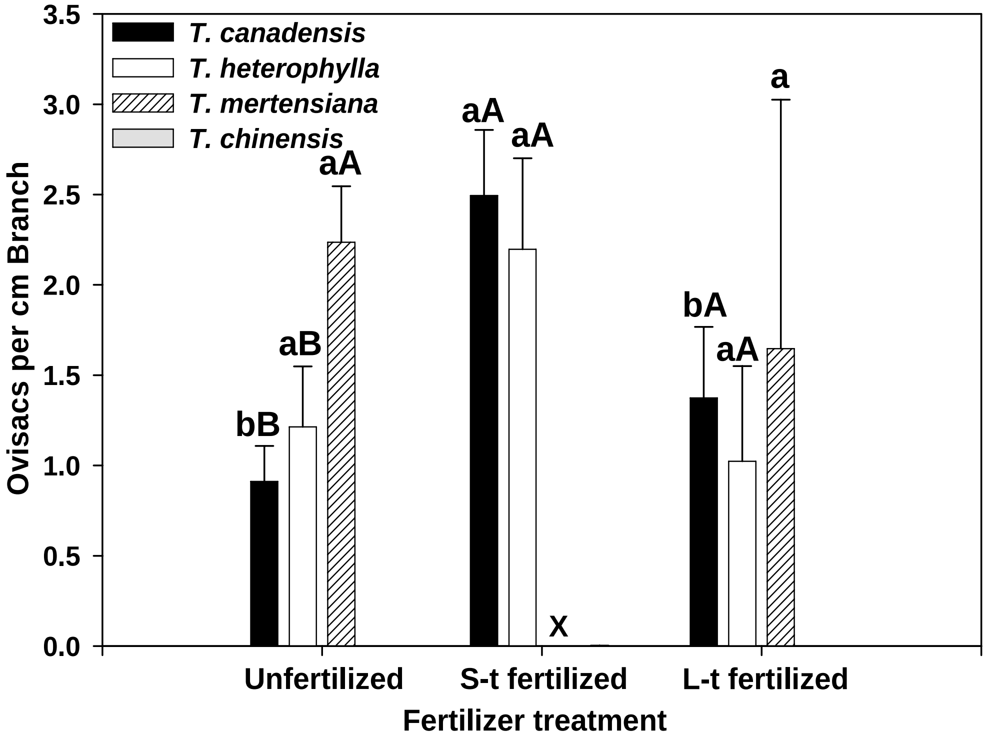

Fig. 4.2. Mean (± SE) number of progredien ovisacs per branch on various hemlock species and fertilizer treatments (N = 10) on June 3 and11, 2009. The abbreviations x = missing *T. mertensiana* treatment, S-t = short-term fertilizer and L-t = long-term fertilizer applications. Upper case letters indicate comparison of hemlock species effects within each fertilizer treatment, while lower case letters indicate comparison of fertilizer treatments effects by hemlock species (bars with same fill). Too few adelgids survived on *T. chinensis*. Same case letters (upper or lower) are not significantly different (LSD, α = 0.05).

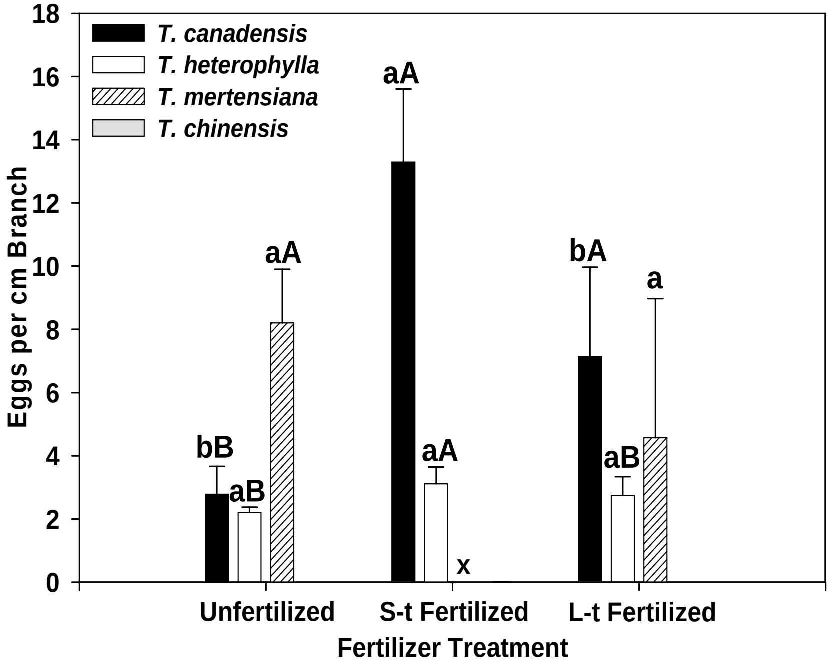

Fig. 4.3. Mean (± SE) number of sisten eggs per branch on various hemlock species and fertilizer treatments (N = 10) on June 3 and11, 2009. The abbreviations x = missing *T. mertensiana* treatment, S-t = short-term fertilizer and L-t = long-term fertilizer applications. Upper case letters indicate comparison of hemlock species effects within each fertilizer treatment, while lower case letters indicate comparison fertilizer treatments effects by hemlock species (bars with same fill). Too few adelgids survived on *T. chinensis*. Same case letters (upper or lower) are not significantly different (LSD, α = 0.05).

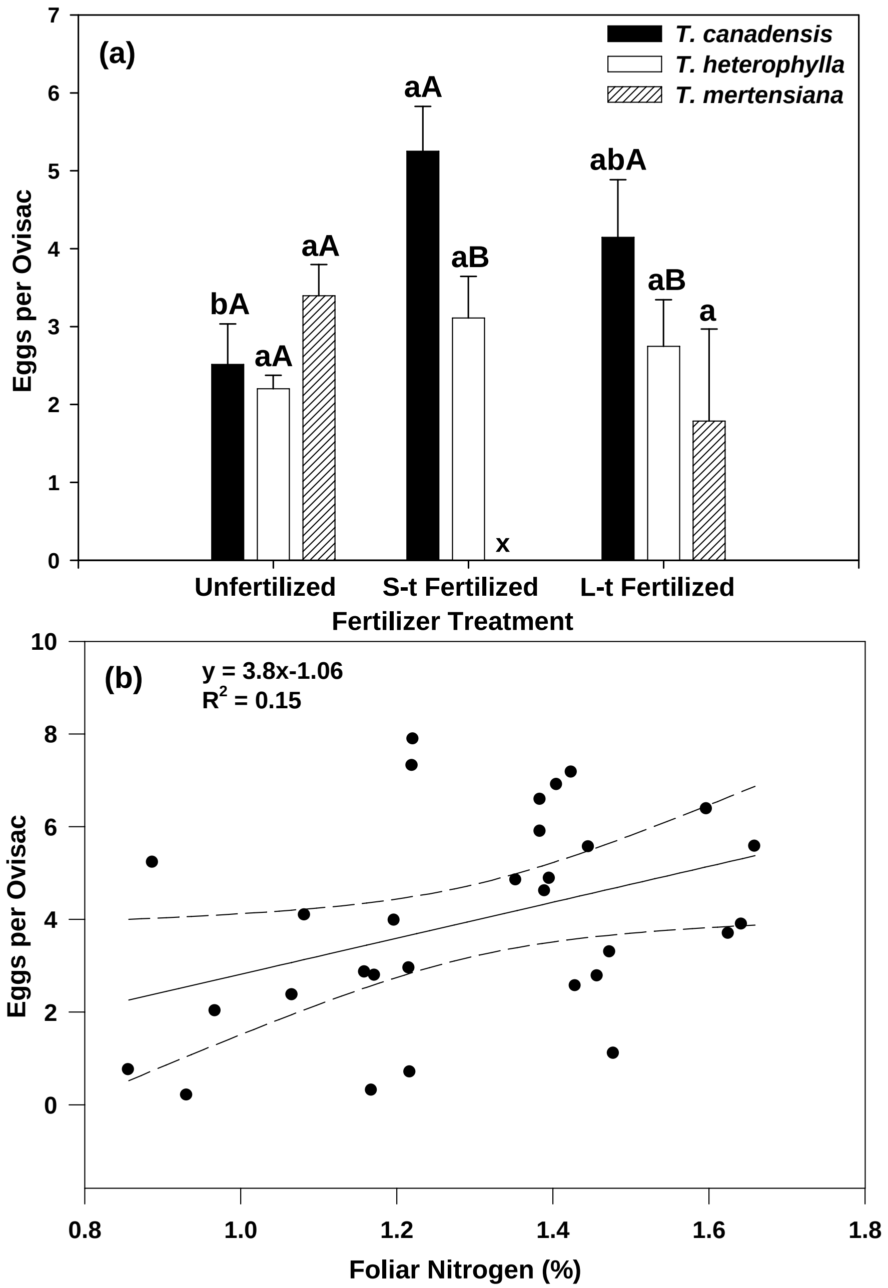

Fig. 4.4. Mean (± SE) (a) number of total sisten eggs per progredien female oviposited on various hemlock species and fertilizer treatments (N = 10) on June 3 and11, 2009. The abbreviations x = missing *T. mertensiana* treatment, S-t = short-term and L-t = long-term. Upper case letters indicate comparison of hemlock species effects within each fertilizer treatment, while lower case letters indicate comparison fertilizer treatments effects by hemlock species (bars with same fill). Too few adelgid survived on *T. chinensis*. Same case letters (upper or lower) are not significantly different (LSD, α = 0.05). (b) Relationship between the progredien fecundity and N content in the *T. canadensis* foliage.

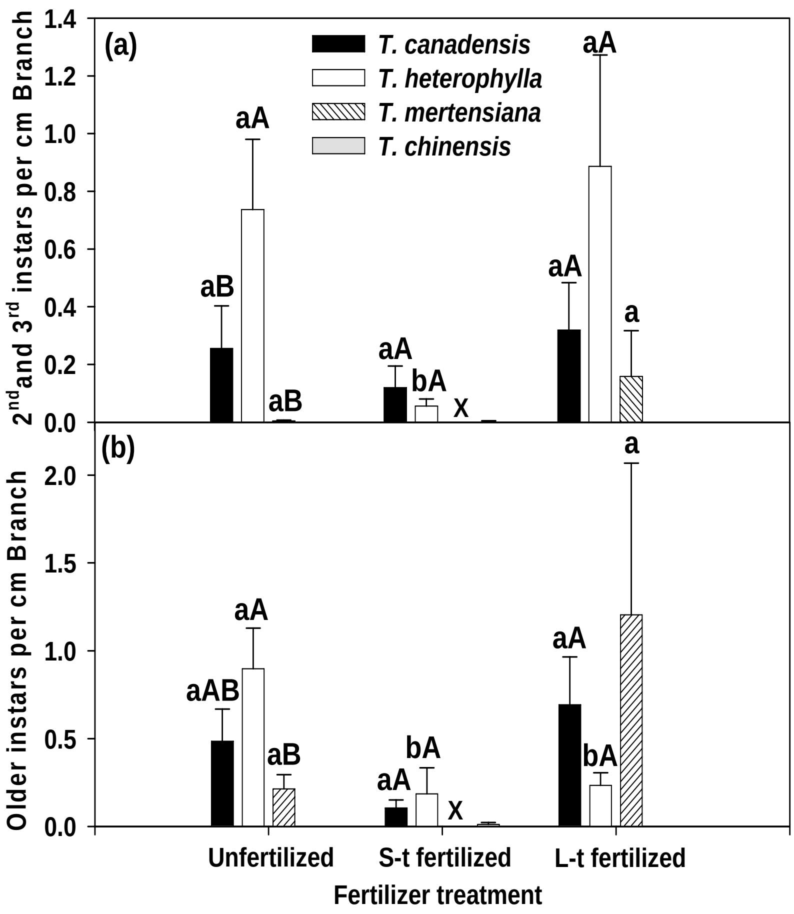

Fig. 4.5. Mean (± SE) (a) number of progredien 2nd- and 3rd-instar nymphs (b) 4th-instar nymphs on various hemlock species and fertilizer treatments (N = 10) on June 3 and11, 2009. The abbreviations x = missing *T. mertensiana* treatment, S-t = short-term and L-t = long-term. Upper case letters indicate comparison of hemlock species effects within each fertilizer treatment, while lower case letters indicate comparison fertilizer treatments effects by hemlock species (bars with same fill). Too few adelgid survived on *T. chinensis*. Too few adelgid survived on *T. chinensis*. Same case letters (upper or lower) are not significantly different (LSD, α = 0.05).

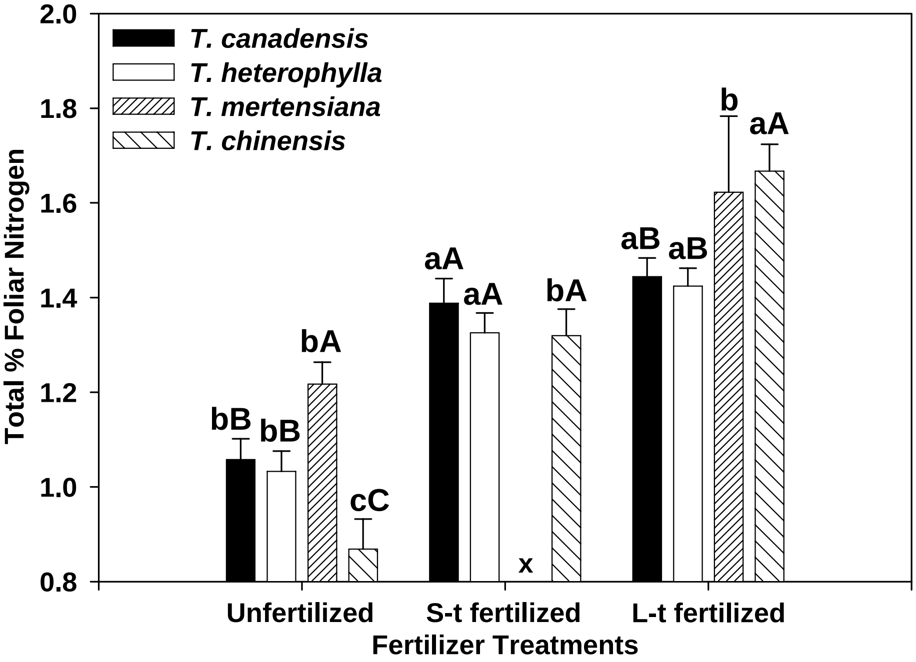

Fig. 4.6. Mean (± SE) content of total foliage N in various hemlock species and fertilizer treatments (N = 10) on June 3 and11, 2009. The abbreviation x = missing *T. mertensiana* treatment, S-t = short-term and L-t = long-term. Upper case letters indicate comparison of hemlock species effects within each fertilizer treatment, while lower case letters indicate comparison fertilizer treatments effects by hemlock species (bars with same fill). Same case letters (upper or lower) are not significantly different (LSD, α = 0.05).

CHAPTER 5

THE RANGE AND RESPONSE OF NEONICOTINOIDS ON HEMLOCK WOOLLY ADELGID (HEMIPTERA: ADELGIDAE)

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Abstract Hemlock woolly adelgid (HWA), *Adelges tsugae* Annand is a serious pest of eastern and Carolina hemlock in the eastern United States. A series of experiments compared commercially available and experimental insecticides, rates, application methods and timing for woolly adelgid control in northern Georgia and North Carolina. Safari 20 SG (dinotefuran) provided an average of 63 to 100% suppression of adelgid populations almost one month after treatment. Arena 50 W (clothianidin) and Merit 75 WP (imidacloprid) were slower acting but provided longer-term adelgid suppression than dinotefuran. Among neonicotinoid insecticides evaluated, dinotefuran provided the quickest suppression of HWA; however, 26 months later HWA re-colonized trees treated with dinotefuran in spring 2006 while imidacloprid treatments were still effective. High volume treatments such as soil drench of dinotefuran did not yield significant additional adelgid control compared to low volume applications such as soil injection. Three months after a fall application, an average of 40 and 76% mortality was observed in comparison with HWA on untreated trees for low and high concentrations of Tristar (acetamiprid) arborjet trunk injection, respectively. Among the Xytect 75 WSP (imidacloprid) soil injection treatments applied during May, August, or November, the May treatment was most effective reducing the population by an average of 92%. Xytect root-flare micro system treatments were also effective as the studied soil injection treatment.

Index words: hemlock woolly adelgid, insecticide, suppression.

Species used in this study: Eastern hemlock, *Tsuga canadensis* L. Carrière.

Chemicals used in this study: Safari 20 SG (Dinotefuran), N-methyl-N‘-nitro-N“-[(tetrahydro-3-furanyl)methyl)]guanidine; Safari 2 G (Dinotefuran); Merit 75 WP (Imidacloprid), (*E*)-1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine; Arena 50 W (Clothianidin), (E)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine; V10170 50 WDG (Clothianidin);

Tristar 30SG (Acetamiprid), (E)-N^1-[(6-chloro-3-pyridyl)methyl]-N^2-cyano-N^1-methyl acetamidine; Xytect 75 WSP (Imidacloprid); Xytect infusible (Imidacloprid).

Significance to the nursery industry

Hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae) is an invasive pest of eastern hemlock, *Tsuga canadensis* L. Carrière, and Carolina hemlock, *Tsuga caroliniana* Engelmann, in the eastern United States. The adelgid injects toxins causing needle drop, reduced shoot growth and branch dieback. Hemlock tree mortality occurs between 4 and 10 years after first infestation depending on the tree health (15). Eastern and Carolina hemlock fill a unique ecological niche in native forests and are valued landscape ornamentals. Hemlock trees are integral components of public sites, farms and private properties. Sustainable management of HWA may only be realized through biological control (2). However, for management of high value hemlock trees, chemical control could be an important tool. This study evaluated the efficacy of neonicotinoid insecticides to suppress hemlock woolly adelgid populations on eastern hemlocks.

Introduction

Hemlocks are long-living shade-tolerant trees that form dense, evergreen, multi-layered canopies that support diverse species of wildlife (12, 28). These stands add aesthetic value, e.g. recreational areas such as parks, trails or general landscape; have genetic value with a need to preserve the gene pool (8); and most importantly ecological value. Since its introduction in 1951, near Richmond VA, hemlock woolly adelgid has become established from southwestern Maine to northeastern Georgia. Adelgids attack newly growing shoots, settle at the needle base, and feed on the cortical parenchyma ray cells of xylem tissues (15).

Adelges tsugae has two generations per year, with adults having a winter generation (the sistens) and a summer generation (the progrediens) (1, 15). The adelgid is a small aphid-like (0.4-1.4 mm) sucking-insect that secretes a white, woolly substance which covers its body and egg masses (10, 13). They only reproduce parthenogenetically in the eastern United States (13, 15). Overwintering adults lay eggs in egg cases (15). The active crawlers hatch from these eggs and subsequently settle at an unoccupied needle base and molt into immobile second instars. In the early spring, the crawlers are actively dispersed by mammals, wind or birds (16). The sisten second-instar nymphs remain inactive throughout the summer until October or November, depending on location, when they molt into older-instars that actively feed on hemlock. Adelgids undergo a polymorphic life cycle on hemlock and spruce (*Picea* spp.) (15). In native hemlock forests, a winged sexuparae generation is produced in late spring (14, 15). The offspring (sexuales) of sexuparae perish because they need a spruce host that does not occur in North America (13, 15).

Management tactics including biological control or increasing host plant resistance (5) might be long-term solutions to bring *A. tsugae* populations into equilibrium on hemlock (2, 3). Meanwhile, chemical control is an important strategy in protecting or rescuing certain high value hemlock stands in forests and landscapes. Various insecticides have already been applied using different delivery mechanisms such as foliar sprays, soil drenches, trunk or soil injections to suppress hemlock woolly adelgid populations (3, 6, 11, 17, 24). Foliar sprays caused greater than 90% mortality of HWA (17) but did not provide uniform insecticide exposure. Chemical applications may be challenging in a forest scenario as it is difficult to reach remote locations; however, landscape trees are relatively accessible. Neonicotinoids, especially imidacloprid, are widely accepted by the nursery and landscaping industries as they are effective against a variety of pests such as aphids (20, 21) and hemlock woolly adelgids (3, 19, 23, 29). Their high oral toxicity to targets and systemic activity make them especially useful for treating trees. More neonicotinoids have become commercially available recently, but information about their efficacy against hemlock woolly adelgid is not known. The objectives of this project were to: (1) evaluate eight insecticides for suppression of hemlock woolly adelgid on eastern hemlock growing in various landscape sites; and (2) assess insecticide efficacy (a) using low or high volume methods, (b) varying sites of application (soil or tree trunk), (c) varying insecticide rates, (d) changing timing (spring versus fall), and (e) evaluating speed and length of residual activity.

Materials and Methods

Hemlock trees were selected at various sites in Macon Co., NC, and at the University of Georgia Mountain Research Station (Union Co.) in Blairsville, GA. The selection of trees was based on the presence and accessibility of branches by pole pruner, and adequate separation between trees. Density-dependent adelgid suppression has been reported on heavily infested trees due to decreasing nutritional suitability and reduced new growth (18). Selected trees at various sites were inspected for moderate populations of adelgid prior to initiation of studies. All the trees were appropriately tagged and recorded by global positioning system (GPS) to facilitate relocation.

Experiment 1 The main objective of this experiment was to compare efficacy, time to control and persistence of Safari® 20 SG (dinotefuran), Merit® 75 WP (imidacloprid) and Arena® 50 W (clothianidin), The trees selected in spring and fall had 24.3 (9.6 in.) ± 2.3 (0.89 in.) (mean ± S. E.), and 20.3 (8.0 in.) ± 2.2 (0.85 in.) cm diameters at breast height (dbh), respectively. Two separate sets of hemlock trees were treated with neonicotinoid products in the spring (May 4, 2006) and fall (November 3, 2006). Six treatments were tested on each date: an untreated control; three rates of Safari 20 SG, 3, 6 or 12 g (AI) /2.5 cm (1 in.) dbh; one rate of Merit 75 WP, 2g (AI) /2.5 cm dbh; and one rate of Arena 50 W, 2 g (AI) /2.5 cm dbh. We had five replications blocked by diameter class and elevation. The absorbing fine-roots of hemlock trees are primarily located in the subsurface soil and within the dripline area of a tree. Insecticides were applied to the subsurface soil layer (6 cm deep) using Kioritz soil injectors (Kioritz Corp. Tokyo, Japan). Numbers of injections per tree were determined based on the insecticide rate and dbh of each tree. Nine sets of branch samples (see description below) were collected to evaluate the efficacy of the spring application for HWA control (April 25 (precount), May 25, June 27 July 25, Sept. 26 and Nov. 3 in 2006, May 29 and Nov. 14 in 2007, and July 17, 2008), and 5 sets of samples were collected to evaluate the fall application (Nov. 3 (precount) and Dec. 3 in 2006, May 29 and Nov.14 in 2007, and July 17, 2008).

Experiment 2 This experiment was designed to comparatively evaluate dinotefuran applied to soil as a drench, low volume injection or granule relative to imidacloprid in the spring. This study also evaluated the effect of application timing and length of activity for adelgid reduction. The selected trees had a Mean ± S. E. of 18.6 (7.33 in.) ± 0.9 (0.36 in.) cm dbh. The six treatments included an untreated control, Safari 20 SG, 6 g/2.5 cm dbh applied by Kioritz soil injection or soil drench, Safari 2 G, 60 g/2.5 cm dbh applied by hand broadcast, and Merit 75 WP, 2 g/2.5 cm dbh applied by Kioritz soil injection or soil drench on May 4, 2007. The application volume for Kioritz soil injection and soil drench were 30 mL (1 fl. oz.)/2.5 cm dbh and 1 L (1 qt)/2.5 cm dbh, respectively, for all the experiments where applicable. These treatments were replicated on five trees blocked by diameter class. Insecticide applications were administered to treatment trees only once. The procedure of Kioritz soil injection was same as previously described. For drench treatments the appropriate quantity of insecticide (WP product) was mixed with water and poured around the tree trunk. This method is widely used by homeowners. Among the application methods, hand broadcasting is simple and inexpensive. The granular insecticide was directly applied to the soil after raking back the mulch and needles at the base of trees. Five sets of branch samples were collected on May 29, June 12, July 12 and November 14 in 2007 and July 16 in 2008 to evaluate efficacy and duration of control.

Experiment 3 Seven treatments were applied to hemlocks at the Georgia Mountain Station on September 7, 2007. Three treatments were similar to those in Experiment 2: an untreated control and two treatments of Safari 20 SG using 6 g/2.5 cm dbh applied by either Kioritz soil injection or soil drench. The other four treatments were: Safari 20 SG 3 g/2.5 cm dbh plus V10170 50 WDG (clothianidin) 2 g /2.5 cm dbh applied by either Kioritz soil injection or soil drench, and V10170 50 WDG 2 g/2.5 cm dbh alone applied by soil drench, and Merit 75 WP 2 g/2.5 cm dbh alone applied by soil drench. Three sets of branch samples were collected using a pole pruner on November 14, 2007, and February 7 and July 9, 2008.

Experiment 4 This study was designed to evaluate the speed and length of activity of Tristar® 30 SG (acetamiprid) applied by Arborjet trunk injection (Arborjet, Woburn, MA) for suppression of adelgid. In addition, this study also evaluated timing, rate, and single or double applications of acetamiprid against hemlock woolly adelgid. Three treatments of two rates of Tristar 30 SG (6 or 12 (AI) mL/ 2.5 cm dbh) and an untreated control were initiated on November 3, 2006. The following spring four additional treatments were initiated. Two groups of trees were selected and each received Tristar 30 SG at either 6 or 12 mL/ 2.5 cm dbh on April 4, 2007. These groups were then subdivided and one set containing both treatments (one low or one high rate) initiated in the spring, received a second dose of either 6 or 12 mL/ 2.5 cm dbh of Tristar 30 SG. The completely randomized design had seven treatments with four single tree replicates.

The arborjet truck injection system was used to deliver the diluted insecticide. Insecticide volume was determined based on the dbh of each tree and was treated at the rate of 4 ml (0.14 oz.)/ 2.5 cm dbh. A hole of 0.74 cm diameter and 1.5 cm in depth was drilled into the tree trunk to reach the sapwood and this hole is closed with a plastic plug. A needle was inserted into the plastic plug through a septum within it. Insecticide solution was actively taken up into the tree under a difference in the pressure gradient though the needle. The site of drilling was within 90 cm above the soil-level (3). Six sets of branch samples were collected and evaluated for hemlock woolly adelgid on November 3 (precount), December 7 in 2006, March 14, May 29, and November 14 in 2007, and July 17 in 2008.

Experiment 5 Xytect 75 WSP and Xytect infusible (imidacloprid) were applied with a HTI 2000 soil-injection probe (Rainbow Treecare Scientific Advancements, Minnetonka, MN) or by root-flare injection with an M3 injection system, respectively. The HTI 2000 soil-injection probe accurately delivers insecticide solution to the root-zone area of trees. This study compared the efficacy of spring and fall applications. Xytect 75 WSP treatment rates were proportional to the dbh of the trees. Rates were 0.75 g (AI) /2.5 cm dbh for 10 to 40 cm (4 to 16 in.) dbh trees, 1 g (AI) /2.5 cm dbh for 42.5 to 50 cm (17 to 20 in.) dbh trees, and 1.5 g (AI) /2.5 cm dbh for 52.5 to 65 cm (21 to 26 in.) dbh trees and were applied on May 3, 2007. Additional summer and fall soil injection treatments were applied to separate trees on August 22 and November 1 2007. A Xytect infusible treatment at the rate 0.75 g (AI) /2.5 cm dbh was applied by root-flare injections with the M3 injection system also in November. There were five single-tree replications per treatment in this design. Trees were sampled on four dates, May 29, August 22 and November 1 in 2007 and July 16 in 2008 to assess hemlock woolly adelgid control.

Sampling procedure and data analysis

Treatments were evaluated using samples that each consisted of four branch terminals (15 cm long) collected (two/height) at 1.5 m (5 ft) and 6.1 m (20 ft) above the ground. Branches were sealed in plastic bags, immediately stored in a cooler and returned to the laboratory. In the laboratory, these samples were temporarily maintained at 27°C, 80% relative humidity and 14:10 (L:D) photoperiod in a growth chamber (Percival Environmental Chambers, Percival scientific Inc., Perry, IA). All available life stages of HWA were counted for all sampling dates under 10X magnification. The variable “total immatures” is the sum of eggs, crawlers, and second- and older-instar nymphs wherever applicable. The adelgid count data were transformed as the square root of adelgid counts and analyzed using the general linear models (GLM) procedure of SAS (22). Means were separated using LSD ($\alpha = 0.05$).

Results and Discussion

Experiment 1 Following spring application, significant suppression of hemlock woolly adelgid was observed at medium (6 g [AI] /2.5 cm dbh) and high (12 g [AI] /2.5 cm dbh) rates of Safari one month after application, relative to the untreated control (Table 5.1). This result was most evident in the second-instars. Egg sac numbers were not significantly different among treatments relative to untreated controls at this time. Trees treated with Safari averaged 81 or 93 % adelgid suppression for medium and high rates, respectively by June (Table 5.1). Compared to control trees, all stages of adelgid were equally suppressed by medium and high rates of Safari. However, treatment with Merit and Arena resulted in low HWA suppression in the first year (Table 5.1). These trends in suppression were consistently apparent in July, September and November samples in 2006. In May of the following year, all the rates of Safari resulted in an average range of 63 to 100% suppression. No egg sacs, eggs, crawlers, or second and older-instars were observed in the high Safari treatment, but a few second-instars were observed on medium Safari-treated trees. Merit and Arena treatments were also effective in reducing the adelgid population by an average of 65% and 71%, respectively, relative to the control. Similarly, Merit showed greater reduction in the adelgid population in the second than first year in previous studies (3). Growth of new shoots was significantly higher (84%) on Safari treated-trees (Table 5.1). By November, both medium and high treatments of Safari, Merit and Arena resulted in 100% adelgid suppression. The effect of Safari appeared to have declined by two years post-application as re-colonization by the adelgid population was noticed on treated trees (Table 5.1). In fall-treated trees, there was a significant reduction (72.1%) in HWA density caused by Merit and Arena 18 months after treatment when compared to the untreated control trees (Fig. 5.1). Analysis of spring treatments indicated Safari was the most rapid acting of the neonicotinoid insecticides tested, but also the least persistent. Merit activity was slow but more persistent. Meanwhile, fall treatment with Merit provided significant suppression of HWA while Safari was less effective (Table 5.1 and Fig. 5.1).

Experiment 2 In May 29 2007, about one month after spring treatments (May 4, 2007) adelgid egg density was noticeably reduced by an average of 92 to 100% in Safari-treated trees as compared to the untreated control regardless of the application methods used (Table 5.2). This result was consistent with the rapid rate of mortality caused by Safari in the previous experiment (Experiment 1). On the same date, Merit- treated trees had significantly more eggs, second, and older-instars relative to the other treatments including Safari. Merit- treated trees also had fewer newly growing branches compared to the other treatments. In June, all Safari treatments significantly reduced total immature-adelgids causing an average range of 74 to 78% mortality compared to untreated trees. New branch growth was comparatively lower on Merit soil-injected trees than on other treated trees. These trends continued with 100% second-instar mortality on soil injected trees as well as 95% and 88% reduction on granular and drenched trees in July. In November 2007 and July 2008, relative to untreated trees, significant adelgid mortality was recorded for all the insecticide products regardless of application method. In the previous study (Experiment 1), spring application (May 4, 2006) of Merit did not result in adelgid suppression until the May 29, 2007 sample. However, in this experiment significant mortality in Merit-treated trees occurred earlier (by November, 2007) and was still evident in the July, 2008 branch samples. Previously, Webb et al. (29) reported fast and remarkable recovery, indicated by disappearance of infestation symptoms and production of new growing tips, of Merit soil-drenched trees after 434 days and 816 days on low and highly severe initial HWA infested trees, respectively.

Experiment 3 HWA mortality was not observed in the fall-treated trees in the November samples (Table 5.3). However, In February 2008, Safari or V10170 soil injections were effective and averaged 100% mortality of females resulting in no eggs compared to untreated trees after a fall application in September of the previous year. July 2008 samples from both soil-injected or soil-drenched Safari trees or V10170 soil-injected trees had significantly fewer older-instars adelgids, in contrast to untreated trees. Nymph densities were similar on trees soil drenched with Safari and V10170 combined, and with insecticide free trees. Adelgid populations in July 2008, echoed the preceding results with a heavy decline in crawlers and second-instars on both single soil injected or soil drenched trees with Safari or V10170 relative to untreated trees.

Experiment 4 Evidence of adelgid suppression was not observed until March, 2007. An average 40 or 76% mortality was seen on trees in the low or high dose treatments of Tristar applied using the arborjet system in November, 2006 (Table 5.4). By May 2007 a decline in egg densities of 78 and 58% in low- or high-dose Tristar treated trees applied in November 2006 was observed relative to adelgid eggs on untreated trees, respectively. Total nymphs also showed the same pattern with an average of 68% and 49% mortality at low and high doses of Tristar, respectively. During this sample period no significant differences in adelgid populations between fall and spring applications were noted. However, adelgid egg mortality in low-dose-spring-applied trees (April 4, 2007) increased by an average of 9% compared with low-dose fall-applied (November 3, 2006) trees. This reduction in adelgid eggs increased to an average range of 27 to 37% between high rate spring treated trees and high rate fall treated trees. Lower percentages of total HWA immature survival could be seen in the high dose, spring Tristar-treated trees than fall high dose on trees. In November 2007 samples, adelgid survival repeated the same pattern as observed on the previous date. However, the highest reduction of 97% in adelgid nymphs occurred in the high double-application of Tristar compared to all other insecticide treated trees in the November branch samples. Evaluation of trunk injection treatments in July 2008 showed surviving second-instar nymphal populations were the same among all treatments of Tristar. No statistically significant difference was observed in older-nymphs between spring and fall or low and high rate Tristar treatments or single and double dose applications. Daccola et al. (6) also reported 85% mortality of HWA with the ready-to-use insecticide, Imicide (10% imidacloprid), applied by Arborjet Viper trunk injection in summer compared to untreated trees which had 37.9% mortality in samples collected the following fall.

Experiment 5 Xytect- treated trees, in May, 2007 did not show significant suppression of second-instar nymphs by August, 2007 when compared to untreated trees (Fig. 5.2). Among the November, 2007 samples, an average 68% and 82% of older-instar nymph mortality was observed relative to control trees in May and August treatments, respectively. Nymphs in July, 2008 samples were significantly reduced compared with controls. On that date, Xytect treatments applied during spring (May 2007) suppressed older-instars by an average of 92%. Xytect root-flare micro system treatments were also effective.

Results from these studies indicate that Safari can play a valuable role when rapid control of HWA is required to rescue trees. Suppression of adelgids was consistent in all soil applied experiments except fall treatments as described in Experiment 1. Many factors such as drought, low temperature or low soil organic matter, might affect insecticide efficacy immediately after application. These factors could delay their mobility and proper root interception in the soil. Low temperature, 5^{o}C (50^{o}F) to 15^{o}C (60^{o}F), especially during winter, and lack of soil moisture, resulting in reduced evapo-transpiration from hemlock needles and decreasing xylem sap flow, could result in inefficient translocation of insecticides (7, 27). We found that treatment with Safari during spring was equally efficacious in controlling adelgid in all studies. Because the active ingredient in Safari had excellent water solubility of 39,800 ppm, Cowles et al. (3) predicted it would be effective against HWA as they found it more effect on armored scales compared with the widely used Merit insecticide. Similarly, an unregistered neonicotinoid, thiamethoxam (active ingredient) was tested for its efficacy against adelgids and yielded immediate suppression as opposed to Merit (9).

Low volume applications of Safari, such as soil injection, have provided rapid and significant suppression in different sites. Moreover, high volume application such as soil drenching with Safari did not yield additional control of adelgid populations. This means that under suitable soil conditions, recommended rates of Safari can be adequately distributed throughout hemlock trees within one month after soil injection. Diminishing densities of HWA remarkably improved overall tree health through increased shoot growth. The shallow placement of insecticide and adequate soil organic matter will minimize the risk of leaching and runoff during heavily rainfall. It is important that plant care professionals carefully consider the most efficacious insecticide and best application techniques that pose negligible risk to non-target organisms and the environment.

Merit in our trials in the southeastern US provided persistent but delayed residual activity on HWA requiring up to one year after application to reach effective levels in the trees. It has been shown that soil injected Merit concentration could take 2 to 3 months (26) or one year after application (3) to be distributed to large tree branches. Merit might have restricted mobility in the soil by forming a strong bond to soil organic matter before coming in contact with the active roots of hemlock trees (4). Interestingly, Xytect having the same active ingredient as Merit (imidacloprid), when applied using the HTI 2000 soil injection system provided adelgid reduction within 6 months after spring treatment in our study. Furthermore, spring treated trees after 13 months had the same adelgid populations as fall treated trees after 7 months. There was no difference in HWA populations on trees treated with root-flare Micro-infusion and HTI 2000 soil injection after 7 months. This indicated that Merit remained stable (3), mobile, and easily translocated to new tips from year to year. Our data and results from others clearly suggest Merit applications are long lasting against key pests in landscapes (25).

Although, trunk injection of Tristar did provide significant suppression it was not 100% effective. Tristar fall Arborjet trunk injection was not effective until after late winter. Instead, immediate adelgid mortality was noticed one month after spring-trunk applications. Previous studies reported that trunk injections with Merit provided less impact on HWA populations compared to soil injection, although insecticide residues were detected in the hemlock tissues (3). Compared to single dose arborjet trunk-injection, adelgid numbers were not statistically different in double dose-treated trees (spring vs. fall) even 9 months after fall treatment. Arborjet trunk microinjected IMA-jet (5% Imidacloprid w/w) insecticide showed distinguishable HWA control only one year after treatment (7). This result may be due to inadequate distribution of insecticide to tree canopy. However, superior long-term adelgid suppression was observed after a second application on the same trees.

These findings on existing and emerging insecticide chemistries, formulations and application tactics for management of HWA will enormously benefit the landscape and nursery industries as they are integrated. The selection of insecticide and feasible strategy of insecticide delivery is often difficult in real landscape environments. Unless a stable biological control or host plant resistance remedy can be achieved to counter this pest problem, insecticide based curative control can be considered as a viable integrated pest management (IPM) tool.

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Table 5.1. Number (Mean ± S. E) of hemlock woolly adelgids and new growth after application of products on 4 May 2006.

Sampling Date	Hemlock woolly Adelgid Life-stages	Untreated Control	Safari 20 SG (L[a])	Safari 20 SG (M)	Safari 20 SG (H)	Merit 75 WP	Arena 50 W	df	F[P]
May 06	Egg sacs	3.66[z] ± 0.54a	3.08 ± 0.28ab	4.58 ± 0.63a	3.12 ± 0.91ab	3.62 ± 0.66a	2.01 ± 031b	5	2.6^{NS}
	Eggs	11.44 ± 3.10a	6.03 ± 0.59ab	3.79 ± 1.38b	5.80 ± 2.84ab	9.53 ± 3.72ab	5.45 ± 0.99ab	5	1.6^{NS}
	Crawlers	4.21 ± 0.98a	3.59 ± 0.98a	3.06 ± 0.64a	3.02 ± 1.10a	4.51 ± 1.01a	2.02 ± 0.88a	5	1.1^{NS}
	Second-instars	8.02 ± 1.12a	3.59 ± 1.75ab	1.42 ± 0.92b	2.09 ± 1.22b	8.97 ± 2.98a	6.79 ± 2.63ab	5	3.1*
	Total immatures	14.81 ± 3.20a	8.48 ± 1.41ab	5.51 ± 1.42b	7.63 ± 2.84b	14.96 ± 3.96a	9.63 ± 2.34a	5	3.1*
	New Growths	1.36 ± 0.66a	1.86 ± 0.86a	1.38 ± 0.57a	1.97 ± 0.35a	2.08 ± 0.82a	1.28 ± 0.73a	5	0.5^{NS}
June 06	Egg sacs	3.15 ± 0.34a	1.26 ± 0.59bc	0.20 ± 0.2c	0.0c	2.22 ± 0.33ab	1.75 ± 0.85b	5	6.7**
	Eggs	7.19 ± 1.94a	2.80 ± 1.83bc	0.0c	0.0c	4.65 ± 0.31ab	3.58 ± 1.67b	5	5.8**
	Crawlers	5.98 ± 1.14a	1.64 ± 0.73b	0.60±0.40b	0.40 ± 0.40b	6.69 ± 1.54a	5.08 ± 1.28a	5	7.9**
	Second-instars	7.19 ± 1.57a	6.22 ± 1.71a	2.28 ± 0.74bc	0.81 ± 0.53c	6.99 ± 1.41a	5.85 ± 1.30ab	5	4.1*
	Older-instars	1.48 ± 0.67ab	0.55 ± 0.35bcd	0.28 ± 0.28cd	0.0d	1.77 ± 0.47a	1.15 ± 0.57abc	5	3.9*
	Total immatures	12.62 ± 1.88a	7.93 ± 1.89b	2.44 ± 0.84c	0.95 ± 0.65c	11.44 ± 1.24ab	9.16 ± 2.00ab	5	9.9***
	New Growths	0.98 ± 0.40a	1.48 ± 0.38a	1.18 ± 0.32a	1.08 ± 0.45a	1.59 ± 0.12a	1.64 ± 0.17a	5	0.7^{NS}
July 06	Egg sacs	1.66 ± 0.51a	0.20 ± 0.20b	0.0b	0.4 ± 0.24ab	1.7 ± 0.76a	0.49 ± 0.49ab	5	2.8*
	Eggs	3.05 ± 1.01a	0.0b	0.0b	0.0b	3.04 ± 1.36a	1.33 ± 1.09ab	5	3.3*
	Crawlers	3.32 ± 1.02a	0.63 ± 0.63 b	0.40 ± 0.40b	0.60 ± 0.40b	1.89 ± 0.98ab	1.18 ± 0.64ab	5	2.2^{NS}
	Second-instars	17.44 ± 2.89bc	11.39 ± 2.12cd	7.67 ± 1.99d	6.13 ± 1.29d	24.89 ± 2.10a	21.89 ± 3.07ab	5	11.3***
	Older-instars	1.78 ± 0.51a	0.0b	0.28 ± 0.28b	0.48 ± 0.30ab	1.66 ± 0.72a	0.35 ± 0.34b	5	3.1*
	Total immatures	18.24 ± 3.07bc	11.47±2.13cd	7.71 ± 2.02d	6.22 ± 1.34d	25.40 ± 2.29a	22.08 ± 3.15ab	5	10.9***
	New Growths	0.95 ± 0.41ab	0.60 ± 0.40abc	0.0c	0.20 ± 0.20bc	1.31 ± 0.35a	0.75 ± 0.46abc	5	2.5^{NS}
Sep. 06	Second-instars	12.70 ± 2.35a	3.18 ± 1.90bc	1.59 ± 0.72c	1.41 ± 0.45c	10.09 ± 1.75a	5.78 ± 1.08b	5	14.6***
	Older-instars	3.28 ± 1.03a	0.57 ± 0.57b	0.0b	0.0b	2.51 ± 0.76a	0.20 ± 0.20b	5	5.9**
	Total immatures	13.22 ± 2.44a	3.26 ± 1.98bc	1.59 ± 0.72c	1.41 ± 0.45c	10.61 ± 1.61a	5.79 ± 1.07b	5	15.2***
	New Growths	1.05 ± 0.48b	2.80 ± 0.97a	1.63 ± 0.19ab	2.07 ± 0.69ab	1.78 ± 0.24ab	1.65 ± 0.31ab	5	1.0^{NS}
Nov. 06	Second-instars	3.28 ± 0.72a	1.52 ± 0.94ab	0.0b	0.0b	1.09 ± 0.67b	0.63 ± 0.63b	5	4.2**
	Older-instars	7.65 ± 2.96a	3.47 ± 3.47ab	0.0b	1.04 ± 1.04ab	7.17 ± 2.08ab	3.12 ± 1.93ab	5	3.1*
	Total immatures	10.41 ± 2.60a	4.87 ± 3.34 ab	0.45 ± 0.45b	1.17 ± 1.17b	11.39 ± 3.64a	4.37± 2.13ab	5	2.9*
	New Growths	0.95 ± 0.42a	1.16 ± 0.33 a	0.68 ± 0.29a	1.23 ± 0.35a	1.31 ± 0.35a	1.26 ± 0.32a	5	0.7^{NS}
May 07	Egg sacs	3.28 ± 0.72a	1.52 ± 0.94 ab	0.0b	0.0b	1.09 ± 0.67b	0.63 ± 0.63b	5	4.2**
	Eggs	1.86 ± 0.99a	1.27 ± 1.26a	0.0a	0.0a	0.4 ± 0.0a	0.87 ± 0.87a	5	1.0^{NS}
	Crawler	1.78 ± 1.38a	1.18±1.18a	0.0a	0.0a	0.0a	0.57 ± 0.57a	5	1.1^{NS}
	Second-instars	5.36 ± 1.33a	1.65 ± 1.33b	0.28 ± 0.28b	0.0b	2.19 ± 0.78b	0.73 ± 0.47b	5	6.1**
	Older-instars	2.03 ± 0.57a	0.55 ± 0.35ab	0.0b	0.0b	0.20 ± 0.20b	1.11 ± 1.11ab	5	2.24^{NS}
	Total immatures	6.87 ± 1.75a	2.58 ± 2.17b	0.28 ± 0.28b	0.0b	2.41 ± 0.78b	1.99 ± 1.50b	5	3.7*
	New Growth	0.83 ± 0.36b	2.81 ± 0.85ab	3.23 ± 0.49ab	5.18 ± 0.63a	3.07 ± 1.34ab	2.66 ± 0.89b	5	2.9*

Nov. 07	Older-instars	9.52 ± 2.99a	3.21 ± 1.68b	0.0b	0.0b	0.0b	0.0b	5	8.5^{**}
July 08	Second-instars	20.31 ± 2.59ab	24.45 ± 1.96a	15.07 ± 4.64b	13.33 ± 3.29b	0.0c	2.89 ± 1.94c	5	11.8^{***}

[a] Rates of Safari L= low; M = Medium; H = High, 3 or 6 or 12 (AI) g/ 2.5 cm dbh, respectively.

[P] Significantly different: $*P < 0.05$; $** P < 0.01$; $*** P < 0.001$; NS, Not significant.

[z] Mean S. E. for adelgid survival and new growth.

Table 5.2. Number (Mean ± S. E) of hemlock woolly adelgids after soil injection, granular application or drenching on 4 May 2007.

Sampling Date	Hemlock Woolly Adelgid Life-stages	Untreated Control	Safari 20 SG Kioritz Soil Injection	Safari 20 SG Soil Drench	Safari 2 G Hand Broadcast	Merit 75 WP Kioritz Soil Injection	Merit 75 WP Soil Drench	df	F^P
May 07	Egg sacs	4.19 ± 0.77a	5.54 ± 0.73a	2.97 ± 0.92a	3.91 ± 0.79a	4.22 ± 1.11a	-	4	1.0^{NS}
	Eggs	3.48 ± 1.29b	0.28 ± 0.28c	0.0c	0.0c	6.65 ± 1.14a	-	4	13.0^{***}
	Crawler	2.51 ± 1.42ab	0.85 ± 0.52b	0.20 ± 0.20b	1.08 ± 1.08b	4.44 ± 0.52a	-	4	4.4^{*}
	Second-instars	4.74 ± 1.57b	4.49 ± 1.57b	5.47 ± 0.51ab	3.81 ± 0.34b	8.85 ± 1.21a	-	4	2.8^{NS}
	Older-instars	0.97 ± 0.61bc	0.20 ± 0.20c	1.59 ± 0.68b	1.32 ± 0.34bc	3.31 ± 0.13a	-	4	6.9^{**}
	Total Adelgid	7.13 ± 2.07b	5.24 ± 1.12b	5.85 ± 0.59b	4.59 ± 0.69b	12.74 ± 0.88a	-	4	8.0^{***}
	New Growth	2.59 ± 0.99a	2.61 ± 0.86a	3.24 ± 0.36a	3.04 ± 0.48a	0.20 ± 0.20b	-	4	3.0^{*}
June 07	Egg sacs	6.41 ± 0.94ab	4.83 ± 1.27b	3.65 ± 1.29b	9.03 ± 1.00a	10.04 ± 1.90a	7.38 ± 1.18ab	5	2.9^{*}
	Eggs	25.11 ± 5.47a	0.0b	2.41 ± 2.41b	3.22 ± 2.88b	18.95 ± 2.48a	21.85 ± 3.47a	5	11.2^{*}
	Crawler	6.56 ± 1.69a	0.40 ± 0.24b	0.83 ± 0.61b	0.55 ± 0.35b	5.93 ± 0.86a	6.15 ± 1.05a	5	10.1^{***}
	Second-instars	14.67 ± 2.29a	6.39 ± 1.34c	6.74±2.25c	4.94 ± 0.80c	9.09 ± 0.96bc	13.65 ± 0.89ab	5	6.6^{**}
	Older-instars	5.45 ± 0.82a	0.28 ± 0.28b	0.72 ± 0.72b	0.63 ± 0.63b	4.72 ± 0.47a	5.48 ± 0.70a	5	15.3^{***}
	Total Adelgid	30.46 ± 6.03a	6.44 ± 1.35b	8.04 ± 2.95b	7.28 ± 2.26b	22.57 ± 2.33a	27.27 ± 3.38a	5	9.5^{***}
	New Growth	3.09 ± 0.46a	3.44 ± 0.38a	3.40 ± 0.24a	3.58 ± 0.49a	1.57 ± 0.49b	2.37 ± 0.55ab	5	3.6^{*}
July 07	Second instars	13.62 ± 1.38a	0.0c	1.67 ± 1.34c	0.72 ± 0.72c	6.26 ± 1.14b	6.52 ± 1.05b	5	21.7^{***}
	New Growth	2.68 ± 0.32a	3.19 ± 0.44a	3.34 ± 0.39a	2.58 ± 0.69a	1.18 ± 0.32b	2.13 ± 0.34ab	5	3.2^{*}
Nov. 07	Older-instars	5.39 ± 0.93a	0.0b	0.0b	0.45 ± 0.45b	0.0b	0.0b	5	31.1^{***}
July 08	Second-instars	24.19 ± 1.58a	0.0b	0.0b	0.72 ± 0.72b	0.57 ± 0.57b	0.0b	5	170.2^{***}

[P] Significantly different: *$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, Not significant.

Table 5.3. Number (Mean ± S. E) of hemlock woolly adelgids after treatment by soil injection or drench on 7 September 2007.

Sampling Dates	Hemlock Woolly Adelgid Life-Stages	Untreated Control	Safari 20 SG Kioritz Soil Injection	Safari 20 SG Soil Drench	Safari 20 SG + V10170 50 WDG Kioritz Soil Injection	Safari 20 SG + V10170 50 WDG Soil drench	V10170 50 WDG Kioritz Soil Injection	V10170 50 WDG Soil Drench	df	F^P
Nov 07	Older-instars	9.75 ± 1.84a	2.56 ± 1.40b	3.50 ± 1.43b	8.35 ± 2.16ab	5.82 ± 2.81ab	7.99 ± 1.54ab	6.29 ± 2.64ab	6	1.6^{NS}
Feb 08	Eggs	11.83 ± 3.69a	0.0c	1.37 ± 1.37bc	8.63 ± 2.68a	12.75 ± 2.46a	0.0c	6.77 ± 2.47ab	6	5.7^{**}
	Older-instars	7.66 ± 1.94a	1.36 ± 0.58b	2.63 ± 1.87b	5.90 ± 1.77ab	8.45 ± 1.43a	1.41 ± 0.59b	4.82 ± 1.89ab	6	3.2^{*}
	Total Immatures	14.26 ± 4.06a	1.36 ± 0.58c	3.05 ± 2.29abc	10.47 ± 3.20ab	15.33 ± 2.79a	1.41 ± 0.59c	8.34 ± 3.09abc	6	4.5^{**}
July 08	Crawlers	1.01 ± 0.47a	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	6	6.5^{**}
	Second-instars	15.8 ± 1.17a	2.53 ± 1.05c	3.52 ± 1.55c	5.64 ± 1.08bc	12.74 ± 1.33a	8.21 ± 1.32b	4.70 ± 0.42c	6	19.5^{***}

^{P} Significantly different: *$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, Not significant.

Table 5.4. Number (Mean ± S. E) of hemlock woolly adelgids after treatment using the Arborjet trunk injection system on 3 November 2006, 4 April 2007 and November 14 2007.

Sampling Dates	Hemlock woolly Adelgid Life-stages	Untreated Control	Tristar Low Fall treatment 2006	Tristar High Fall treatment 2006	Tristar Low Spring treatment 2007	Tristar Low Spring and Fall treatment 2007	Tristar High Spring treatment 2007	Tristar High Spring and Fall treatment 2007	df	F^P
Nov 06	Second-instars	7.27 ± 4.68a	5.14 ± 1.79a	5.41 ± 3.51a	-	-	-	-	2	0.1^{NS}
	Older-instars	8.69 ± 3.63a	9.67 ± 2.20a	10.28 ± 4.11a	-	-	-	-	2	0.0^{NS}
	Total Adelgid	11.57 ± 5.77a	11.04 ± 2.72a	11.79 ± 5.27a	-	-	-	-	2	0.1^{NS}
	New Growth	1.64 ± 0.14a	1.47 ± 0.17a	1.79 ± 0.14a	-	-	-	-	2	3.7^{NS}
Dec 06	Second-instars	2.45 ± 1.15a	2.06 ± 0.71a	3.89 ± 1.62a	-	-	-	-	2	0.5^{NS}
	Older-instars	8.18 ± 2.19a	8.12 ± 1.24a	7.59 ± 1.29a	-	-	-	-	2	0.0^{NS}
	Total Adelgid	8.61 ± 2.39a	8.45 ± 1.29a	8.97 ± 1.29a	-	-	-	-	2	0.0^{NS}
	New Growth	1.79 ± 0.14a	1.53 ± 0.22a	1.79 ± 0.14a	-	-	-	-	2	1.1^{NS}
Mar 07	Egg sacs	4.24 ± 0.71a	4.22 ± 1.07a	5.12 ± 1.59a	-	-	-	-	2	0.2^{NS}
	Eggs	9.65 ± 2.09a	7.91 ± 1.37a	5.00 ± 2.22a	-	-	-	-	2	0.9^{NS}
	Second-instars	7.44 ± 2.15a	4.43 ± 2.37b	1.80 ± 1.04b	-	-	-	-	2	13.3^{**}
	Total Adelgid	13.05 ± 1.31a	9.63 ± 2.00ab	5.56 ± 2.26b	-	-	-	-	2	4.2^{NS}
May 07	Egg sacs	8.70 ± 2.83a	4.19 ± 0.48ab	4.14 ± 1.24ab	5.96 ± 1.53ab	4.48 ± 1.82ab	4.23 ± 3.06ab	1.62 ± 1.31b	6	1.2^{NS}
	Eggs	16.90 ± 2.22a	3.72 ± 2.66b	7.05 ± 4.83b	2.24 ± 1.08b	3.36 ± 1.55b	2.56 ± 2.56b	0.81 ± 0.53b	6	4.9^{**}
	Crawlers	9.16 ± 2.31a	0.79 ± 0.79b	3.57 ± 3.11b	0.0b	0.93 ± 0.36b	1.53 ± 0.98b	0.0 ± 0.0b	6	5.1^{**}
	Second-instars	13.79 ± 2.79a	5.28 ± 2.72b	8.03 ± 2.69ab	8.61 ± 2.03ab	5.72 ± 2.05b	4.21 ± 0.76b	4.31 ± 0.55b	6	2.5^{NS}
	Older-instars	7.33 ± 1.69a	2.78 ± 0.42b	3.26 ± 1.57b	0.75 ± 0.48b	1.43 ± 0.48b	1.39 ± 0.66b	0.60 ± 0.36b	6	5.1^{**}
	Total Adelgid	25.45±3.11a	8.12 ± 3.15b	12.92 ± 5.74b	9.40 ± 1.62b	7.08 ± 2.43b	6.18 ± 2.31b	4.55 ± 0.60b	6	5.0^{**}
	New Growth	3.94 ± 0.65ab	2.99 ± 0.72abc	4.41 ± 0.78a	1.98 ± 1.09bc	2.81 ± 0.78abc	0.66 ± 0.66c	0.68 ± 0.42c	6	3.4^{*}
Nov 07	Older-instars	13.21 ± 2.61a	2.35 ± 1.47b	5.99 ± 4.51b	1.62 ± 0.94b	2.50 ± 1.78b	1.87 ± 1.87b	0.43 ± 0.43b	6	4.1^{**}
Jul 08	Second-instars	12.64 ± 0.75a	0.83 ± 0.82b	5.73 ± 4.23ab	5.87 ± 2.31ab	2.15 ± 1.84b	6.26 ± 3.71ab	3.06 ± 3.06b	6	2.1^{NS}

^{P} Significantly different: *$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, Not significant.

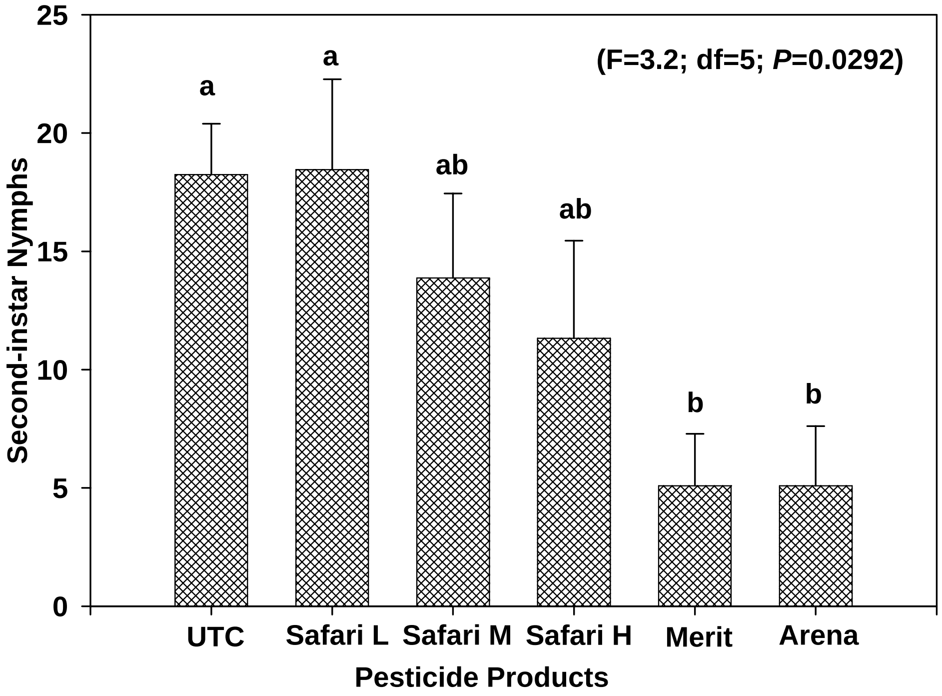

Figure 5.1. Number (Mean ± S. E.) of adelgids in July 2008 branch samples from the fall-treated trees (Nov. 06) in Highlands, NC. The rates of Safari represent; UTC= untreated control; L = low (3 g/ 2.5 dbh); M = Medium (6 g/ 2.5 dbh); H = High (12 g/ 2.5 dbh).

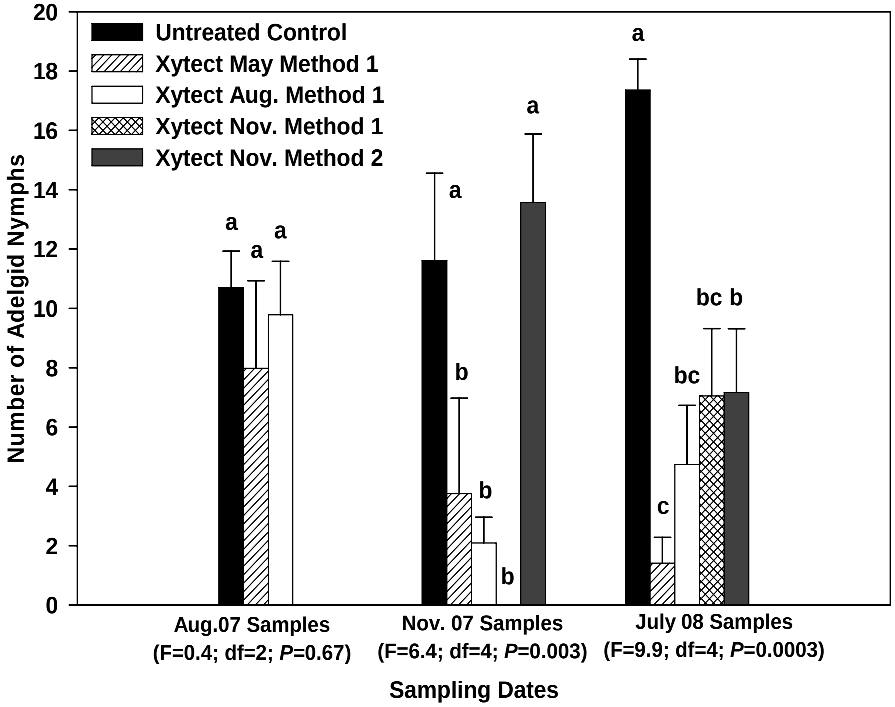

Figure 5.2. Number (Mean ± S. E.) of adelgids on Xytect- treated trees in Highlands, NC. The terms: method 1 or method 2 represent application method used to treat insecticide as HTI 2000 soil injection or root-flare injections by M3 injection system, respectively.

CHAPTER 6

OLFACTORY RESPONSE OF HEMLOCK WOOLLY ADELGID (HEMIPTERA: ADELGIDAE) PREDATORS TO HEMLOCK SPECIES (*TSUGA* SPP.)

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Abstract Hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), is causing widespread mortality of eastern hemlock, *Tsuga canadensis* (L.) Carrière, and Carolina hemlock, *Tsuga caroliniana* Engelmann, in the eastern US. Non-native predators, *Laricobius nigrinus* Fender (Derodontidae) and *Sasajiscymnus tsugae* (Sasaji and McClure) (Coccinellidae) have been released to control this invasive species. However, there is limited information on how they find prey. Thus, we examined the olfactory response of *L. nigrinus* and *S. tsugae* adults to the odor of *T. canadensis* branches in a Y-tube olfactometer. In addition, we assessed predator preference for various uninfested hemlock species by providing a choice between *T. canadensis* and three Asian hemlock (Japanese hemlocks, *Tsuga diversifolia* (Maxim.) Mast. and *Tsuga sieboldii* Carrière, and the Chinese hemlock, *Tsuga chinensis* (Franch.) E. Pritz) branches to assess if host tree influences behavior. Seventy-three percent of the time *L. nigrinus* and *S. tsugae* beetles showed no response when no odors were present in either chamber. However in February trials with host odors present, *Sasajiscymnus tsugae* adults responded but moved toward the "no odor" control chamber rather than the chamber containing *T. canadensis*. The same response was also noted in March when *T. sieboldii* was present in one of the chambers. In arena bioassays, a greater number of *S. tsugae* were recovered from the *T. canadensis* branch when these beetles were given a choice between *T. canadensis* and *T. diversifolia* foliage. This response was not noted when *S. tsugae* were provided with a choice between *T. canadensis* and *T. sieboldii* or *T. chinensis*. Similarly, *L. nigrinus* did not show any specific attraction when given choices between *T. canadensis* and *T. diversifolia*, *T. sieboldii* or *T. chinensis*.

Key words Hemlock woolly adelgid, olfactometer, *Sasajiscymnus tsugae*, *Laricobius nigrinus*, Y-tube olfactometer

Hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), stands out as one of the most destructive pests of two native hemlock species; eastern hemlock, *Tsuga canadensis* (L.) Carrière, and Carolina hemlock, *Tsuga caroliniana* Engelmann, in eastern North America. This pest causes heavy tree mortality within as few as 2-3 years in the southern Appalachian Mountains (Trotter and Shields 2009). Low tree resistance to the pest and lack of effective natural enemies provide ideal conditions for *A. tsugae* to rapidly expand its range (McClure and Cheah 1999). Moreover, the biology of *A. tsugae,* specifically its parthenogenetic reproduction and high fecundity, help it to adapt to novel ecosystems. Among various strategies used to manage the pest in the forest or ornamental landscape, chemical and biological control tactics have been widely adopted. Use of chemical control has practical limitations especially in the forests (Cowles et al. 2006) so deployment of the predaceous beetles *Laricobius nigrinus* Fender (Derodontidae) and *Sasajiscymnus tsugae* (Sasaji and McClure) (Coccinellidae) has been widely practiced to control *A. tsugae* (Lamb et al. 2006, McClure and Cheah 1999).

Previously, terpenoid levels of seven *Tsuga* species were studied and the asian [*T. chinensis* (Franch.) E. Pritz., *T. diversifolia* (Maxim.) Mast. and *T. sieboldii* Carrière] and western hemlocks [*T. heterophylla* (Raf.) Sarg., and *T. mertensiana* (Bong.) Carriere] were found to have high levels of monoterpenes such as α-pinene, β-caryophyllene, or α-humulene. In addition to having similar monoterpenes, the susceptible eastern hemlock species had a high level of a distinctly different monoterpene, isobornyl acetate (Lagalante and Montgomery 2003). Broeckling and Salom (2003b) observed higher levels of volatile compounds from matured previous-year *T. canadensis* growth than in current-year growth. Volatile chemical profiles derived from adelgid infested or uninfested *T. canadensis* branches were similar. However, *α*-pinene emissions were slightly lower from infested branches (57%) than uninfested ones (66%)

(Broeckling and Salom 2003b). Lagalante et al. (2006) suggested that the phenology of *A. tsugae* is adapted to evade the toxic levels of terpenoids found in young eastern hemlock branches. Despite this knowledge about the chemical composition of hemlock foliage, how specialist predators imported from the western U.S. or Asia are affected by these or other chemical constituents of hemlocks is unknown.

In their native range of southern Japan the derodontid predator *L. nigrinus* attacks *A. tsugae* developing on *T. heterophylla* and the coccinellid predator, *S. tsugae*, consumes *A. tsugae* on *T. sieboldii*. These predators have been widely released to control *A. tsugae* populations on *T. canadensis* in the eastern US. Several studies have shown that predaceous coccinellids such as *Harmonia axyridis* (Pallas), *Hippodamia convergens* (Guérin-Méneville), *Adalia bipunctata* L., *Exochomus flaviventris* Mader, and *Cryptolaemus montrouzieri* Mulsant (Hamilton et al. 1999, Heidari and Copland, 1992, Obata, 1997, Raymond et al. 2000) use olfactory cues to detect their prey or prey host plants. Based on antennal morphology, Broeckling and Salom (2003a) suggested that *L. nigrinus* might have a greater inherent olfactory mediated behavioral affinity to *A. tsugae* than the coccinellid *S. tsugae* but provided no olfactory response data. We tested whether *S. tsugae* and *L. nigrinus* responded to odors of hemlock branches and, if given a choice, which tree species they preferred.

Materials and Methods

Insect Culture and Plant Material. *Sasajiscymnus tsugae* were obtained from Young Harris College's predator rearing laboratory (Young Harris, GA) and *L. nigrinus* were obtained from the biocontrol rearing laboratory at the University of Georgia (Athens GA). In our laboratory, *S. tsugae* adults were maintained in ventilated one-liter plastic containers at 26 ± 1°C and provided with 2 to 3 freshly cut hemlock woolly adelgid infested *T. canadensis* terminal branches (5 cm long). *Laricobius nigrinus* adults were provided with adelgid-infested hemlock branches and were temporarily stored at the ambient temperature of 12°C and 50% RH. After every bioassay beetles were replaced with new beetles from the rearing laboratories. Cut branches of eastern hemlock were collected from trees at the University of Georgia Mountain Research Station, Blairsville, GA. *Tsuga chinensis* and *T. sieboldii* foliage was obtained from the U.S. National Arboretum (Washington DC) and *T. diversifolia* from Washington Park Arboretum (Seattle, WA). They were inserted into Oasis® foam (Advantage plus Floral Foam, Kent, OH) to reduce desiccation, stored at 12°C and used in bioassays within 30 days.

To test the effectiveness of the Y-olfactometer we collected adult granulate ambrosia beetles, *Xylosandrus crassiusculus* (Mot.) (Coleoptera: Curculionidae) from naturally infested camphor (*Cinnamomum camphora* (L.) J. Presl) tree logs. Ethyl alcohol is an attractant to *X. crassiusculus* in natural settings widely used to trap these beetles (Miller and Duerr 2008) so we used it as the attractant in our tests.

Olfactometry Design and Assays. Olfactory response of the predators was tested using a 1 cm diameter glass Y-tube olfactometer (Analytical Research Systems Inc., Gainesville, FL) ,with a 12 cm long stem and two 5.5 cm arms with a 78° angle. The ends of the two arms of the Y-tube were connected to two individual airflow meters (1.6 L per minute max.) that in turn coupled to two 11.5 L glass chambers. These glass chambers were linked to two 300 ml glass humidifiers each containing 100 ml distilled water. Two vacuum pumps (Cole-Parmer, model: L- 79200-00, Vernon Hills, IL) were used to pull air through activated charcoal filters (max. inlet dry airflow of 1034.250 kPa) to purify it and then the air entered the humidifier before entering the glass chamber with or without host material. All of these accessories were connected using Teflon® tubing.

Glass chambers and the Y-tube were thoroughly rinsed using water, then 99.5% acetone and dried at 110^{o}C for 1 h in a laboratory oven prior to every assay. During the assay, both airflow meters were regulated at 0.3 L per minute and a uniformly diffused 25W red-filtered incandescent light bulb was placed between the forking arms of the Y-tube. Hemlock branches weighing between 3.6 to 150 g were introduced into one of the glass chambers and the other left empty. Assays were conducted at room temperature (26 ± 1°C).

Adult beetles were starved for at least 24 h in ventilated plastic containers (500 mL) prior to testing. Afterwards beetles were introduced at the base of the Y-tube and given 5 minutes to respond. When beetles moved 2 cm or further into one of the arms of the Y and remained there for at least 20 s, it was recorded as a choice for the odor source connected to that arm. After 5 beetles were tested the Y-tube was washed in acetone to remove any residual odor and the tubing connected to the arms of the Y-tube were switched to insure that lighting or other factors were not influencing beetle choices. We also tested beetles with both chambers empty as an additional check for positional bias in the olfactometer. Since our initial results showed no predator response using hemlock branches with and without adelgids, we only used uninfested branches in the later tests.

Because of unusual responses from the predators we also tested *X. crassiusculus* adults (within one day after emergence) using 400 mL of 95% ethyl alcohol in one chamber. Unlike *L. nigrinus* or *S. tsugae*, *X. crassiusculus* adults were given 20 minutes to respond.

Arena Choice Assays. Predator preference between *T. canadensis* and *T. chinensis* or *T. diversifolia* or *T. sieboldii* foliage was tested in a rectangular (41 x 46 cm) 7.5 cm deep wooden box arena covered with nylon mesh. None of the foliage used in the bioassays was infested with adelgids. A trial included at least 10 adult beetles of one species (*L. nigrinus* or *S. tsugae*), starved in individual containers for 24 h prior to each test. Once the beetles were introduced to the test arena it was sealed using nylon mesh for the 15 h test period. All tests were conducted from 6:00 PM to 9:00 AM EST at 26 ± 1°C, RH ≈50-55% and no light.

Statistical Analyses. Olfactometer data were analyzed using the PROC FREQ procedure of SAS (SAS Institute 2003). Only beetles that responded were included in the analyses. Chi-square for goodness of fit was used to test for beetle response. A paired t-test was used to examine the preference of predators for *T. canadensis* or alternative hemlock species (PROC TTEST).

Results and Discussion

Neither predator demonstrated a clear olfactory preference for cut branches of *T. canadensis* when given a choice between branches and no host material (Table 6.1). When both chambers were empty 26.3% of *L. nigrinus* and 27% of *S. tsugae* responded by moving into an arm of the Y-olfactometer. Initial *S. tsugae* responses, when branches were present, were weak with less than 40% responding in August and September but *S. tsugae* activity improved remarkably from October through March (Table 6.1). However, despite the increased responsiveness of the beetles, trials in February and March 2009 were the only ones in which there were significant differences, and then higher proportions of beetles moved toward the control or empty chamber. Even when exposed to *T. sieboldii* from their native range in Japan in March 2009 *S. tsugae* went toward the empty chamber.

Laricobius nigrinus were also more active when one of the chambers contained *T. canadensis* branches but they failed to respond consistently toward the chamber with host tree branches (Table 6.1). The amount of foliage used in the test chamber did not influence the response. Even when only 3.6 g was used 82.5% of the beetles responded but 58.6% of those went to the control side of the Y.

Most ‘no decision’ observations by *L. nigrinus* and *S. tsugae* adults resulted from the predators remaining inactive within the stem of the Y-tube. Upon exposure to odors of *T. canadensis*, *S. tsugae* beetles moved spirally along the glass stem of the Y-tube but made slow progress towards the Y-tube junction. *Laricobius nigrinus*, on the other hand, moved toward the Y junction without much spiral movement around the interior of the tube. Our observations are consistent with previous studies, where *L. nigrinus* and *S. tsugae* did not demonstrate obvious significant attraction to *T. canadensis* odor (Broeckling 2002).

Although we switched tubes on the olfactometer every 5 beetles to make sure that lighting or some other factor wasn’t affecting beetle response, we tested Asian ambrosia beetles, *X. crassiusculus,* to make sure the olfactometer was working rightly. We ran three trials of approximately 20 beetles each and found that 80 – 94% of the beetles responded by moving toward the chamber containing ethanol in all three (Table 6.2). These results clearly demonstrate that the olfactometer was working properly.

There might be several possible reasons that caused these contradictory results. First, most of the *S. tsugae* beetle colonies were derived from few initial beetles imported from Japan (Flowers et al. 2007) and were reared over multiple generations on adelgid-infested branches in rearing cages. Over successive generations, the selection pressure likely favored beetles that could survive under these conditions. The combination of initially low genetic variability plus extended rearing under very artificial conditions could have resulted in beetles that have poor host finding abilities. However, *L. nigrinus* beetles were consistently field-collected, maintained for egg production in the laboratory and then deployed to eastern hemlock forests (Flowers et al.

2007), so the same argument doesn't apply but they responded in a similar manner. Perhaps these predators use other cues in combination with olfactory cues to find hosts. Broeckling (2002) concluded that olfactory cues might be less important in host searching over a long distance in *S. tsugae* adults because these beetles have low densities of pore sensilla on antennal walls. In addition, Bahlai et al. (2008) reported that adult coccinellid predators, *Harmonia axyridis,* responded to visual cues derived from silhouettes of horizontal or vertical rods and leaves of the host plant buckthorn, *Rhamnus cathartica* L. However, they could not differentiate between horizontal and vertical rods or between leaves of buckthorn and domestic apple, *Malus domestica* Borkh, without host volatiles. *Harmonia axyridis* responsed better when *R. cathartica* odor and visual cues were present at the same time (Bahlai et al. 2008). Likewise, both *L. nigrinus* and *S. tsugae* may use a combination of stimuli to find hosts. We clearly showed that the presence of host material (hemlock foliage) elicits a response and increases beetle activity. However, it is possible that without visual cues the beetles are unable to orient their response in the appropriate direction.

It is unclear whether concentration of *T. canadensis* volatiles influences repellency or attraction of *A. tsugae* predators. Beetles showed no distinguishable preference when the quantity of hemlock branch material was manipulated by weight or when airflow in the olfactometer was reduced or increased. However, there are several examples where low levels of volatiles acted as an attractant and high levels functioned as a repellent in diverse groups of insects (Broeckling 2002 and references therein).

Studies of diurnal behavior of *L. nigrinus* and *S. tsugae* showed that *L. nigrinus* was more active at night while *S. tsugae* was active during the daytime (Flowers et al. 2007). However, in our initial trials neither beetle was inactive during the day or night (SVJ unpublished) so we chose to conduct the test under nocturnal conditions to be consistent with Flowers et al. (2007) observations for *L. nigrinus*.

In our choice arena trials, more *S. tsugae* were recovered from *T. canadensis* branches when beetles were given a choice of *T. canadensis* and *T. diversifolia* foliage (Table 6.3). However, in trials where *T. sieboldii* or *T. chinensis* were provided as a choice along with *T. canadensis* the beetles showed no preference. We expected *S. tsugae* to prefer one or both of the Japanese hemlock species but instead they settled more on eastern hemlock or showed no preference. These results suggest that either *S. tsugae* cannot differentiate between hemlock species or that long term rearing of *S. tsugae* on eastern hemlock has resulted in a change in host tree preference. More research is required to understand how host trees affect hemlock woolly adelgid predator behavior.

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Table 6.1. Response of the predators *S. tsugae* and *L. nigrinus* in a Y-tube olfactometer when provided a choice of odors from *T. canadensis* and *T. sieboldii* branches and nothing

Date	Comparison		No. of trials	Ave. Foliage wt. (g)	No. of Beetles Tested	% Response	N	% Preference		X^2	P
	Test	Control						Test	Control		
Sasajiscymnus tsugae											
Aug. 2008	*T. canadensis*	blank	4	148.5	48	39.6	19	57.89	42.11	0.4737	0.4913
Sep. 2008	*T. canadensis*	blank	3	135.3	38	31.6	12	33.33	66.67	1.3333	0.2482
Oct. 2008	*T. canadensis*	blank	2	113.0	30	70.0	21	57.14	42.86	0.4286	0.5127
Dec. 2008	*T. canadensis*	blank	5	131.8	90	50.0	45	42.22	57.78	1.0889	0.2967
Feb. 2009	*T. canadensis*	blank	4	72.4	81	86.4	70	32.86	67.14	8.2286	0.0041*
Mar. 2009	*T. sieboldii*	blank	4	85.9	81	64.2	52	32.69	67.31	6.2308	0.0126*
Feb. 2009	blank	blank	2	-	37	27.0	-	-	-	-	-
Laricobius nigrinus											
Dec. 2008	*T. canadensis*	blank	1	150.8	19	47.3	9	33.33	66.67	1.0000	0.3173
Jan. 2009	*T. canadensis*	blank	4	123.8	87	85.1	74	43.24	56.76	1.3514	0.2450
Feb. 2009	*T. canadensis*	blank	2	26.0	40	80.0	32	43.75	56.25	0.5000	0.4795
Dec. 2009	*T. canadensis*	blank	3	3.6	35	82.5	29	41.38	58.62	0.8621	0.3532
Feb. 2009	blank	blank	3	-	57	26.3	-	-	-	-	-

The notations indicate the significant difference (*$P < 0.05$).

Table 6.2. Response of *Xylosandrus crassiusculus* (Mot.) in a Y-tube olfactometer when provides a choice of 95% ethyl alcohol and nothing

Comparison		No. of Beetles Tested	% Response	N	% Preference		X^2	*P*
Test	Control				Test	Control		
95% ethyl alcohol	Blank	22	90.9	20	80	20	7.200	0.0073*
95% ethyl alcohol	Blank	20	90	18	94.4	5.5	14.222	0.0002*
95% ethyl alcohol	Blank	20	90	18	94.4	5.5	14.222	0.0002*

The notations indicate the significant difference (**P* < 0.05).

Table 6.3. Response of *S. tsugae* and *L. nigrinus* in an enclosed arena bioassay when provided a choice of branches from *T. canadensis* and various alternative hemlock species for 24 h

Predator	Alternative hemlock spp.	No. of trials	No. of Beetles Tested	% Ave. Response	N	% Preference		*t-test*	*P*
						T. canadensis	Alternative hemlock spp.		
Sasajiscymnus tsugae									
	T. sieboldii	6	78	71.1	56	43.2 ± 5.1	27.7 ± 7.6	1.28	0.2565
	T. diversifolia	5	50	66	33	50.0 ± 10.0	18.0 ± 3.7	3.72	0.0205*
	T. chinensis	5	51	56.9	29	38.9 ± 10.0	18.0 ± 8.0	1.37	0.2439
Laricobius nigrinus									
	T. sieboldii	5	63	65.6	42	35.5 ± 2.5	30.0 ± 6.3	1.06	0.3504
	T. diversifolia	5	46	72.4	34	52.1 ± 11.9	20.3 ± 10.4	1.51	0.2049
	T. chinensis	4	40	47.5	19	25.0 ± 5.0	22.5 ± 7.5	1.00	0.3910

The notations indicate the significant difference (*$P < 0.05$).

CHAPTER 7

SUMMARY

Healthy hemlock trees, *Tsuga canadensis* (L.) Carrière, and adelgid, *Adelges tsugae* Annand (Adelgidae) populations should favor retention and population growth of adelgid predators like *Laricobius nigrinus* Fender (Derodontidae) and *Sasajiscymnus tsugae* (Sasaji & McClure) (Coccinellidae). Sixty eastern hemlock trees between 15.2 and 38.1 cm diameter at breast height (dbh) were selected in the Chattahoochee National Forest, Helen GA. Trees were treated with 0, 10 or 25% of 1.5g imidacloprid (Merit 75WP) per 2.5 cm of tree dbh and were either fertilized or not, in a 3 x 2 factorial design. After two years, more ovisacs and eggs were found on trees that received imidacloprid in the order 0% > 10% > 25%. Fertilized trees had greater adelgid fecundity which was positively correlated with total foliar N in both winter generations. In February 2009, N concentration and *A. tsugae* fecundity were higher on fertilized trees receiving 10% insecticide compared to unfertilized trees receiving the same amount of insecticide. In addition, N content was higher in insecticide treated trees than in untreated trees regardless of fertilizer in June 2009 samples. Tree growth parameters such as new shoots or needles and length of new shoots per unit length of branch exhibited a dose response (25% > 10% > 0%) to insecticide in June 2009 samples. Concentrations of N, P and K were higher in the foliage of trees treated with insecticide. Foliar aluminum concentrations were consistently lower in trees with higher insecticide dosages. Likewise, foliar calcium, zinc, manganese and boron were also higher in unhealthy trees that did not receive imidacloprid. Trees treated with low rates of imidacloprid were healthier than untreated trees, but only trees treated with 10% of the recommended rate of imidacloprid had sufficient adelgids to possibly sustain predators over extended periods of time.

We studied the distribution of hemlock woolly adelgid within hemlock trees for three summer (progredien) and two winter (sisten) generations in the Chattahoochee National Forest north of Helen, GA. In November 2006, sixty eastern hemlock, *T. canadensis* trees were treated with either 0, 10 or 25% of 1.5g of imidacloprid insecticide per 2.5 cm of tree dbh and two levels of fertilization (fertilized or not) in the summer of 2007 and 2008. *Adelges tsugae* ovisacs per cm branch were significantly more abundant for consecutive generations from June 2007 to June 2008 in the upper tree crown of untreated trees and when all trees were combined, and that was the general trend for most comparisons. However, adelgid ovisacs were more abundant in the lower crown of insecticide treated trees in June 2008. More sisten nymphs were found settled on the upper crown branches than on the lower ones, in the summers of 2007 and 2008. Higher adelgid fecundity was observed on the upper tree crown in February 2008 and in both the winter and summer of 2009. In contrast, *A. tsugae* were more fecund in the lower tree crown region regardless of insecticide rate they received in June 2008. On fertilized trees, *A. tsugae* fecundity was significantly higher in the upper crown region in February 2008. In the following summer in June 2008 fecundity was higher in the lower tree crown strata then bounced back to upper tree crown strata by summer 2009. New growth of branches also varied among sample dates. Greater density of sisten ovisacs were observed near branch tips than on the region closest to the tree trunk in summer 2007. These data demonstrate the variable distribution of hemlock woolly adelgid and hemlock growth in the tree crown over time and suggest that sampling only one crown area will not provide accurate estimates of adelgid densities or predator recovery.

We evaluated host plant resistance to *A. tsugae* among five hemlock species under three different fertilizer regimes to see whether or not fertilizer affected resistance, and to examine if fertilizer and host tree species affected prey quality. The three treatments included a long-term fertilizer application (June 2008 to June 2009) a short-term fertilizer application (March to June 2009), and an unfertilized group. On March 31, 2009, all plants were artificially infested. Among unfertilized hemlocks, foliar N content was highest in the *Tsuga mertensiana* (Bong.) Carrière, lowest in *T. chinensis* (Franch.) E. Pritz and intermediate in the *T. canadensis* and *T. heterophylla* (Raf.) Sarg. A greater number of progredien ovisacs or sisten eggs per cm shoot were noted on *T. mertensiana* than on other hemlock species, an intermediate density was noted on *T. canadensis* and *T. heterophylla,* and none developed on unfertilized *T. chinensis*. Compared to unfertilized *T. canadensis*, recently-fertilized trees had more ovisacs, eggs and adelgid fecundity. Fertilizing *T. heterophylla* had no effect on *A. tsugae* feeding on them. However, densities of developing *A. tsugae,* especially older-instar nymphs, were greater on unfertilized *T. heterophylla* than on *T. heterophylla* regardless of fertilizer. In the spring, long-term fertilized *T. canadensis* or *T. heterophylla* plants produced abundant new growth and had a higher dry weight biomass relative to unfertilized plants. Feeding preference of adelgid predators was tested by providing equal numbers of adelgid eggs developed on various hemlock species that received fertilizer or not, in a small choice arena. More eggs from fertilized *T. canadensis* were consumed by the predators *L. nigrinus* and *S. tsugae* than from unfertilized ones. No predator interaction-effects were noted on any other choices.

A series of experiments compared commercially available and experimental insecticides, rates, application methods and timing for woolly adelgid control in northern Georgia and North Carolina. Safari 20 SG (dinotefuran) treated trees provided an average range of 63 to 100%

suppression of adelgid populations almost one month after treatment. Arena 50 W (clothianidin) and Merit 75 WP (imidacloprid) were slower acting but provided longer-term adelgid suppression. Among neonicotinoid insecticides evaluated, dinotefuran provided the quickest suppression of HWA however, 26 months later HWA re-colonized trees treated in spring 2006 while Merit treatments were still effective. High volume treatments such as soil drench of Safari did not yield significant additional adelgid control compared to low volume applications such as soil injection. Three months after a fall application an average of 40 and 76% mortality was observed in comparison with HWA on untreated trees for low and high concentrations of Tristar (acetamiprid) arborjet trunk injection, respectively. Among the Xytect 75 WSP (imidacloprid) soil injection treatments applied during May, August, or November, the May treatment was most effective reducing the population by an average of 92%. Xytect root-flare micro system treatments were also effective as the soil injected treatment.

Non-native predators, *L. nigrinus* and *S. tsugae* have been released to control this invasive. However, there is limited information on how they find prey. Thus, we examined the olfactory response of *L. nigrinus* and *S. tsugae* adults to the odor of *T. canadensis* branches in a Y-tube olfactometer. In addition, we assessed predator preference for various uninfested hemlock species by providing a choice between *T. canadensis* and three Asian hemlocks (Japanese hemlocks, *Tsuga diversifolia* (Maxim.) Mast. and *Tsuga sieboldii* Carrière, and the Chinese hemlock, *T. chinensis* branches to see if host tree influences behavior. Seventy-three percent of the time *L. nigrinus* and *S. tsugae* beetles showed no response when no odors were present in either chamber. However in February trials with host odors present, *S. tsugae* adults responded but moved toward the “no odor” control chamber rather than the chamber containing *T. canadensis*. The same response was also noted in March when *T. sieboldii* was present in one of the chambers. In arena bioassays, a greater number of *S. tsugae* were recovered from the *T. canadensis* branch when these beetles were given a choice between *T. canadensis* and *T. diversifolia* foliage. This response was not noted when *S. tsugae* were provided with a choice between *T. canadensis* and *T. sieboldii* or *T. chinensis*. Similarly, *L. nigrinus* did not show any specific attraction when given choices between *T. canadensis* and *T. diversifolia*, *T. sieboldii* or *T. chinensis*.