Effects of Fertilization of Four Hemlock Species on *Adelges tsugae* (Hemiptera: Adelgidae) Growth and Feeding Preference of Predators

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ABSTRACT   Understanding how fertilization affects host resistance to hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), is important because fertilizers are often used to grow resistant selections to a suitable size for testing. We evaluated four hemlock species (*Tsuga*) under three different fertilizer regimes to assess whether fertility affected resistance to the adelgid and to determine whether it affected feeding preferences of the adelgid predators *Laricobius nigrinus* Fender and *Sasajiscymnus tsugae* (Sasaji & McClure). Treatments were long-term fertilization (from June 2008 to June 2009), short-term fertilization (from March to June 2009), and no fertilizer. Fertilizer was applied biweekly with 240 ppm N by using water-soluble fertilizer (N–P–K, 20:20:20). Plants (>1 yr old) were artificially infested with adelgids on 31 March 2009. Among unfertilized hemlocks ($n = 10$ per species), foliar N was highest in *Tsuga mertensiana* (Bong.) Carrière and lowest in *T. chinensis* (Franch.) E. Pritz. Significantly more progredien ovisacs or sisten eggs were present on *T. mertensiana* than on the other hemlock species with none on unfertilized *T. chinensis*. *A. tsugae* adults on *T. heterophylla* (Raf.) Sarg. were unaffected by fertility, but densities of developing *A. tsugae* nymphs were higher on unfertilized *T. heterophylla* plants than on fertilized *T. heterophylla* plants regardless of fertilizer treatment. Both *L. nigrinus* and *S. tsugae* consumed more adelgid eggs that developed on fertilized *T. canadensis* than from unfertilized plants. The predators did not exhibit this preference for adelgid eggs from females that developed on *T. heterophylla* or *T. mertensiana*.

KEY WORDS   hemlock woolly adelgid, fertility, *T. canadensis*, *T. heterophylla*, *T. mertensiana*

The hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), is an exotic insect pest that has caused widespread mortality to eastern, *Tsuga canadensis* (L.) Carrière, and Carolina hemlock, *Tsuga caroliniana* Engelmann, in the eastern United States (McClure 1987). 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 2009). 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 1991b) as adelgids consume stored nutrients in the xylem parenchyma cells (Shields et al. 1996). In general, tree mortality occurs ≈10 yr after initial infestation. However, tree death may occur within 2–3 yr in the southeastern United States depending on tree size and level of infestation (Trotter and Shields 2009).

Hemlock woolly adelgid has a bivoltine life cycle, which includes sisten (from June to March) and pro-

gredien (from March to June) generations on hemlocks in eastern North America. The progredien generation has polymorphic (both winged and wingless) life stages. Both *A. tsugae* generations develop parthenogenetically on a secondary hemlock host. The primary host of *A. tsugae* in Japan is *Picea torano* (Koch) Koehne (Montgomery et al. 2009) so in North America winged females fly off and die because the primary host is unavailable (McClure 1987). The severity of impact in the eastern United States is attributed to a high replacement rate (≈50 nymphs per sisten female), lack of effective native natural enemies, easy dispersal by mammals, birds, or wind (McClure 1990), and low hemlock resistance (Trotter and Shields 2009). Although use of insecticides is effective in managing this pest, it is only feasible in recreational areas or various ornamental landscapes with valuable hemlocks, or on a limited basis in forests (Cowles et al. 2006). Widespread use of insecticides in forests is too expensive, impractical, and has unacceptable environmental risks (Cowles et al. 2006, Cowles 2009). However, biological control might provide a feasible and sustainable solution to this pest problem in forest systems (Cheah and McClure 1998).

Eight of nine hemlock species are reportedly susceptible to *A. tsugae*, including two western North American hemlocks, western hemlock, *Tsuga heterophylla* (Raf.) Sarg. and mountain hemlock, *Tsuga*

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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 (McClure 1987). Although hemlock woolly adelgid is common on western and mountain hemlock in the Pacific Northwest, it does not damage hemlock in this region (Furniss and Carolin 1977) unless the tree is severely stressed (McClure 1992b). *A. tsugae* survival and fecundity was lower on 1.8-m-tall *T. heterophylla, T. mertensiana* and *T. sieboldii* relative to *T. canadensis* or *T. caroliniana* in Connecticut (McClure 1992b). 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 (38% of the total) in the Northwest. These studies indicate uncertainty regarding the range of resistance among Pacific Northwest hemlock species.

The Asian hemlocks *T. chinensis* and *T. diversifolia* are highly resistant to hemlock woolly adelgid (Bentz et al. 2002, Del Tredici and Kitajima 2004) and the nature of resistance has been reported as nonpreference or antixenosis (Montgomery et al. 2009). However, dense populations of *A. tsugae* were reported on *T. chinensis* in China (Havill and Montgomery 2008). Phylogenetic analysis based on mitochondrial and nuclear DNA sequences of *A. tsugae* populations around the world showed that the hemlock woolly adelgid infesting eastern hemlock is closely related to those infesting *T. sieboldii* in Japan. Adelgids found on *T. chinensis* in China were not closely 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 change either positively or negatively on resistant western and Asian hemlock species planted in the eastern United States.

Several breeding projects involving inter-specific hybrids, intraspecific crosses and vegetative propagation have been conducted (Del Tredici 1985, Wang et al. 1997, Bentz et al. 2002, Pooler et al. 2002, Jetton et al. 2005, Bentz et al. 2008, Montgomery et al. 2009). 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 in the eastern United States by hybridizing Asiatic, adelgid-resistant hemlocks (*T. diversifolia, T. sieboldii,* or *T. chinensis*) with susceptible eastern hemlock have been unsuccessful (Bentz et al. 2002). Phylogenetically, eastern American hemlocks (*T. canadensis* and *T. caroliniana*) are not closely related to each other; instead, susceptible *T. caroliniana* is more closely related to resistant *T. diversifolia* (Havill and Montgomery 2008). Montgomery et al. (2009) tested hybrids by artificially infesting them with *A.*

tsugae crawlers and observed intermediate adelgid population growth (two to four adelgids per cm branch) relative to the resistant-Asian (less than one adelgid per centimeter of branch) and susceptible eastern North American hemlock parents (10–12 adelgids per centimeter of branch).

Fertilizer is commonly applied to nursery trees and shrubs, and in ornamental landscapes (Braman et al. 1998), where its effects on host plant resistance are widely debated (Herms 2002). In most breeding programs, plants have been grown under a scheduled fertilizer regime and tested for adelgid resistance (Bentz et al. 2008, Caswell et al. 2008, Montgomery et al. 2009), but McClure (1992a) showed that fertilizing *T. canadensis* increased *A. tsugae* egg production by two-fold and densities by five-fold relative to unfertilized trees. Nevertheless, effects of fertility have not been examined on resistant hemlock species. In addition, *Laricobius nigrinus* Fender (Derodontidae) and *Sasajiscymnus tsugae* (Sasaji & McClure) (Coccinellidae) are two predators widely released against *A. tsugae* in the forest (Cheah and McClure 1998, Zilahi-Balogh et al. 2003a, Lamb et al. 2006). Healthy adelgid eggs are vital for both predators as food or oviposition sites for *L. nigrinus* (Palmer and Sheppard 2002, Lamb et al. 2006). Therefore, it is important to understand how fertility influences hemlock resistance to *A. tsugae* and its acceptability for predators. Our objectives were to 1) evaluate the host plant resistance among various hemlock species, 2) determine whether hemlock resistance to hemlock woolly adelgid is affected by fertility, and 3) determine whether fertility affects adelgid egg quality for the predator beetles.

Materials and Methods

Plant Material and Treatment. This experiment was conducted at the Mountain Research Station in Blairsville, Union Co., GA, from June 2008 to June 2009. Four species of 1-yr-old hemlocks: *T. canadensis* (Forestfarms, Williams, OR, and Porcupine Hollow Tree Nursery, Central Lake, MI), *T. heterophylla* (Brooks Tree Farm, Brooks, OR), *T. mertensiana* (Forestfarms, Williams, OR, and Parry Tree Farm Nursery, Forest Grove, OR), and *T. chinensis* (Mineral Springs Ornamental Plant Nursery, OR) were purchased. Eastern and western American hemlocks were received as bare-root seedlings and *T. chinensis* as potted plants. The bare-root plants were planted into 3.8-liter pots with unfertile growing medium (Sun-Gro Horticulture, Metro-Mix 300 series, Bellevue, WA) and the *T. chinensis* were replanted into similar-sized pots and growing medium. Plants were maintained in a screen house with screen mesh (Progress Growers, Canton, GA) allowing 50% sunlight as done in the nurseries and irrigated every 2 d. During the winter (from November to February), the average minimum temperature was −1.4°C with a low of −16.1°C on 16 January 2009, so the area between the plants was mulched with hay to reduce the risk of root injury from frost.

Table 1. Number of hemlocks surviving from June 2008 to June 2009 out of 10 planted for each species and treatment

Hemlock species	Long-term fertilized plants, June 2008–June 2009	Short-term fertilized plants, Mar.–June 2009	No. plants not fertilized, June 2008–June 2009	
	Infested (surviving)	Infested (surviving)	Infested (surviving)	Replaced[a]
T. canadensis	10 (10)	10 (10)	10 (10)	0
T. heterophylla	6 (6)	9 (9)	10 (6)	4
T. mertensiana	2 (2)		10 (1)	9
T. chinensis	10 (10)	10 (10)	10 (10)	0

Plants were infested with *A. tsugae* on 31 March 2009.

[a] Dead hemlock plants were replaced by healthy ones from a nursery maintained under the same conditions as the experimental plants throughout the study and no plants were replaced after 31 March 31 2009.

The experiment was a 4 by 3 factorial design with 10 single plant replications per treatment per hemlock species. The fertilizer treatments were long-term fertility (4 June 2008–2 June 2009), short-term fertility (31 March 2009–2 June 2009) and no fertility. The long-term fertility regime was used to simulate the current practices in the nurseries or how selected plants were maintained for screening studies. However, plants may allocate nutrients for plant growth rather than for production of secondary metabolites with continuous fertility (Herms 2002). Thus, an additional short-term fertility treatment was evaluated. Fertilized plants received the recommended label rate of 240 ppm nitrogen (N) of a water-soluble fertilizer (Scott Peter Professional 20-20-20 General purpose, Marysville, OH) at biweekly intervals from 4 June 2008 to 2 June 2009, except November through February when they were fertilized once per month. We selected a biweekly interval for fertility because hemlocks are woody conifers and they need more time to absorb and translocate fertilizer unlike nonwoody plants (e.g., annuals). Plants received fertilizer 20 and six times for the long- and short-term treatments, respectively. This fertilizer had an N composition of 3.94% ammonia, 6.05% nitrate, and 10.01% urea and was applied as a drench using 200 ml of an aqueous solution of 240 mg of fertilizer per plant. Although all 120 plants were initially established after planting based on overall plant vigor and new growth, *T. heterophylla* and *T. mertensiana* suffered mortality regardless of fertilizer treatment (Table 1). This was consistent with a previous finding that suggested *T. heterophylla* and *T. mertensiana* do not grow well even in containers in eastern North America (Bentz et al. 2002). Unfertilized dead plants were replaced with plants that were maintained under the same environmental conditions throughout the course of the experiment (Table 1).

Artificial Infestation and Measurements. Hemlock plants were artificially infested on 31 March 2009 by loosely attaching woolly sisten ovisacs along with hemlock branches collected from nearby infested trees by 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 averaged 142 progredien eggs per sac after randomly assessing ten ovisacs. Branches with ovisacs remained on the plants for 3 wk to facilitate settling of emerged crawlers. Settled adelgids can be quantitatively assessed by counting. 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 higher when adelgid-infested shoots were loosely tied. Settlement rate was not affected by increasing densities of adelgid crawlers (Butin et al. 2007, Jetton et al. 2008b).

On 1 May, plants were evaluated for sedentary first-instar progredien nymphs on the hemlock twigs. Four branches were nondestructively examined under a stereomicroscope (10×), and settled first-instar nymphs counted.

Two destructive samples were collected to capture the peak adelgid fecundity or the time frame when most females mature. On 3 and 11 June 2009, two 15-cm-long terminal branches were cut from each plant and stored at 12°C. Because these plants were small and destructive sampling may induce unintended effects on adelgid development, we did not assess developing adelgids between 1 May and 3 June. Total length of the branch (including length of side-branches), number of winged exuviae, live apterous and pterous nymphs, ovisacs, and eggs were recorded. Adelgid fecundity was calculated by dividing total number of eggs with total number of female adelgids counted. Number of growing hemlock tips and their length were also measured from 10-cm-long branches because fertility may impact plant growth.

Finally, the entire plant was cut ≈1 cm above the growing medium and stored at 12°C. The 97 plants were then oven-dried at 40°C for 48 h, and total plant biomass was recorded. The four 15-cm-long hemlock branches destructively sampled from each plant also were included with the respective plant sample before oven drying. Dried needles were removed and analyzed for total percentage of N by the combustion method of Colombo and Giazzi (1982) and other essential elements such as manganese (Mn), iron (Fe), aluminum (Al), boron (B), copper (Cu), zinc (Zn), sulfur (S), phosphorous (P), potassium (K), calcium (Ca), and magnesium (Mg) were analyzed using an inductively coupled plasma emission spectrograph (Isaac and Johnson 1985, AOAC 1995) at the Plant and Soil Testing Laboratory (University of Georgia, Athens, GA).

Predator Choice Tests. Adult *S. tsugae* and *L. nigrinus* were obtained from the predator-rearing laboratories at Young Harris College (Young Harris, GA) and the University of Georgia (Athens, GA), respectively. Adult beetles, 50 *S. tsugae* and 40 *L. nigrinus*, were brought to the USDA-Forest Service laboratory (Athens, GA) for a beetle preference experiment and were maintained under 26 ± 1°C, 45–55% RH; and a photoperiod of 12:12 (L:D) h in cages.

Beetle response was tested in a 9-cm-diameter petri dish with moistened tissue paper on the bottom as done by Butin et al. (2004). Twenty *A. tsugae* eggs deposited by adults having fed on long-term fertilized or unfertilized hemlocks of three species (*T. canadensis*, *T. heterophylla*, or *T. mertensiana*) were placed on uninfested shoots (1 cm long) collected from each hemlock species, except *T. chinensis* due to lack of *A. tsugae* development on this species. One shoot with eggs from adelgids fed on fertilized trees and one with eggs from adelgids fed on unfertilized trees was placed on opposite sides of the petri dish arena, and one adult beetle (starved 24 h) was introduced into the center. Intact eggs and those damaged by predators were quantified after 24 h, which is sufficient time for beetles to consume eggs (Butin et al. 2004). All the tests were conducted at 26–27°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 in adelgid fed 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. An analysis of variance (ANOVA) using the generalized linear model (GLM) procedure in SAS (SAS Institute 2003) was used to analyze the effects of hemlock species and fertilizer treatments on adelgid densities. Data for the 3 and 11 June assessments were combined because we were interested in assessment of all developing stages of adelgids. Because adelgids are sedentary their density is proportional to branch length (Cowles 2009, Montgomery et al. 2009), so adelgid counts and new shoots and their lengths were standardized by dividing by the total hemlock branch length. Data, associated with the new shoots and lengths were transformed by natural $\log(x + 1)$ to attain homogeneity of variances. However, the dry weight and nutrient content data were not transformed. Few or no adelgids developed on *T. chinensis* plants; therefore, to prevent unnecessary inflation of experimentwise error (type I) rates, adelgid counts from this species were not included in the multiple comparisons of hemlock species for each fertilizer treatment (Montgomery et al. 2009, Reeve and Strom 2004). Also, only two *T. mertensiana* plants in the long-term fertility group survived. Thus, adelgid counts obtained from the two *T. mertensiana* plants were not included in the analysis. Correlations between adelgid density and foliar nutrient content were assessed using the PROC CORR procedure in SAS. The multicolinearity, if it existed among the elements, was removed by considering the partial correlations and adding a PARTIAL statement to the CORR procedure. Regression analysis using the PROC REG procedure in SAS determined the relationship between fecundity and percent foliar N concentration. The predator feeding choice tests were analyzed by paired *t*-tests using PROC TTEST in SAS.
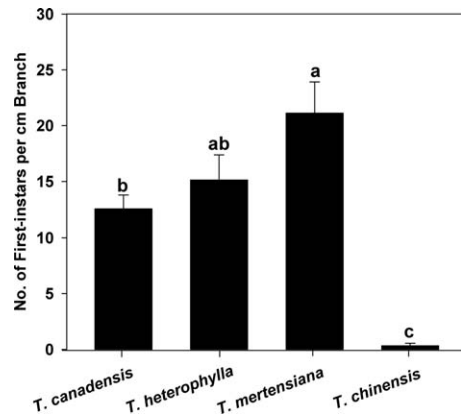

Fig. 1. Mean ± SE number of progredien settled first-instars observed on various hemlock species ($N = 20$) (both unfertilized and long-term fertilized treatments) May 2009. Bars with similar letters are not significantly different ($\alpha = 0.05$; LSD test).

Results

Effect of Hemlock Species. We did not observe any significant difference in adelgid settlement between long-term fertilized and unfertilized plants during our nondestructive assessment on 1 May 2009 (1 mo after being infested), so data from those treatments (long-term fertilized and unfertilized) were combined for comparisons among hemlock species for this date. More settled first instars were present on *T. mertensiana* than on *T. canadensis*, and the fewest were on *T. chinensis* ($F = 99.6$; df = 3, 51; $P < 0.001$) (Fig. 1). The number of settled first-instars on *T. heterophylla* was not significantly different from *T. canadensis* or *T. mertensiana*.

In June 2009, 2 mo after infestation, there were no significant interactions between hemlock species and fertilizer for any adelgid life stage. Unfertilized *T. heterophylla* plants had significantly more progredien nymphs than the other hemlock species ($F = 3.9$; df = 2, 18; $P = 0.039$) (Fig. 2a). Progredien ovisacs per cm branch were greater on *T. mertensiana* than on *T. canadensis* or *T. heterophylla* and none were present on *T. chinensis* ($F = 5.6$; df = 2, 18; $P = 0.012$) (Fig. 2b). However, the total progredien adelgids present on the unfertilized plants were not significantly different among North American hemlock species ($F = 2.9$; df = 2, 18; $P = 0.076$) (Fig. 2c).

The number of sisten eggs laid by progredien females was greater on *T. mertensiana* than on unfertilized *T. canadensis* or *T. heterophylla* ($F = 7.3$; df = 2, 18; $P = 0.005$) (Fig. 3). No eggs were present on *T. chinensis* plants. Progredien fecundity was similar among the hemlock species ($F = 2.4$; df = 2, 16; $P = 0.125$) (Fig. 4). Significantly more sisten first-instar nymphs settled on *T. mertensiana* (4.42 ± 1.04 [mean ± SE]nymphs per cm) than on *T. canadensis* (1.66 ± 0.48 nymphs per cm) and *T. heterophylla* (1.70 ± 0.59 nymphs per cm) ($F = 3.6$; df = 2, 18; $P = 0.047$).

Effect of Fertilizer Treatments. The total foliar N content was significantly greater for both the short-

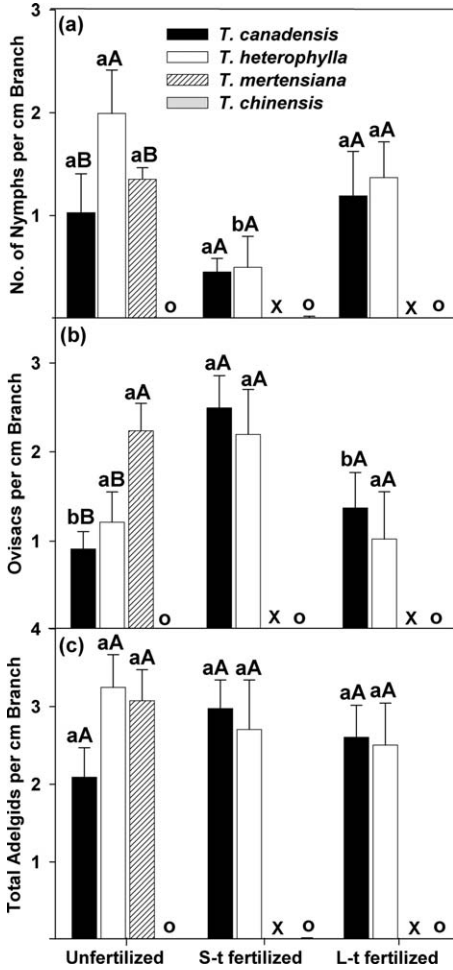**Fig. 2.** Mean ± SE number of total progredien nymphs (a), progredien ovisacs (b), and total progredien adelgids (c) per branch for hemlock species and fertilizer treatments (replicates, N = 10) in June 2009. Abbreviations are as follows: x, missing *T. mertensiana* treatment; o, few adelgids survived on *T. chinensis*; S-t, short-term fertility; and L-t, long-term fertility. Uppercase letters indicate comparisons of hemlock species within each fertilizer treatment, whereas lowercase letters indicate comparisons of fertilizer treatment effects by hemlock species. Bars with similar-case letters (upper or lower) are not significantly different (α = 0.05; LSD test).

term and long-term fertilized *T. canadensis* (F = 21.2; df = 2, 11; P < 0.001) or *T. heterophylla* (F = 17.9; df = 2, 13; P < 0.001) plants than their respective unfertilized plants (Fig. 5). In *T. chinensis*, a higher foliar N content was recorded for long-term fertilized plants followed by short-term fertilized plants then nonfertilized plants (F = 38.4; df = 2, 18; P < 0.001). Only two *T. mertensiana* plants survived in the long-term fertility group so they were not included in the analysis.

Within species, short-term fertilization significantly increased fecundity of adelgids on *T. canadensis* (F = 4.4; df = 2, 18; P = 0.027) compared with plants receiving no fertilizer (Fig. 4). Adelgid fecundity on
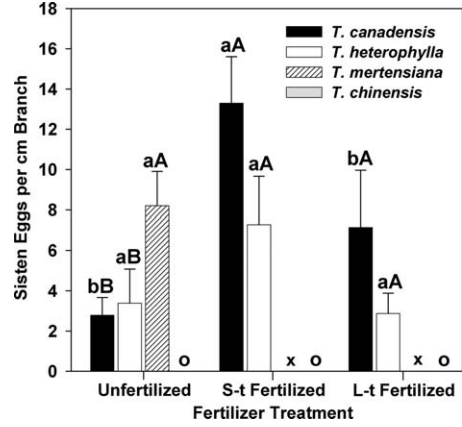

Fig. 3. Mean ± SE number of sisten eggs per branch for hemlock species and fertilizer treatments (replicates, N = 10) in June 2009. Abbreviations are as follows: x, missing *T. mertensiana* treatment; o, few adelgids on *T. chinensis*; S-t, short-term fertility; and L-t, long-term fertility. Uppercase letters indicate comparisons of hemlock species within each fertilizer treatment, whereas lowercase letters indicate comparisons of fertilizer treatments by hemlock species. Few adelgids survived on *T. chinensis*. Bars with similar-case letters (upper or lower) are not significantly different (α = 0.05; LSD test).

long-term fertilized *T. canadensis* was not significantly different from the other treatments. Fecundity on *T. heterophylla* was not affected by fertilizer treatment. Progredien adult (F = 5.8; df = 2, 18; P = 0.011) and egg (F = 7.0; df = 2, 18; P = 0.005) densities were greater on short-term fertilized *T. canadensis* than on

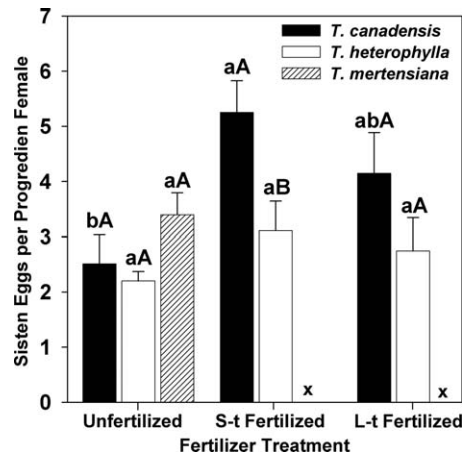**Fig. 4.** Mean ± SE number of total sisten eggs per progredien female present on hemlock species associated with each fertilizer treatment (replicates, N = 10) in June 2009. Abbreviations are as follows: x, missing *T. mertensiana* treatment; S-t, short-term fertility; and L-t, long-term fertility. Uppercase letters indicate comparisons of hemlock species within each fertilizer treatment, whereas lowercase letters indicate comparisons of fertilizer treatments by hemlock species. Few adelgids survived on *T. chinensis*. Bars with similar-case letters (upper or lower) are not significantly different (α = 0.05; LSD test).
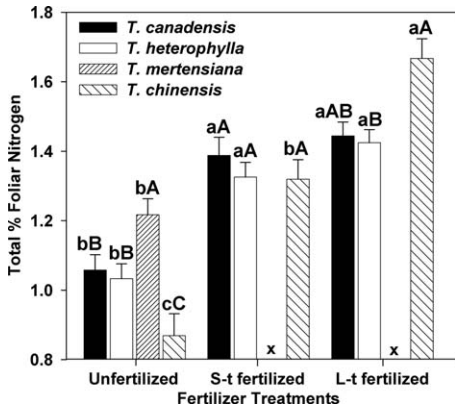
Fig. 5. Mean ± SE total percentage of foliar N content among hemlock species and fertilizer treatments ($N = 10$) in June 2009. Abbreviations are as follows: x, missing *T. mertensiana* treatment; S-t, short-term fertility; and L-t, long-term fertility. Uppercase letters indicate comparisons of hemlock species within each fertilizer treatment, whereas lowercase letters indicate comparisons of fertilizer treatments by hemlock species. Bars with similar-case letters (upper or lower) are not significantly different ($\alpha = 0.05$; LSD test).

unfertilized or long-term fertilized plants (Figs. 2b and 3). Regression analysis showed a significant correlation between adelgid fecundity and N content for *T. canadensis* ($r^2 = 0.15$; $F = 5.04$; df = 1, 28; $P < 0.033$) when all the treatments were combined.

More progredien nymphs were present on unfertilized and long-term fertilized *T. heterophylla* plants than on short-term fertilized plants ($F = 7.1$; df = 2, 13; $P = 0.008$) (Fig. 2a). In June, within the progredien nymphs, settled first and second instars were significantly more abundant on unfertilized *T. heterophylla* plants ($F = 6.3$; df = 2, 13; $P = 0.012$) (0.74 ± 0.24 [mean ± SE] nymphs per cm) than on short-term fertilized plants (0.06 ± 0.02 nymphs per cm) but not long-term fertilized plants (0.89 ± 0.38 nymphs per cm). In addition, still older progredien nymphs (older

than second instars) were significantly more abundant on unfertilized *T. heterophylla* ($F = 4.7$; df = 2, 13; $P = 0.029$) (0.89 ± 0.23 nymphs per cm) than on short-term (0.19 ± 0.15 nymphs per cm) or long-term fertilized plants (0.23 ± 0.07 nymphs per cm).

In comparisons between species within the same treatment, female fecundity was significantly higher on *T. canadensis* than on *T. heterophylla* receiving short-term fertilization ($F = 9.2$; df = 1, 6; $P = 0.023$) (Fig. 4). In both the short- and long-term treatments, ovisacs per centimeter were not significantly different among the hemlock species (Fig. 2b). Although ovisac ($F = 11.6$; df = 1, 5; $P = 0.019$), egg ($F = 34.1$; df = 1, 5; $P = 0.002$), and fecundity ($F = 11.5$; df = 1, 5; $P = 0.019$) data were significantly different within the long-term fertilization treatment, the least significant difference (LSD) did not show significant differences between the means (Figs. 2b, 3, and 4). This may have been a result of the unequal sample sizes of *T. canadensis* and *T. heterophylla* (Table 1). Only three ovisacs were present on *T. chinensis*.

Fertilizer Effects on Tree Growth. Long-term fertilized *T. canadensis* plants had more new shoots and needles, greater shoot length, and higher total dry weight than unfertilized or short-term fertility plants (Table 2). Long-term fertilization of *T. heterophylla* plants resulted in greater needle and total biomass compared with unfertilized or short-term fertility plants which were similar. *T. heterophylla* receiving short-term fertilization had more new shoots than unfertilized trees but not more than long-term fertilized trees. Both fertilizer regimes produced equal lengths of new shoots that were longer than those on unfertilized plants.

Among unfertilized trees (Table 2), numbers of new shoots were significantly higher on unfertilized *T. chinensis* ($F = 17.5$; df = 3, 27; $P < 0.001$) (0.54 ± 0.04 [mean ± SE]) than the other unfertilized hemlock species (*T. canadensis*, 0.17 ± 0.03; *T. heterophylla*, 0.27 ± 0.0; and *T. mertensiana*, 0.17 ± 0.05). Unfertilized *T. chinensis* also had longer new shoots ($F = 47.4$;

Table 2.   Effect of fertilizer treatments (mean ± SE) on various hemlock species growth parameters measured 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	7.6	2, 18	0.004
	Length of new shoots	0.30 ± 0.06b	0.23 ± 0.07b	0.88 ± 0.20a	6.4	2, 18	0.008
	Needle dry wt	12.11 ± 1.33b	13.83 ± 1.52b	26.42 ± 2.26a	22.5	2, 18	<0.001
	Total dry wt	26.79 ± 3.09b	36.45 ± 6.77b	50.93 ± 4.70a	6.9	2, 18	0.006
T. heterophylla	New shoots	0.26 ± 0.03b	0.91 ± 0.41a	0.42 ± 0.04ab	3.7	2, 13	0.053
	Length of new shoots	0.41 ± 0.07b	0.90 ± 0.17a	1.03 ± 0.11a	6.3	2, 13	0.013
	Needle dry wt	4.83 ± 0.70b	3.80 ± 0.31b	8.52 ± 2.00a	8.1	2, 13	0.005
	Total dry wt	12.60 ± 1.85b	8.77 ± 0.76b	19.72 ± 3.68a	10.9	2, 13	0.002
T. mertensiana	New shoots	0.16 ± 0.04a	–	0.09 ± 0.09a	32.3	1, 1	0.111
	Length of new shoots	0.07 ± 0.02a	–	0.04 ± 0.04a	3.3	1, 1	0.322
	Needle dry wt	2.52 ± 0.24a	–	4.14 ± 2.57a	1.1	1, 1	0.485
	Total dry wt	7.08 ± 0.49a	–	12.84 ± 0.96a	16.6	1, 1	0.153
T. chinensis	New shoots	0.54 ± 0.04a	0.40 ± 0.09a	0.36 ± 0.05a	2.1	2, 18	0.155
	Length of new shoots	1.12 ± 0.08a	1.11 ± 0.26a	1.07 ± 0.16a	0.2	2, 18	0.857
	Needle dry wt	22.65 ± 2.84a	13.58 ± 1.41b	18.91 ± 2.47ab	3.6	2, 18	0.047
	Total dry wt	50.88 ± 6.48a	33.45 ± 3.72a	41.19 ± 4.84a	2.7	2, 18	0.092

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

Table 3. Mean ± SE foliar element concentrations among four unfertilized hemlock species measured June 2009

Element	Foliage nutrient concn				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	7.2	3, 27	0.001
Ca	0.52 ± 0.03b	0.39 ± 0.02c	0.65 ± 0.02a	0.68 ± 0.06a	11.5	3, 27	<0.001
K	0.44 ± 0.03c	0.74 ± 0.06a	0.43 ± 0.03c	0.56 ± 0.02b	14.4	3, 27	<0.001
P	0.26 ± 0.02b	0.26 ± 0.01b	0.28 ± 0.03b	0.36 ± 0.01a	5.1	3, 27	0.006
Mg	0.31 ± 0.02a	0.33 ± 0.01a	0.31 ± 0.02a	0.23 ± 0.01b	5.4	3, 27	0.005
S	0.21 ± 0.02a	0.11 ± 0.01b	0.26 ± 0.04a	0.12 ± 0.01b	7.5	3, 27	<0.001
Al	166.32 ± 12.83b	190.01 ± 39.25b	282.99 ± 27.21a	79.31 ± 7.19c	10.6	3, 27	<0.001
B	29.97 ± 2.02c	49.91 ± 2.47a	40.16 ± 2.96b	41.88 ± 4.30ab	7.2	3, 27	0.001
Cu	3.78 ± 0.54a	2.60 ± 0.55a	2.60 ± 0.56a	2.65 ± 0.39a	1.2	3, 26	0.338
Fe	148.91 ± 10.78a	385.94 ± 190.02a	220.88 ± 21.85a	102.57 ± 8.89a	1.7	3, 27	0.195
Mn	86.26 ± 12.34bc	223.16 ± 21.67b	659.44 ± 94.05a	61.99 ± 9.21c	32.9	3, 27	<0.001
Zn	27.45 ± 12.34bc	36.67 ± 6.92ab	49.43 ± 5.04a	22.90 ± 1.31c	6.2	3, 27	0.002

Means for Ca, K, Mg, N, P, and S are expressed as total percentage concentration (of dry weight), whereas Al, B, Cu, Fe, Mn, and Zn are in ppm. Means in a row followed by different letters are significantly different ($P < 0.05$; LSD test). Analyses of variance were performed on the untransformed data.

df = 3, 27; $P < 0.001$) (1.12 ± 0.08), whereas *T. mertensiana* had the shortest (0.07 ± 0.02) relative to the other hemlock plants (*T. canadensis*, 0.30 ± 0.06; and *T. heterophylla*, 0.42 ± 0.07). Needle biomass was highest ($F = 32.3$; df = 3, 27; $P < 0.001$) on *T. chinensis* plants (22.66 ± 2.84) followed by *T. canadensis* (12.11 ± 1.33) and then *T. heterophylla* (4.83 ± 0.71) or *T. mertensiana* (2.52 ± 0.25). Similarly, total biomass was greatest in *T. chinensis* ($F = 27.9$; df = 3, 27; $P < 0.001$) (50.88 ± 6.48 [mean ± SE]) followed by *T. canadensis* (26.79 ± 3.09) and then in either *T. heterophylla* (12.61 ± 1.85) or *T. mertensiana* (7.08 ± 0.49).

Plant Nutrient Analyses. Most of the elements varied significantly with species in the unfertilized treatment. Percentage of total N and Al were highest in *T. mertensiana* foliage and lowest in *T. chinensis* for the unfertilized treatment (Table 3). The P content was significantly higher in *T. chinensis* compared with the other hemlock species. *T. heterophylla* had more K than the other species, whereas the lowest levels were detected in *T. canadensis* and *T. mertensiana*. Magnesium 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 than *T. heterophylla* and *T. chinensis*. The highest and lowest Mn and Zn contents were detected in *T. mertensiana* and *T. heterophylla* foliage, respectively. Boron levels in the foliage were highest in *T. heterophylla* and lowest in *T. canadensis*. Significantly more

Ca was recovered from *T. mertensiana* and *T. chinensis* plant foliage than *T. canadensis* and *T. heterophylla*.

The partial correlation analysis revealed a significant relationship associated with N, P, Mg, S, Al, B, and Mn and settled crawlers or the sedentary population derived from them (Table 4). Adelgid nymphs developing on hemlock trees were positively correlated with Mg and Al and negatively correlated with P and S content. Increasing levels of Mn were associated with increased numbers of ovisacs and their eggs and there was a positive relationship between Al or B content and ovisac density ($P < 0.05$). Conversely, there was a negative association between P content and ovisac and egg densities. The number of eggs produced by progredien females was positively related with S content in the foliage. Furthermore, N, P, S, and B contents were positively correlated with adelgid fecundity. However, fecundity was negatively correlated with foliar Fe content ($P < 0.1$). There was a significant positive correlation between Mn content and sisten nymphs. In addition, sisten nymphs were negatively associated with P or B contents ($P < 0.1$).

Choice Arena Tests. After 24 h, significantly more *A. tsugae* eggs derived from adelgids that fed on long-term fertilized *T. canadensis* were consumed by *L. nigrinus* adults than eggs from adelgids that fed on unfertilized plants ($t = -2.6$, df = 9, $P = 0.031$). *S. tsugae* also consumed more eggs from females that developed on long-term fertilized *T. canadensis* than on unfertilized plants ($t = -2.3$, df = 9, $P = 0.051$).

Table 4. Pearson's correlation coefficients between adelgid life stages and foliar nutrient concentration of hemlock species

Pairwise partial correlations variable	Foliar nutrient concn											
	N	Ca	K	P	Mg	S	Al	B	Cu	Fe	Mn	Zn
Pro. second or third instars					0.24*							
Total Pro. nymphs				−0.28**	0.35**	−0.23*	0.25*					
Pro. ovisacs				−0.32**			0.21[a]	0.17[a]			0.39**	
Sis. eggs				−0.26*		0.26*		0.19[a]			0.40***	
Sis. eggs/pro. ovisac	0.28*			0.29*		0.42**		0.28*	−0.26[a]			
Sis. nymphs	0.18[a]			−0.19[a]				−0.14[a]			0.26*	

The notations indicate the significant correlation (*P*: [a], <0.1; *, < 0.05; **, < 0.01; and ***, < 0.001) between variables and foliar nutrient concentrations. Pairwise partial correlations accounts for the multiple correlation effects among the nutrients. pro., progrediens; sis., sistens.

However, neither predator showed a preference for *A. tsugae* eggs that developed from females fed on long-term fertilized versus unfertilized *T. heterophylla* or *T. mertensiana*.

Discussion

As in previous studies (Bentz et al. 2002, Del Tredici and Kitajima 2004, Montgomery et al. 2009), we found *T. chinensis* was the most resistant species. Almost no first-instar nymphs were present suggesting that crawlers either failed to settle or died shortly after. Montgomery et al. (2009) reported similar results for *T. chinensis* seedlings. One of our goals was to determine whether fertility altered hemlock resistance to *A. tsugae*. Pontius et al. (2006) concluded that higher foliar N content would result in higher *A. tsugae* infestation levels. Based on our results, in the case of *T. chinensis*, fertility had no effect. Long-term and short-term fertilized plants had more foliar N, more new growth, and larger plants, but none of these factors resulted in more adelgids. These results indicate that the resistance mechanism of *T. chinensis* is not associated with the nutritional quality of the plant, but with an external physical or chemical barrier to crawler establishment or the inability of adelgid crawlers of Japanese lineage (Havill et al. 2006) to recognize them as acceptable hosts. Hemlock woolly adelgids found in eastern North America are more closely related to those 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 that we used in our study. Thus, genetic differences in *A. tsugae* further complicate interaction of the adelgid with various hemlock species and our ability to understand host resistance (Havill and Montgomery 2008).

Conversely, our data showed that *A. tsugae* were present on unfertilized, young *T. canadensis*, *T. heterophylla*, and *T. mertensiana*. Mausel (2005) reported that fecundity of *A. tsugae* sistens on mature *T. heterophylla* in Washington state was higher than on *T. canadensis*, whereas McClure (1992a) and Jetton et al. (2008b) observed the opposite. The data of Mausel (2005) indicate that the western population of *A. tsugae* is adapted to western hemlock. The results of McClure (1992a) and Jetton et al. (2008b) suggest that the Japanese adelgid population, which occurs in the eastern United States, is not well adapted to western hemlock. We found fecundity and total adelgids per centimeter of branch similar among unfertilized *T. canadensis* and *T. heterophylla*, suggesting that *T. heterophylla* may not be resistant. Our data and previous work (McClure 1992a, Mausel 2005, Pontius et al. 2006, Jetton et al. 2008b) indicates that determining resistant/susceptible hemlock among and within the various species of North American may be difficult and likely dependent upon adelgid population (western, eastern, Chinese, or Japanese), size and tree age, nutritional quality, and possibly climate and geographic

location. 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 *T. mertensiana* plants, including the bark, on the stems, and in branchlet regions. More adult females were observed on *T. mertensiana* than the other hemlock species, but they had fewer nymphs compared with *T. heterophylla* (Fig. 2a and b). Because all three species had similar numbers of adelgids when nymphs and adults were combined, our findings suggest that *A. tsugae* developed more quickly on *T. mertensiana* than on *T. heterophylla* but not *T. canadensis*. However, these results are consistent with our nutrient content analyses that showed that unfertilized *T. mertensiana* had higher foliar N than unfertilized *T. canadensis* or *T. heterophylla* (Fig. 5). *T. chinensis*, which had almost no adelgids that either settled or survived, had the lowest foliar N content among unfertilized plants but very few developed on long- or short-term fertilized plants, which had higher N contents, so nutritional quality may not have been a factor in adelgid development on this species.

N is an important nutrient to piercing-and-sucking insects, which often respond to higher N levels in host plants (McClure 1992a, Petitt et al. 1994, Kytö et al. 1996, van Emden 1996, Nevo and Coll 2001, Hogendorp et al. 2006, Chen et al. 2010). Pontius et al. (2006) suggested that the N content of host plants is involved in hemlock resistance or susceptibility. *T. canadensis* under both short- and long-term fertility had higher levels of foliar N compared with unfertilized plants (Fig. 5) but only the long-term fertilized trees had more plant biomass (Table 2). Interestingly, short-term fertilized *T. canadensis* had more mature ovisacs and eggs per centimeter branch than both unfertilized and long-term fertilized plants (Figs. 2 and 3). This was evident with fecundity although females on long-term fertilized plants did not produce more eggs than the other two treatments (Fig. 4). McClure (1991a) noted an immediate increase (within 3 mo) in *A. tsugae* fecundity when feeding and developing on young *T. canadensis* trees after a spring fertilizer application. However, it is unclear why there was no significant difference in adelgid density between long-term fertilized and unfertilized *T. canadensis* because long-term fertilized plants had more and longer new shoots, a greater biomass of needles and greater total plant biomass.

Fertilizer applications may reduce the production of carbon- and N-based 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 under nutrient-limited conditions would produce more secondary metabolites. Production of secondary metabolites would decrease as nutrient availability increased and shift resources to growth. Our data showed that *A. tsugae* fecundity on reportedly resistant *T. heterophylla* plants (McClure 1992b, Jetton et al. 2008b) was similar regardless of fertilizer regime. Unlike both long- or

short-term fertilized *T. canadensis* or *T. heterophylla* plants, fertilized *T. chinensis* plants were not less susceptible to *A. tsugae.* However, we recovered three ovisacs with 13 eggs and 23 developing nymphs from two fertilized *T. chinensis* plants. No adelgids were recovered from unfertilized *T. chinensis*, which was consistent with the previous reports (Del Tredici and Kitajima 2004, Montgomery et al. 2009).

In spring 2009, long-term fertilized *T. canadensis* or *T. heterophylla* plants produced more new growth and greater biomass than plants that were not fertilized. Conversely, despite the comparatively high levels of foliar N in fertilized *T. chinensis* plants, they did not produce more new growth than unfertilized plants. Because we artificially infested hemlock plants with high egg densities (average of 14,200 eggs per plant), a dilution effect due to increased surface area for crawlers to settle and mature, resulting from increased growth of fertilized plants, should not have been a problem.

Our data showed that a high Al content was associated with high numbers of developing nymphs (Fig. 2a). Similarly, Mg content was positively correlated with developing nymphal densities, and Mg content was uniformly higher among susceptible North American hemlocks than resistant *T. chinensis* plants. Retnakaran and Beck (1967) reported that pea aphid, *Acyrthosiphon pisum* (Harris), requires low quantities of Mg for reproduction. Interestingly, Mn content was correlated with adelgid abundance. Whether Mn content affects tree resistance is unknown. *A. tsugae* infestations seem to decline when P is present in higher concentrations in hemlocks (Pontius et al. 2006), and our data concur with this finding, although we found P content to be positively correlated with adelgid fecundity. Higher S or B contents were found to be associated with an increase in adelgid fecundity; however, how they are related to adelgid reproduction is not clear. When the fertilizers having N, P, and S were applied to *Pinus radiata* (D. Don), tree basal area growth improved but it reduced colonization by the aphid, *Essigella californica* (Essig) (Hopmans et al. 2008). Hopmans et al. (2008) concluded that the availability of P and S helped trees to rectify these elemental deficiencies and produce abundant new shoot growth. In our experiment, correlation analysis showed that increasing concentrations of N, Al, and S were associated with increasing adelgid densities, whereas P and K concentrations were associated with decreasing densities.

Tritrophic studies that determine interactions of biological control agents with prey affected by host plant quality may help in understanding bottom-up factors influencing predator populations (Chen et al. 2010). Host plant quality could be influenced by availability of nutrients and production of secondary metabolites. For *T. canadensis*, our choice test results showed that *L. nigrinus* and *S. tsugae* consumed more eggs that developed on adelgids that fed on long-term fertilized plants than eggs of females that developed on unfertilized ones. Also, fertilized *T. canadensis* had high levels of N, which increased *A. tsugae* fecundity.

L. nigrinus larvae and adults prefer *A. tsugae* eggs compared with other life stages (Zilahi-Balogh et al. 2003b). Zilahi-Balogh et al. (2003b) reported that ≈300 *A. tsugae* eggs are required for *L. nigrinus* larvae to complete development. It has been shown that consumption of at least one egg is vital for *S. tsugae* adults to begin oviposition (Butin et al. 2003). Previous studies have demonstrated an increase in numbers of the egg predator *Tytthus vagus* Knight (Miridae) on the delphacids *Prokelisia dolus* Wilson and *Prokelisia marginata* (Van Duzee) on fertilized $(60–120\,\mathrm{g\,N/m^2})$ perennial cordgrass, *Spartina alterniflora* (Lois.) (Claudio and Denno 2003). Several other examples of the effect of fertility on top-down factors are discussed in Chen et al. (2010). However, *L. nigrinus* and *S. tsugae* adults in our trials did not feed more on eggs from females that fed on fertilized plants when the host was *T. heterophylla.* Although the exact reason for this phenomenon is not clear, Lagalante and Montgomery (2003) reported considerable variation in terpenoid levels between *T. heterophylla* and *T. canadensis*, which could affect predator feeding if they are incorporated into the eggs.

Our study suggests that performance of hemlock woolly adelgid on various hemlock species is difficult to determine when hemlocks are grown outside of their native range. Additional studies are required to determine hemlock species susceptibility or resistance or tolerance to *A. tsugae*, specifically on popular cultivars, even if resistance is known in the native range. Also, performance of hemlock woolly adelgid may be affected by nutritional status of hemlock species as per our experiment. Although changes in fertility did not disrupt resistance of Chinese hemlock to *A. tsugae*, it is likely that resistance in this species is at the host recognition or crawler establishment stage because very few crawlers settled (Fig. 1). Levels of nutrients and endogenous compounds vary with fertility; in particular, this variation is more intense in young plants than in mature trees (Kytö et al. 1996). In addition, our experiments demonstrated that the nutritional value of adelgid eggs may influence predator behavior but more work is needed to determine whether the nutritional value of hemlock trees in the field affects predators.

Acknowledgments

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