Evaluation of Hemlock (*Tsuga*) Species and Hybrids for Resistance to *Adelges tsugae* (Hemiptera: Adelgidae) Using Artificial Infestation

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ABSTRACT Hemlock (*Tsuga*) species and hybrids were evaluated for resistance to the hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae). The adelgid was accidentally introduced from Asia to the eastern United States, where it is causing widespread mortality of the native hemlocks, *Tsuga canadensis* (L.) Carrière and *Tsuga caroliniana* Engelm. These two native species plus the Asian species *Tsuga chinensis* (Franch.) E. Pritz and *T. dumosa* (D.Don) Eichler and *Tsuga sieboldii* Carrière, and the hybrids *T. chinensis* × *T. caroliniana* and *T. chinensis* × *T. sieboldii*, were artificially infested with the crawler stage of *A. tsugae* in the early spring 2006 and 2007. After 8 or 9 wk—when the spring (progrediens) generation would be mature—counts were made of the adelgid. In both years, the density of *A. tsugae* was highest on *T. canadensis*, *T. caroliniana*, and *T. sieboldii*; lowest on *T. chinensis*; and intermediate on the hybrids. On *T. chinensis* and the *T. chinensis* hybrids, fewer adelgids settled, fewer of the settled adelgids survived, and the surviving adelgids grew slower. Thus, the nature of the host resistance is both nonpreference (antixenosis) and adverse effects on biology (antibiosis). Tree growth (height) was associated with resistance, but no association was found between time of budbreak and resistance that was independent of the taxa. Many of the hybrids grow well, have attractive form, and are promising as resistant landscape alternatives for the native hemlocks.

KEY WORDS *Adelges tsugae*, hemlock woolly adelgid, hybrid hemlock, host plant resistance

The eastern North American hemlocks, *Tsuga canadensis* (L.) Carrière and *Tsuga caroliniana* Engelm., are highly susceptible to injury by the hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae). The adelgid was first observed in the eastern United States in 1951 in Richmond, VA, and now occurs in 17 eastern states from Maine to Georgia (USDA–Forest Service 2008). *A. tsugae* also occurs in western North America on *Tsuga heterophylla* (Raf.) Sargent and *Tsuga mertensiana* (Bong.) Carrière; in China on *Tsuga chinensis* (Franch.) E. Pritz and *T. dumosa* (D.Don) Eichler; and in Japan on *Tsuga diversifolia* (Maxim.) Mast. and *Tsuga sieboldii* Carrière; however, these species are not damaged by the adelgid. Mitochondrial DNA analysis suggests a long evolutionary history between *A. tsugae* and hemlocks in western North America and Asia, whereas *A. tsugae* in the eastern United States is a recent introduction from southern Japan (Havill et al. 2006; Havill et al. 2007). The lack of host resistance and/or natural enemies has been suggested as a reason for the severe impact of *A. tsugae* on hemlocks in the eastern United States (Cheah and McClure 1996).

Only the hemlock species in eastern North America are believed to be susceptible to *A. tsugae;* however, formal experimental comparisons of the resistance of hemlock species are limited. In a 1-yr-old nursery planting in Connecticut, adelgid survival was 74–89% on *T. canadensis* and *T. caroliniana* and only 1–4% on *T. diversifolia, T. heterophylla,* and *T. mertensiana* (McClure 1992). Based on their own observations and those from several sources, Del Tredici and Kitajima (2004) concluded that *T. chinensis* was highly resistant, but they were uncertain whether *T. heterophylla* and *T. sieboldii* were resistant.

Hemlock is the secondary host of *A. tsugae* on which parthenogenic generations occur; like all adelgids, its primary host is spruce (*Picea*) on which sexual reproduction and gall formation occurs. The primary host in Japan is *Picea torano* (=*P. polita*) (Koch) Koehne (Inouye 1953) and recently adelgids in galls on two spruce species in China have been confirmed as *A. tsugae* by using DNA (Foottit et al. 2009). In North America, *A. tsugae* migrants were observed to deposit eggs on both native and exotic spruce species, nymphs from these did not survive past the first instar (McClure 1987). The hemlock woolly adelgid has two wingless (apterous) parthenogenic generations on hemlock each year. In Maryland, where the experiments reported here were conducted, the spring (progrediens) generation begins with eggs laid in April and ends in early July after the adults have laid the eggs

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that begin the overwintering (sistens) generation. The sistens eggs hatch quickly and the first instar nymphs (neosistens) enter diapause after settling at a feeding site. The neosistens do not resume growing until the cool months of autumn and complete their development in early spring. A generation of winged adults (alates), called sexupara, is produced along with the progrediens generation, but matures about a week earlier and flies away in futile search for a suitable primary host.

The only mobile stage on hemlock is the first-instar nymph, which crawls about on the tree and can be carried between trees by wind or animals (McClure 1990). Once these crawlers settle at feeding sites at the base of the needles, they do not change location. The crawlers of the progrediens generation emerge from eggs in the woolly ovisacs over a period of 4 wk—beginning before and ending after most of the vegetative buds of *T. canadensis* have broken—and usually settle on the terminal internode of branches produced the previous year. There is some evidence that the earlier hatching crawlers are more successful in establishment (Butin et al. 2007). The crawlers of the sistens generation occur in late June and early July and settle on the new, current year's growth.

T. canadensis is a popular ornamental landscape tree that is widely planted within and outside its native range, which extends from Nova Scotia west to Wisconsin and south to Georgia. It has dense foliage that can be heavily sheared and is extremely shade tolerant. The somewhat smaller and slower growing *T. caroliniana,* native to the southern Appalachian Mountains, is also a desirable landscape tree but is rare in horticultural commerce and not widely planted, probably because it does not transplant well (Swartley 1984, Dirr 1998). The horticultural industry would welcome an alternative for *T. canadensis* that is both resistant to the hemlock woolly adelgid and retains its shade tolerance and its esthetic form and foliage. The western North American hemlocks—found at high elevations or in cool, moist areas of the Pacific Northwest—are not well adapted to the eastern North American climate (Dirr 1998, Del Tredici and Kitajima 2004), even though *T. heterophylla* from Idaho have survived >30 yr at the Arnold Arboretum near Boston, MA. The southern and northern Japanese hemlocks (*T. sieboldii* and *T. diversifolia,* respectively) have been used to a limited extent in ornamental plantings in the eastern United States (Dirr 1998, Swartley 1984). Chinese hemlock, (*T. chinensis*) is showing good environmental tolerance as well as resistance to the adelgid and recently was suggested as a replacement for eastern hemlock in landscapes (Del Tredici and Kitajima 2004). The creation of hybrids between the eastern North American species and the Asian species is another approach to acquire hemlocks with resistance to *A. tsugae,* desirable form and habit, and superior adaptability to the eastern United States This article is the first to evaluate hemlock hybrids experimentally for resistance to *A. tsugae.*

The U.S. National Arboretum began a breeding program to determine the potential for hybridization between the susceptible eastern North American hemlocks and the Asian species in the 1990s. Interspecific hybrids as well as intraspecific crosses were successfully produced from controlled pollination of several hemlock species (Bentz et al. 2002). Amplified fragment-length polymorphism analysis confirmed >50 authentic hybrids between *T. caroliniana* and *T. chinensis, T. sieboldii,* and *T. chinensis,* and between *T. sieboldii* and *T. diversifolia* (Pooler et al. 2002). Several attempts to hybridize *T. canadensis* with the three Asian species were not successful (Bentz et al. 2002). These controlled crosses and selfed progeny from parental species were grown in containers for 4–10 yr and out planted for long-term evaluation in fall 2002 and by 2006, most trees in the field planting were of sufficient size to evaluate for resistance to *A. tsugae* and horticultural qualities.

The objectives of this study were to 1) evaluate resistance of the hemlocks by artificial infestation with *A. tsugae* progrediens crawlers in the early spring, 2) determine whether nonpreference (antixenosis) or adverse effects on growth (antibiosis) are components of any resistance observed, and 3) determine whether resistance is correlated with the growth and budbreak of the trees.

Materials and Methods

Plant Material. Seedlings from interspecific and intraspecific controlled pollinations (see Bentz et al. 2002) were out planted in 2002 in sandy-loam soil at the USDA South Farm, Beltsville, MD. A randomized block design was used. Trees were spaced 3 m apart, lightly fertilized initially, and drip-irrigated as needed.

Table 1 shows the hemlock taxa planted, survival as of 2007, and the number used in each year of the experiment reported herein. Following convention, interspecific hybrids are listed with the female parent first followed by the male. Because of high mortality of *T. caroliniana* and its importance for comparison with the hybrids, three additional trees of *T. caroliniana* of the same age and parentage, from another planting, were used in the 2007 trial. If trees of sufficient size and in good health were available, up to 10 replicates were chosen randomly for 2006; in 2007, additional hybrid accessions were added to better determine differences among the hybrids. *T. diversifolia* and its hybrids were not included in the experiment because of limited numbers and their small size.

Screening for Resistance. We evaluated 72 trees in 2006 and 100 trees in 2007 and assessed adelgid density in more detail in 2007. Before the start of the trials, the hemlocks in the plantation were carefully screened for the presence of adelgids and none were found. Several of the trees were infested with scale insects, but these caused no visible damage to the foliage.

Artificial infestation of the trees with *A. tsugae* followed the same procedure both years. Ovisacs produced by the overwintering generation of *A. tsugae,* each containing from 50 to 125 eggs just starting to hatch, were harvested from heavily infested trees located at the U.S. National Arboretum, Washington,

Table 1. Hemlock (*Tsuga*) taxa planted at USDA South Farm, Beltsville, MD, with 6-yr tree survival and number trees tested each year

Parentage[a]	Coding for taxa used in trials	Planted (no.)	Survival (%)	No. tested	
				2006	2007
T. canadensis self [A]		20	90		
T. canadensis self [B]		20	70		
T. canadensis × *canadensis* [A]		20	100		
T. canadensis × *canadensis* [B]	*T. canadensis*	20	95	10	11
T. caroliniana self	*T. caroliniana*	21	14		2
T. caroliniana × *caroliniana*	*T. caroliniana*	20	15	3	5[b]
T. caroliniana × *chinensis*	*T. car* × *chi*	41	83	12	21
T. chinensis self		2	100		
T. chinensis × *chinensis*	*T. chinensis*	19	90	10	10
T. chinensis × *caroliniana*	*T. chi* × *car*	15	100	10	14
T. chinensis × *diversifolia*		10	100		
T. chinensis × *sieboldii*	*T. chi* × *sie*	27	93	9	15
T. diversifolia self		2	100		
T. diversifolia × *diversifolia*		20	100		
T. diversifolia × *chinensis*		7	100		
T. diversifolia × *sieboldii*		8	88		
T. sieboldii × *sieboldii*	*T. sieboldii*	15	93	10	10
T. sieboldii × *chinensis*	*T. sie* × *chi*	20	75	8	12

[a] Crosses show the female parent listed first, followed by the male parent.
[b] Includes three trees from a nearby planting.

DC, 10 km from the experimental site. On 7 April 2006 and 11–12 April 2007, small branches, ≈0.2 m in length, were collected and divided into bundles of two to four branches, so that each bundle contained about the same number of *A. tsugae* ovisacs. Based on counts of 10 randomly selected bundles, the estimated average number of egg masses per bundle was 554 and 626, in 2006 and 2007, respectively. One bundle was placed on each tree at mid-level on an average-sized branch and enclosed in a nylon mesh bag (29 by 46 thread count/ 2.54 cm) (National Filter Media, Wallingford, CT). Bags remained on trees until July of each year to impede dispersal of the next generation to adjacent branches or trees.

In 2006, *A. tsugae* infestation density was evaluated by randomly removing three twigs from each bagged branch on 30 May–2 June. We counted the live adelgids on each internode of the previous year's growth under a dissecting microscope. The number of live adelgids was defined as number of the developing nymphs, adult aptera, and the alates that had been on the twig. At the time of sampling, most of the alates had matured and were dead in the bottom of the bag; these were not counted; instead we counted their former presence on the twig as indicated by the residue of wax coupled with molted exuviae. The internode length was measured to the nearest 0.5 cm, and the presence and length of new foliage on the twig were noted.

In 2007, we examined five twigs from each bag collected on 6–11 June. Twigs were measured and adelgids counted as in 2006, except that the numbers of several developmental stages and forms were recorded—dead first-instar nymphs, alates, immatures (nymphal instars II–IV, and adults without eggs), and apterous adults with eggs on the previous year's twig. Additionally, the aestivating first-instar sistens (neosistens) were counted on the current year's new growth, which was the only stage present on the new growth. In November, the artificially infested branches were removed from the trial trees and brought to the laboratory, where a random sample of 10 terminal shoots was removed from each branch for measurement and counting of adelgids.

Tree Measurements. Tree height and spread at the widest diameter were recorded to the nearest centimeter during each winter, beginning in January 2005. Beginning the last week of March, all the hemlocks in the nursery were surveyed for vegetative budbreak at biweekly (2005) and weekly (2006 and 2008) intervals until budbreak was observed on every tree. Budbreak was defined as the emergence of >10% of the buds from their bud scales on two or more quadrants of the tree. The relative budbreak of individual trees was calculated as the deviation from the median day of budbreak for all the hemlocks in the planting.

Statistical Analyses. Although the trees were planted in a randomized block design, exploratory analyses indicated that blocking had no effect and was not included in final models. The data for *A. tsugae* abundance on the taxa are characterized by a hundredfold range between means, with variance increasing with mean size. Hence, the data were transformed for statistical analysis—log + 1 for adelgid densities and the arcsine square root for percentages. Tree growth parameters were not transformed for analysis. After the appropriate transformation, a univariate General Linear model (SPSS, Inc. 2005) was run followed by the Waller–Duncan range test for unequal sample sizes using the harmonic mean sample size, controlling for type I and type II error equally. The harmonic mean was used for range tests because *T. caroliniana* was under represented in the samples and the variances of the transformed data are homogeneous. All tables and figures are presented with untransformed means and their standard errors.

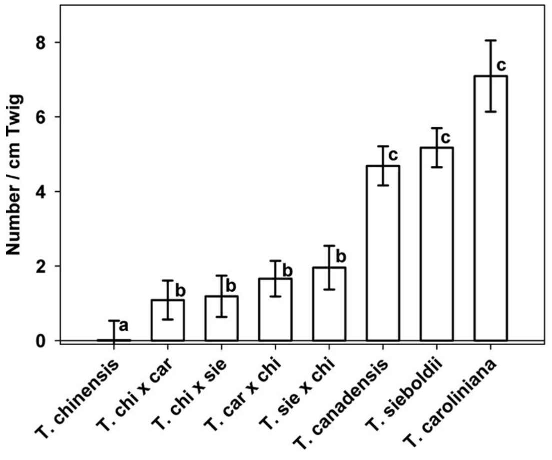

Fig. 1. Density (mean ± SE) of live *A. tsugae*, June 2006, on artificially infested hemlock taxa. Different letters above columns designate significantly different means using Waller–Duncan range test ($P < 0.05$) on log-transformed data.

Table 2. Proportion (mean ± SE) of *A. tsugae* in mid-June 2007 in each category

Taxon	No.	Live (% of settled)	Immature (% live)	Alates (% live)
T. chinensis	10	2.2 ± 6.9a	0.0	100.0
T. chi × *car*	14	16.1 ± 5.5ab	37.3 ± 5.0bc	59.9 ± 4.2
T. chi × *sie*	15	21.0 ± 5.4b	33.6 ± 4.2bc	56.6 ± 3.6
T. sie × *chi*	12	21.2 ± 6.3b	43.4 ± 5.0c	53.1 ± 4.2
T. car × *chi*	21	26.9 ± 4.6b	43.5 ± 3.3c	54.6 ± 2.8
T. sieboldii	10	62.6 ± 6.6c	26.5 ± 4.2ab	56.9 ± 3.6
T. caroliniana	7	68.9 ± 7.8c	24.4 ± 5.0ab	60.7 ± 4.2
T. canadensis	11	61.8 ± 6.3c	19.5 ± 4.0a	51.3 ± 3.4

Means in a column followed by different letters are significantly different ($P < 0.05$; Waller–Duncan range test on arcsine square root-transformed data).

Results

First Trial (2006). In late June, the density of live *A. tsugae* that had developed on the infested branches was highest on *T. canadensis, T. caroliniana,* and *T. sieboldii;* intermediate on the hybrids; and least dense on *T. chinensis,* which had only 0.01 live adelgids per cm twig (Fig. 1). The settled first-instar nymphs that did not develop were not counted.

Second Trial (2007). Density of *A. tsugae* in 2007 (Fig. 2) followed the pattern observed in 2006 on the taxa. In 2007, we not only counted the live adelgids but also recorded if these were immature, winged (alate), or wingless (aptera), and also counted the dead first-instar nymphs. The density of settled adelgids (the total of live and dead adelgids) on the previous year's growth was highest on *T. canadensis, T. caroliniana,* and *T. sieboldii,* with means of 9 to 10 adelgids per cm; intermediate on the hybrids, two to four adelgids per cm; and lowest on *T. chinensis,* 0.2 adelgid per cm. The density of live adelgids per cm was six to seven on *T. canadensis, T. caroliniana,* and *T. sieboldii;* 0.5–1.4 on the hybrids; and only 0.01 on *T. chinensis.*

The proportion of settled adelgids surviving and reaching maturity generally followed the rank order of the adelgid density on the taxa, whereas the proportion of alates was independent of the adelgid density (Table 2). The percentage of survival past the first instar was lower on the taxa on which fewer adelgids settled: 60–70% survived on *T. canadensis, T. caroliniana,* and *T. sieboldii,* whereas 16–27% survived on the hybrids, and only 2% survived on *T. chinensis.* The percentage of the adelgids that were immature was lower on *T. canadensis, T. caroliniana,* and *T. sieboldii* than on the hybrids; statistical contrast of these three species as a group with the four hybrids as a group indicates that the two groups are different (P = 0.00003). (*T. chinensis* was not included in the statistical comparison because there were only three indi-

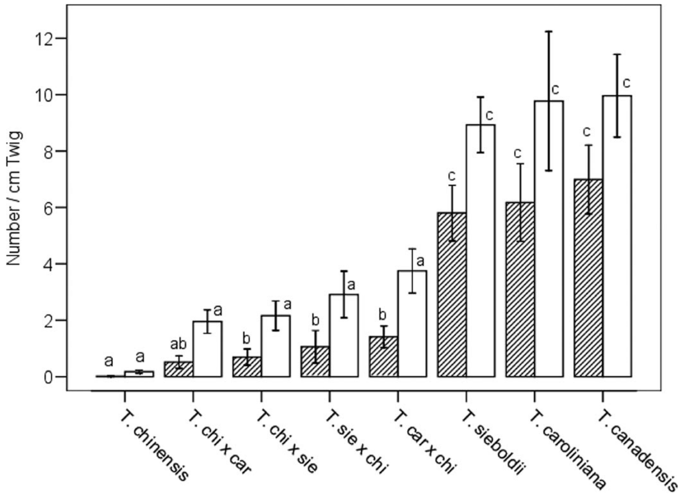

Fig. 2. Density (mean ± SE) of settled (open bars) and live (slashed bars) *A. tsugae*, June 2007, on artificially infested hemlock taxa. Different letters above each category of bars designate significantly different means using Waller–Duncan range test ($P < 0.05$) on log-transformed data.

Table 3. Density of *A. tsugae* (mean ± SE/cm twig) generations in June and November 2007

Taxon	No.	Progrediens Aptera (June)	Sistens (June)	Sistens (Nov.)	Per capita change[a] (June Aptera–Nov.)
T. chinensis	10	0.00 ± 0.38a	0.00 ± 0.18a	0.03 ± 0.56a	0.010 ± 0.066 (0.20)
T. chi × car	14	0.25 ± 0.27ab	0.11 ± 0.15a	0.08 ± 0.47a	−0.050 ± 0.055 (0.20)
T. chi × sie	15	0.32 ± 0.26ab	0.34 ± 0.14a	0.44 ± 0.46a	0.034 ± 0.054 (0.36)
T. sie × chi	12	0.46 ± 0.29ab	0.76 ± 0.16a	0.62 ± 0.51a	0.030 ± 0.060 (0.56)
T. car × chi	21	0.68 ± 0.22b	0.00 ± 0.17a	0.30 ± 0.38a	−0.088 ± 0.045 (0.04)
T. sieboldii	10	2.50 ± 0.32c	0.36 ± 0.18b	3.87 ± 0.56b	0.121 ± 0.066 (0.14)
T. caroliniana	7	2.55 ± 0.38c	0.97 ± 0.21c	2.97 ± 0.67bc	0.052 ± 0.078 (0.68)
T. canadensis	11	3.30 ± 0.30c	0.92 ± 0.17bc	5.53 ± 0.53c	0.122 ± 0.063 (0.29)

Means in a column followed by different letters are significantly different ($P < 0.05$; Waller–Duncan range test on log transformed data).

[a] Per capita change in adelgid density (log November sistens − log June aptera) is not significantly different between taxa ($P = 0.100$); numbers in parentheses are "*t*-test" probabilities that the change is equal to zero (=no change in density).

viduals in all the samples, all mature alates.) Thus, both lower survival and slower development occurred on the hybrid taxa compared with the three susceptible species. Excluding *T. chinensis,* the mean percentage of alates on the taxa was homogeneous, ranging from 51 to 61% ($P = 0.567$). The uniformity in percentage alates likely reflects maternal influences rather than the influence of the taxa, because the mothers of the adelgids used to infest the test trees were a homogeneous random sample from two nearby, heavily infested trees.

The density of the egg-laying, progrediens aptera was compared with their progeny, the sistens generation (Table 3). When counting the progrediens on the previous year's growth in mid-June, we also counted the diapausing first-instar nymphs (neosistens) on the new, current year's growth. Because egg laying and emergence of crawlers had not been completed and was more advanced on the susceptible species, we felt that the June count did not adequately represent the density of the sistens generation. Therefore, we reassessed the sistens generation in November. Although the density of settled sistens in November is higher than the density of aptera in June on six of the eight taxa, the per capita change in density—which is the log of the density of progrediens aptera in June minus the log of the density of the sistens in November on each tree—was not different between taxa ($P = 0.100$) and not different from zero for all but one taxa. Although the fall sample was taken in November—when the sistens generation should have broken diapause and resumed development—in every sample the sistens were still in the first instar and seemed dead. The cause of this mortality is unclear, but the sleeve cages over the infested branches were not removed until the end of July and the cages may have affected survival.

Effect of Parental Gender. Whether the sex of the *T. chinensis* parent influenced the density of live adelgids on the hybrids was examined in a statistical model that included both 2006 and 2007 data (Table 4). The model's fixed variables were sex of the *T. chinensis* parent, species of the other parent, and year. Interactions were not significant and omitted in the final model. Effects of year and species of the other parent were not significant. Sex of the *T. chinensis* parent was significant ($P = 0.031$). The least-square marginal means for density of live *A. tsugae* estimated by the model for female and male *T. chinensis* parentage are 0.870 ± 0.22 adelgids and 1.54 ± 0.21 adelgids per cm, respectively.

Association with Tree Parameters. The size of the hemlocks varied considerably both within and among the taxa (Table 5). *T. caroliniana* plants were much smaller than plants of other taxa, and only six of the initial 41 *T. caroliniana* survived. Survival (Table 1) of the other taxa used in the trials ranged from 75 to 100% ($\chi^2 = 8.5$, df = 6, N.S.). The crowns of all trees extended to ground-level and were upright pyramidal in shape, except for *T. chinensis,* which was broadly pyramidal. *T. chinensis* was shorter than *T. canadensis* and *T. sieboldii.* The average height of the hybrids between *T. chinensis* and *T. caroliniana* were greater than their parents, although these hybrids had both the tallest and shortest individual trees. Tree height was associated negatively with the density of live adelgids censused in June 2007 as a covariate in statistical models with all the taxa or only the hybrid taxa as the independent variables ($P = 0.016$ and 0.030, respectively). The effect does not seem to be simply a dilution effect, because the length of the internodes (data not shown) did not influence adelgid density ($P > 0.4$).

The phenology of vegetative budbreak also varied considerably among the taxa (Fig. 3). Plants of *T. chinensis* broke bud earliest (median, 14 April), whereas *T. sieboldii* broke bud last (8 May). Plants of *T. canadensis* and *T. caroliniana* had intermediate dates of budbreak, 27 April and 3 May, respectively. The median budbreak dates of the hybrids were between those of their parents but closer to the date of *T. chinensis* than the other parent. Statistically, the me-

Table 4. Influence of sex of *T. chinensis* parent on the density of live adelgids (mean/cm ± SE, number samples in parentheses) on hybrids

Yr	Other parent	Sex of *T. chinensis* parent	
		Female	Male
2006	*T. caroliniana*	0.52 ± 0.41 (14)	1.41 ± 0.34 (21)
	T. sieboldii	0.69 ± 0.40 (15)	1.06 ± 0.45 (12)
2007	*T. caroliniana*	1.09 ± 0.49 (10)	1.66 ± 0.47 (12)
	T. sieboldii	1.19 ± 0.52 (9)	1.96 ± 0.55 (8)

No significant effect on adelgid density for year or other parent ($P = 0.103$ and 0.953, respectively); sex of *T. chinensis* parent affected adelgid density significantly ($P = 0.031$).

Table 5. Tree height above ground and crown spread of *Tsuga* seedlings measured February 2008 after five growing seasons in the field

Taxon	No.	Ht (m)		Spread (m)	
		Mean	Range	Mean	Range
T. caroliniana	4	1.57 ± 0.28a	0.86–2.16	1.31 ± 0.17a	1.00–1.64
T. chinensis	11	2.06 ± 0.17ab	1.35–2.42	2.07 ± 0.11b	1.41–2.74
T. sie × *chi*	13	2.52 ± 0.16bc	1.50–3.08	2.03 ± 0.10b	1.49–2.70
T. car × *chi*	21	2.64 ± 0.12c	1.05–4.70	2.15 ± 0.08bc	1.34–3.16
T. chi × *sie*	15	2.81 ± 0.15c	2.14–3.58	1.99 ± 0.09b	1.48–2.98
T. canadensis	12	2.86 ± 0.16c	2.04–3.51	2.11 ± 0.10bc	1.70–2.73
T. sieboldii	10	2.90 ± 0.18cd	2.25–3.17	1.87 ± 0.11b	1.52–2.22
T. chi × *car*	14	3.38 ± 0.15d	1.48–4.69	2.40 ± 0.09c	1.71–3.13

Means in a column followed by different letters are significantly different ($P < 0.05$; Waller–Duncan range test).

dian budbreak dates of *T. chinensis* and *T. caroliniana* hybrids were not different from the budbreak date of *T. chinensis;* however, the median budbreak of *T. chinensis* and *T. sieboldii* hybrids was different from that of either parent. The hybrids of *T. chinensis* and *T. sieboldii* showed considerable range in budbreak, with about the same number of individuals in each quartile.

The rank order of the taxa (data in Figs. 2 and 3) gives the impression of a relationship between budbreak phenology and hemlock resistance. The density of live *A. tsugae* was lowest on *T. chinensis,* the taxon that broke bud earliest; the hybrids were intermediate in both adelgid density and date of budbreak, whereas adelgid density was highest on *T. canadensis, T. caroliniana,* and *T. sieboldii,* the taxa that broke bud last. However, budbreak was not significant ($P > 0.3$) in statistical models with density of live *A. tsugae* as the dependent variable and budbreak as a covariate with all taxa, with the three susceptible species only, or with the hybrid crosses only.

Discussion

In this study, *T. canadensis*, *T. caroliniana,* and *T. sieboldii* had high populations of *A. tsugae* (>8 per cm), whereas *T. chinensis* had extremely low populations (<0.2 per cm), and hybrids between *T. chinensis* and *T. caroliniana* or *T. sieboldii* had intermediate populations (0.5–2.0 per cm). McClure (1991) reported that—in a forest situation where *A. tsugae* was in its initial outbreak—the adelgid totally inhibited growth of new foliage the following spring, except on three trees where the density of *A. tsugae* was <4 per cm. With 4 per cm as a threshold, our results indicate that *T. chinensis* and its hybrids are resistant and *T. sieboldii* is susceptible. Two types of resistance were observed on *T. chinensis* and its hybrids: nonpreference (antixenosis), expressed as fewer adelgids settling on the plants, and antibiosis, expressed as slower growth and lower survival of the settled adelgids. For each density parameter, the hybrids were intermediate, but tended toward the resistant parent *T. chinensis,* which indicates dominant or partial dominant inheritance of resistance traits (Fritz 1999).

Although *T. sieboldii* is susceptible to the adelgid, this species is tolerant of infestations. In Japan, *A. tsugae* is generally sparse on hemlock in forests but does attain high densities on *T. sieboldii* in landscape plantings (McClure 1995). One of us (M.E.M.) has observed densities of *A. tsugae* >4 per cm on *T. sieboldii* in Japan but saw little damage to the trees. In the

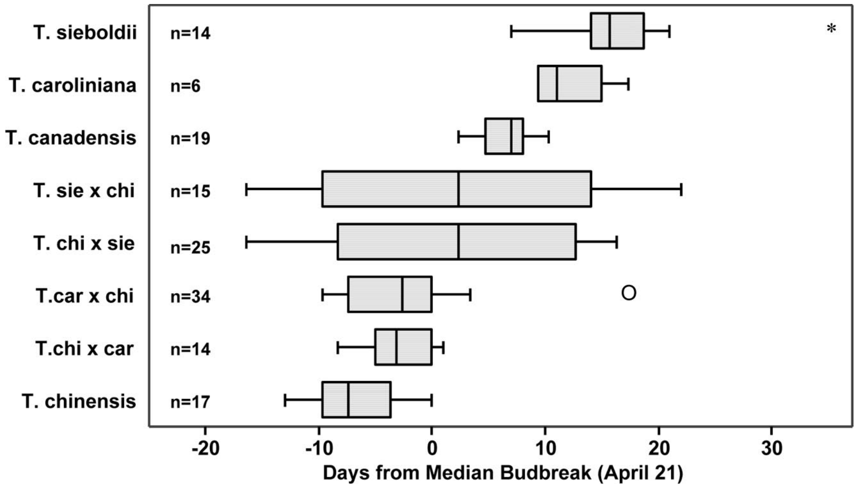

Fig. 3. Budbreak phenology for *Tsuga* species and their hybrid crosses in a field planting at USDA South Farm, Beltsville, MD. Boxplots show the median, a box around the central 50% of the values, and the range of data, excluding outliers (○) and extreme outliers (*).

eastern United States, we have observed *A. tsugae* at high densities on *T. sieboldii* in landscape plantings (S.E.B., personal observation) and in forested situations (M.E.M., personal observation). In both locations nearby *T. canadensis* with similar adelgid densities were in decline, whereas the *T. sieboldii* had considerable new growth and seemed healthy. The introduction of *A. tsugae* in eastern North America has been traced to populations in southern Japan collected from *T. sieboldii* (Havill et al. 2006). There are two distinct lineages of adelgid in Japan, one lineage associated with *T. sieboldii* and the other lineage associated with *T. diversifolia.* There are additional distinct lineages in China and in western North America (Havill et al. 2007). Although *T. sieboldii* and *T. chinensis* diverged from a common ancestor fairly recently (Havill et al. 2008), the adelgids in China diverged some time ago and are sufficiently distinct genetically to be considered separate species (Havill et al. 2007). Considering that the *A. tsugae* introduced to eastern North America is genetically similar to the *A. tsugae* on *T. sieboldii* in Japan, it is not surprising to see the same host relationship where they are both alien.

The hemlocks in eastern North America (*T. canadensis* and *T. caroliniana*) lack resistance or tolerance to *A. tsugae.* The traits that make these two species vulnerable probably evolved independently, because *T. caroliniana* shares a more recent common ancestor to the Asian species than to *T. canadensis* (Havill et al. 2008). Differences in ancestry may explain why *T. caroliniana* can hybridize with *T. chinensis* but not with *T. canadensis* and why *T. canadensis* cannot hybridize with *T. caroliniana* or any Asian species (Bentz et al. 2002). Although the two eastern North American hemlocks are not closely related, their terpenoid chemistry is remarkably similar (Lagalante and Montgomery 2003) and may have undergone similar, but independent, changes that provide protection against endemic defoliators, but left them vulnerable to *A. tsugae* (Lagalante et al. 2007). Havill and Montgomery (2008) provide additional perspective on worldwide phylogenetic relationships of *A. tsugae* and its hemlock hosts.

Although we did not find a relationship between date of budbreak and density of live adelgids, the relationship of budbreak phenology and *A. tsugae* survival should be examined further. The synchrony between budbreak and herbivory is often critical and varies with the specific insect and host association because nutrients are often highest in young tissues, but these may have higher secondary, defensive chemicals (Herms and Mattson 1992); hence, it is difficult to predict the role of phenology on insect host relationships. The silver fir adelgid, *Adelges nordmannianae* (Eckstein), progrediens generation was more abundant on the families of Nordman fir that broke bud later (Nielsen et al. 2002), whereas the balsam fir twig aphid, *Mindarus abietinus* Koch, did better on trees that broke bud earlier (Fondren and McCullough 2003). *A. tsugae* prefers to feed in mature tissue and change in terpenoid chemistry as the new hemlock growth matures has been related to its temporal feeding pattern (Lagalante et al. 2006). The very resistant *T. chinensis* breaks bud 2–3 wk earlier than the susceptible species T. *canadensis, T. caroliniana*, and *T. sieboldii. T. diversifolia,* another species reported to be resistant to *A. tsugae* (McClure 1992), broke bud ≈10 d earlier than *T. canadensis* in the South Farm, Beltsville, plantation (data not shown). There is some evidence that adelgids artificially infested on *T. canadensis* early in the year establish at a higher rate than adelgids artificially infested a few weeks later (Butin et al. 2007). Our infestations were done early—eggs were placed on the hemlocks when natural hatch was just beginning. Experiments using a series of infestation times on trees within a taxon with a wide range of budbreak may clarify the influence of budbreak on adelgids acceptance and survival on hemlock.

The significant maternal effect on resistance of *T. chinensis* hybrids to *A. tsugae* is important to note for future breeding efforts and the study of host plant resistance. Differential cytoplasm inheritance occurs in the Pinaceae, where mitochondria are maternally inherited and chloroplasts are paternally inherited (Hipkins et al. 1994). Although organelle inheritance has not been studied in *Tsuga,* it seems to follow the inheritance pattern found in other members of Pinaceae (Wang et al. 1997) In maize (*Zea mays* L.), significant maternal effects occur for the resistance of corn kernels to the maize weevil, *Sitophilus zeamais* Motschulsky, including the interaction between nuclear and cytoplasmic organelles (Dhliwayo et al. 2005). Further crosses, including the generation of F2 hybrids and backcrosses, are necessary to determine the exact mode of inheritance for resistance in hybrid *Tsuga.*

This study demonstrated that interspecific hybridization of *T. chinensis* with the susceptible species *T. caroliniana* and *T. sieboldii* is a promising method of producing hemlocks that are resistant to *A. tsugae* and have good growth characteristics. Additional hybridization to develop resistant hemlocks may be more efficient using *T. chinensis* as the female parent rather than the male parent. Clonal propagation of selected hybrids is proceeding so that plantings may be made at various locations. This will make it possible to examine, preferably under natural infestation, the three-way relationship between host plant, herbivore, and environment that is crucial to assessing host resistance. In the shorter term, we hope to more extensively challenge the hybrids and investigate their relative resistance to the sistens generation of the adelgid. We also will investigate the relative susceptibility of the hemlock taxa in the planting to scale insects. As our first field plantation of hybrids matures, we plan to evaluate the flowering phenology of the hybrids to determine whether there is potential to interbreed naturally with native hemlocks.

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