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False Ring Formation in Eastern Hemlock Branches: Impacts of Hemlock Woolly Adelgid and Elongate Hemlock Scale

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ABSTRACT Herbivores can alter plant physiology through the induction of abnormal wood formation. Feeding by some insects induces the formation of false rings, a band of thick-walled latewood cells within the earlywood portion of the tree ring that reduces water transport. Hemlock woolly adelgid (*Adelges tsugae* Annand) and elongate hemlock scale (*Fiorinia externa* Ferris) are invasive insects that both feed on eastern hemlock [*Tsuga canadensis* (L.) Carrière]. *Adelges tsugae* has a greater effect on tree health than *F. externa*, but the mechanism underlying their differential effect is unknown. We explored the effects of these herbivores by assessing growth ring formation in branches of trees that had been experimentally infested for 4 yr with *A. tsugae*, *F. externa*, or neither insect. We measured false ring density, ring growth, and earlywood: latewood ratios in the two most recently deposited growth rings. Branches from *A. tsugae*-infested trees had 30% more false rings than branches from *F. externa*-infested trees and 50% more than branches from uninfested trees. In contrast, branches from *F. externa*-infested trees and control trees did not differ in false ring formation. Radial growth and earlywood: latewood ratios did not differ among treatments. Our results show that two invasive herbivores with piercing-sucking mouth parts have differing effects on false ring formation in eastern hemlock. These false rings may be the product of a systemic plant hypersensitive response to feeding by *A. tsugae* on hemlock stems. If false rings are responsible for or symptomatic of hemlock water stress, this may provide a potential explanation for the relatively large effect of *A. tsugae* infestations on tree health.

KEY WORDS *Adelges tsugae*, *Fiorinia externa*, *Tsuga canadensis*, herbivory, false rings

Herbivores can alter plant physiology directly through tissue and nutrient removal and indirectly through the induction of increased chemical (Bezemer et al. 2003, Kaplan et al. 2008), morphological defenses (Levin 1973), or both. Although such responses vary between herbivores, alterations in plant physiology are especially likely with invasive or other species that reach high densities on their host plants (Sakai et al. 2001). Although herbivore-induced changes to plant structure are most commonly thought to involve architectural shifts resulting from bud/branch mortality or altered height and radial increments (Traw and Dawson 2002, Sopot et al. 2003), herbivory also may induce changes in woody plant tissues in the stems of conifers and other woody plants (Fernandes 1990).

In conifers, false rings are thick-walled xylem cells that appear as dark bands of latewood flanked on both sides with earlywood (Copenheaver et al. 2006). False rings occur within an annual ring but, although they resemble the end of an annual ring, do not occur on a yearly or seasonal basis. Normal rings are composed of large, thin-walled cells formed early in the growing season and small, thick-walled cells formed later in the

year. These true rings are characterized by an abrupt increase in cell size at the start of the new growing season, whereas false rings are identified by a slow increase in cell diameter and decrease in cell wall thickness adjacent to the false ring (Copenheaver et al. 2006). Like compression wood, false rings have thick-walled xylem cells that increase resistance to water flow (Bolton and Petty 1978). False rings are associated with water stress and insect infestation, and have been observed in conifers such as *Pinus sylvestris* L. and *P. banksiana* Lamb. (Hollingsworth and Hain 1992, Cherubini et al. 2003, Copenheaver et al. 2006). Drought may induce false rings by reducing photosynthesis and stopping cambial activity during the summer (Cherubini et al. 2003). During periods of water stress, small, thick-walled cells are formed in the wood; if conditions become more favorable, subsequent cells will be larger with thinner walls (Wimmer et al. 2000). This alternation in cell size may appear as a false ring. In support of this, Wimmer et al. (2000) found that false rings were associated with periods of alternating wet and dry months.

Although not all herbivores induce changes in ring formation (Priya and Bhat 1997, Hejari et al. 2010), certain insects have been linked to their occurrence. Increased densities of the balsam woolly adelgid [*Adelges piceae* (Ratzeburg)] are correlated with the

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formation of rotholz rings, a type of abnormal wood similar to compression wood, in Fraser fir [*Abies fraseri* (Pursh) Poir.] (Hollingsworth and Hain 1992), and rotholz rings are only found near areas of adelgid feeding. Because these rings contain cells that do not conduct water and balsam woolly adelgid feeding also is associated with an increase in nonconducting heartwood (Arthur and Hain 1986), the resulting water stress eventually may kill the tree (Hollingsworth and Hain 1991). The formation of rotholz rings may defend against low-density adelgid infestations by forming necrotic tissue around the feeding site that isolates and starves the insects (Arthur and Hain 1985). With many points of adelgid feeding, however, so much of the stem may become nonconductive that the increased water stress actually kills the tree (Arthur and Hain 1985, McClure 1988).

The hemlock woolly adelgid [*Adelges tsugae* Annand (Hemiptera: Adelgidae)] is an invasive hemipteran herbivore that is causing high mortality of eastern hemlock [*Tsuga canadensis* (L.) Carrière] across the eastern United States (Orwig et al. 2002). *Adelges tsugae* first was reported in Virginia in the 1950s (Souto et al. 1996) and since has spread rapidly along the east coast, now ranging from northern Georgia to Maine (McClure and Cheah 1999, USFS 2008). *Adelges tsugae* completes two generations per year in its invaded range (McClure 1989), and feeds on eastern hemlock at the base of the needle petiole by inserting its stylet bundle into xylem ray parenchyma tissue (Young et al. 1995). Feeding by *Adelges tsugae* devastates hemlock stands; mature hemlocks infested with *A. tsugae* can die within 4 yr of infestation (McClure 1991), and seedlings appear similarly vulnerable (Preisser et al. 2011).

The elongate hemlock scale [*Fiorinia externa* Ferris (Hemiptera: Diaspididae)] is another invasive sessile herbivore that feeds on eastern hemlock (McClure 1978). *Fiorinia externa* arrived in New York in the early 1900s but remained in the mid-Atlantic until the 1970s, when its range and population density began increasing rapidly. It can now be found in over 14 states from northern Georgia to southern Maine (McClure 1978, Preisser and Elkinton 2008, Abell 2010). *Fiorinia externa* produces two generations per year (as it does in its native range) in southern states, but only one generation per year in New England (McClure 1978, Abell 2010). It feeds on mesophyll cells from its location on the underside of the needles and reduces hemlock growth while causing needle discoloration and loss (McClure 1980). Although *A. tsugae* infestation usually is lethal to hemlocks, *F. externa* infestation only results in tree death with heavy, sustained infestations (McClure 1980, 1991). Experimental and survey work has confirmed that *A. tsugae* is much more harmful than *F. externa* to eastern hemlock (Preisser and Elkinton 2008, Preisser et al. 2008, Miller-Pierce et al. 2010).

Although little is known about why these species differ in their impact, there is evidence that *A. tsugae* induces an especially pronounced hypersensitive response in the tree (Radville et al. 2011). The hyper-

sensitive response is a plant defense response that increases the levels of reactive oxygen species such as superoxide anions, hydroxyl radicals, and hydrogen peroxide (H_2O_2), thereby inducing cell death in herbivore-colonized areas to isolate and starve feeding organisms (Heath 2000, Liu et al. 2010). The cue for this response is often the presence of a foreign substance indicative of herbivore feeding (reviewed in Cornelissen et al. 2002), and the ensuing localized tissue death is a particularly effective response to sessile herbivores (Karban and Baldwin 1997). This response has been shown to reduce plant damage caused by balsam woolly adelgids, bark beetles, and a host of other herbivore species (Fernandes 1990, Ollerstam and Larsson 2003). In the case of *A. tsugae*, Radville et al. (2011) found that infestation caused a larger localized hypersensitive response (measured as an increase in H_2O_2 concentrations) than was present in either *F. externa*-infested or uninfested trees. Although both *F. externa* and *A. tsugae* produced a localized hypersensitive response, *A. tsugae* infestation also caused a systemic hypersensitive response not observed in the other treatments. Hypersensitivity has been linked to abnormal wood formation and water stress in other adelgid-conifer interactions (Arthur and Hain 1985), and Gómez et al. (2012) found that *A. tsugae*-infested trees had increased levels of proline, an amino acid associated with drought conditions. Recent research (Walker-Lane 2009) on mature hemlocks in the field found significantly more false rings in *A. tsugae*-infested hemlocks than in hemlocks treated with insecticide. Walker-Lane (2009) also determined that the *A. tsugae*-infested hemlocks were drought stressed, but was unable to establish whether *A. tsugae* caused this or simply was more abundant on drought-stressed trees. Because of the insects' differing ability to induce a systemic hypersensitive response in eastern hemlock, we hypothesize that *A. tsugae* infestation will induce a greater degree of false ring formation than will infestation with *F. externa* or neither insect (control). Through this research, we hope to provide insight into why feeding by *A. tsugae* is much more damaging than feeding by *F. externa*.

Methods

In April 2007, we collected uninfested *T. canadensis* saplings 0.7–1 m in height from Cadwell Forest (Pelham, MA), a forest research facility managed by the University of Massachusetts, Amherst. This site was past the northern range limit of both *A. tsugae* and *F. externa* at the time the plants were collected, and careful examination before collection confirmed that neither insect was present on any of the saplings or in the surrounding stand. The trees were planted at East Farm, a research facility managed by the University of Rhode Island (Kingston, RI), in a rectangular grid in an open field. The experiment used a randomized complete block design, with each row containing each treatment. The experimental grid originally contained eight rows (spaced 4 m apart) and 13 trees per row (spaced 2 m apart); most of the trees in each row were

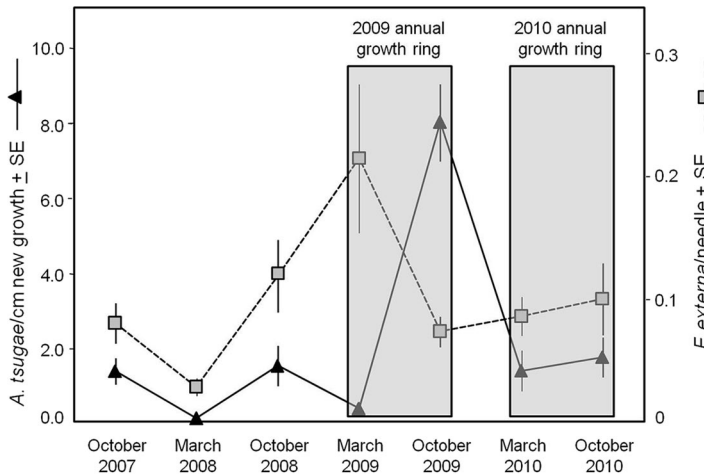


Fig. 1. Densities of the hemlock woolly adelgid *A. tsugae* (left axis, triangular symbols) and the elongate hemlock scale *F. externa* (right axis, square symbols) at the whole-tree level in the *A. tsugae*-only and *F. externa*-only treatments over the course of the experiment. March sampling was conducted before yearly tree inoculations and measures the number of surviving overwintered adults in each treatment. Gray bars indicate the approximate formative period for the 2009 and 2010 annual growth rings; the space between the bars corresponds to the winter period of hemlock dormancy. NOTE: These data were initially presented in online resource one in Gómez et al. (2012), and are reprinted here to confirm that insects were present in each treatment throughout the experiment.

used in an unrelated experiment (Miller-Pierce et al. 2010). Within each row, the trees used in our experiment were assigned randomly to one of three treatments: *A. tsugae* only, *F. externa* only, or neither herbivore (control). Trees in the herbivore treatments were inoculated using hemlock foliage infested with *A. tsugae* or *F. externa* each spring from 2007 to 2011, five yearly infestations in total, using standard protocols (Butin et al. 2007). Details on the experimental design, including the source locations of the herbivore populations used in the inoculation, the precise timing of infestation, and the infestation protocols are reported elsewhere (Miller-Pierce and Preisser 2010). Insect densities in each treatment were recorded in the fall and spring of each year to confirm that each insect was present throughout the experiment (Fig. 1). To minimize cross-contamination between treatments, each tree was enclosed in a 1- by 1- by 2-m (length \times width \times height) plastic PVC pipe frame cage covered with mosquito netting (100 holes/cm² mesh size; Barre Army-Navy, Barre, VT). Although the experiment began with a fully-balanced design, over the 4-yr study a combination of transplant shock, herbivore-induced mortality, and cross-contamination reduced the number of replicates per treatment to eight no-herbivore trees (controls), seven *F. externa*-only trees, and six *A. tsugae*-only trees, for 21 experimental replicates in total.

On 31 May 2011, we collected one branch (\approx 0.6 cm in diameter) per cardinal direction from each of the 21 experimental trees (84 branches in total). After their removal from the tree, each branch immediately was submerged in deionized water. After 24 h, we mounted the base of the branch on a sliding microtome and cut two 60- μ m sections per branch. Sections were placed in a 0.1% safranin O stain solution for

3 min, rinsed for 2 min and mounted on slides. We counted the number of false rings formed in the last 2 yr of branch growth (2009 and 2010) by using a light microscope. Our decision to assess the most recent 2 yr of branch growth was motivated by the fact that although branches varied in age, all of them had at least two distinct growth rings. Each growth ring consisted of both earlywood and latewood; the two wood types easily were distinguishable by their distinctive colors and by the smaller, thick-walled cells characteristic of latewood. False rings easily were identifiable using standard diagnostic characteristics (i.e., a gradual change from earlywood to a band of latewood with earlywood on the opposite side) (Copenheaver et al. 2006). After counting the false rings in the 2009 and 2010 growth rings, we photographed each section (Fig. 2) and used imageJ 1.44 (Abràmoff et al. 2004) to measure the total number of annual growth rings, the branch radius, and the thickness of the earlywood and latewood in the 2009 and 2010 tree rings of each branch. Because every section was asymmetrical, we measured branch radius in three different axes and averaged them to calculate a mean branch radius. We followed the same procedure to calculate the mean thickness of the 2009 and 2010 growth rings and the mean thickness of latewood in the 2009 and 2010 tree rings of each branch. We calculated the width of each ring's earlywood by subtracting the latewood thickness from the mean ring width, and the earlywood:latewood ratio by dividing the thickness of each ring's earlywood by its latewood.

Statistical Analysis. The unit of replication for our analyses was the mean response per tree per treatment (21 replicates). Data were square-root transformed when necessary to improve normality; variances were homogenous between treatments. When analyzing

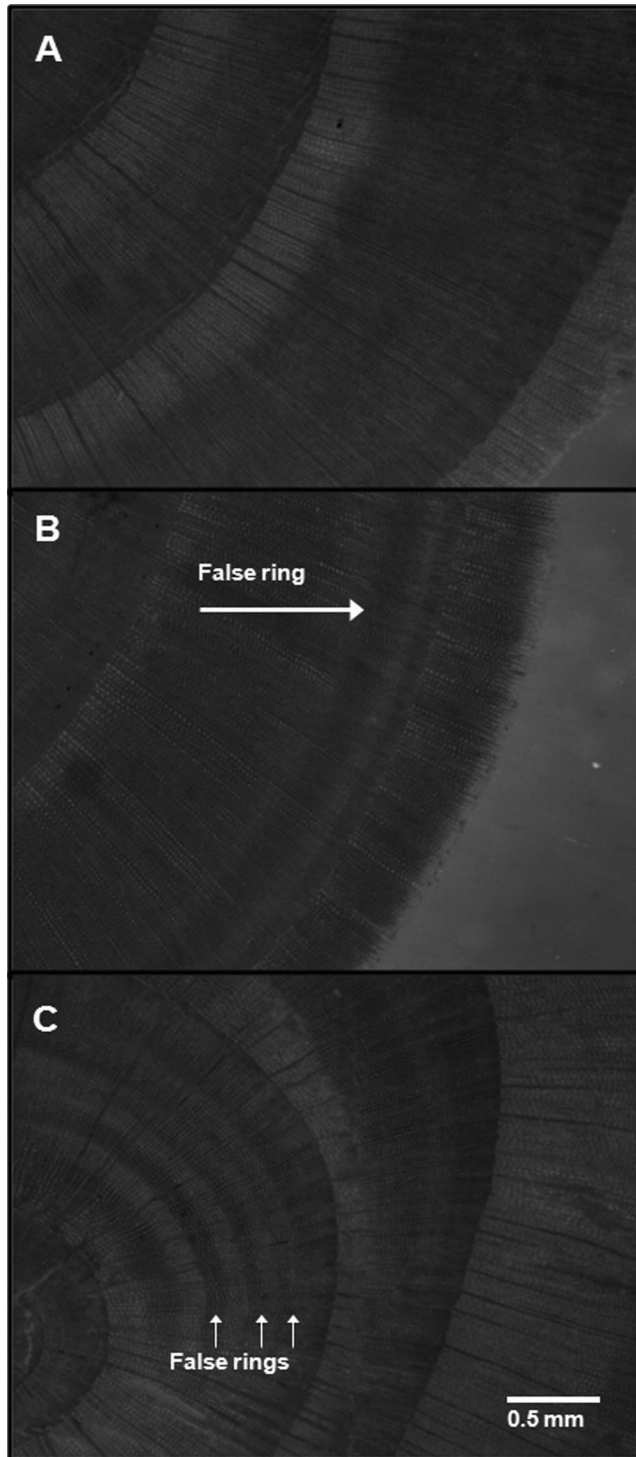


Fig. 2. Panel (A): Cross-section of *T. canadensis* branch. Earlywood is distinguishable by larger thin-walled earlywood cells (located in the left side of each growth ring) and latewood is distinguishable by smaller thick-walled latewood cells (located in the right side of each growth ring). Panel (B): Cross-section of *T. canadensis* branch with false ring (indicated by arrow) located to the left of the true ring. The false ring is a diffuse band of latewood flanked by earlywood. In contrast, the true ring (located to the right of the indicated false ring) is identified by the abrupt alteration from latewood cells in the prior years' growth to earlywood cells. Panel (C): Cross-section of *T. canadensis* branch with multiple false rings (indicated by arrows). All photographs were taken using a PL-A662 camera (Pixelink, Ottawa, Ontario, Canada) attached to an SZX12 microscope (Olympus, Center Valley, PA) at 400 \times magnification.

Table 1. Mean branch radius, number of annual growth rings, earlywood width in 2009 and 2010 growth rings, latewood width in 2009 and 2010 growth rings, ring width and earlywood: latewood ratio in 2009 and 2010 growth rings (± 1 SD) of uninfested trees, *F. externa*-infested trees, or *A. tsugae*-infested trees

	Control	<i>F. externa</i>	<i>A. tsugae</i>
Branch radius (cm)	0.313 (0.074)	0.296 (0.050)	0.290 (0.078)
Number of annual growth rings	4.792 (2.340)	5.097 (1.777)	4.964 (1.666)
Earlywood width 2009 (cm)	0.041 (0.023)	0.034 (0.015)	0.037 (0.023)
Earlywood width 2010 (cm)	0.054 (0.029)	0.050 (0.018)	0.052 (0.029)
Latewood width 2009 (cm)	0.050 (0.022)	0.039 (0.020)	0.037 (0.017)
Latewood width 2010 (cm)	0.063 (0.030)	0.056 (0.025)	0.055 (0.027)
Ring width 2009 (cm)	0.091 (0.033)	0.073 (0.024)	0.074 (0.031)
Ring width 2010 (cm)	0.117 (0.035)	0.105 (0.025)	0.108 (0.037)
Earlywood: latewood 2009 (cm)	0.956 (0.751)	1.171 (0.958)	1.110 (0.626)
Earlywood: latewood 2010 (cm)	1.100 (0.859)	1.190 (0.852)	1.233 (0.864)

There were no significant differences across treatments in all variables.

data on branch diameter and total annual growth rings, we used analysis of variance (ANOVA) to test for the main effects of treatment (*A. tsugae*-only, *F. externa*-only, and control) and location within the experimental grid (included as a blocking variable), and for their two-way interaction. All other data were analyzed using repeated measures ANOVA to test for the main effects of treatment, location, time (either the 2009 or 2010 growth ring), and their interactions. We performed means separation tests, where appropriate, by using Tukey’s honestly significant difference (HSD). Statistical analyses were performed using JMP 9.0.0 (SAS Institute 2010). When initial *P* values are significant, we report both the initial *P* value as well as the *P* value corrected for multiple comparisons at $\alpha = 0.05$ using step-up FDR, a sequential Bonferroni-type procedure (Benjamini and Hochberg 1995).

Results

Branch Size and Age. There were no treatment-level differences in either branch radius or age, measured as the number of annual growth rings (Tables 1, 2A-B). Branches averaged 0.61 ± 0.036 [SE] cm in diameter and had similar numbers of annual growth rings. There was no effect of tree location within the experimental grid (Tables 2A-B), and no significant treatment*location interactions.

False Ring Density. *Adelges tsugae*-infested branches had significantly more false rings than either *F. externa*-infested or control branches (Table 3A; Fig. 3). *Adelges tsugae*-infested trees averaged 0.96 false rings per growth ring, significantly more than in either control or *F. externa*-infested trees (0.48 and 0.66,

respectively; Tukey’s HSD, $P < 0.05$). In contrast, *F. externa*-infested trees did not differ from the uninfested controls (Fig. 3). There was a marginally significant effect of tree location within the experimental grid (Table 3A), but no significant change in false ring density across time (Table 3A). All two- and three-way interactions were nonsignificant (Table 3A).

Earlywood, Latewood, and Ring Width. There were no treatment-level differences in the width of earlywood (Tables 1, 3B), latewood (Tables 1, 3C), or the annual rings (Tables 1, 3D). There also was no effect of treatment on the earlywood: latewood ratio (Tables 1, 3E). These four variables did not change over time and were unaffected by tree location within the experimental grid (Tables 1, 3B-E). There were no significant two- or three-way interactions (Tables 1, 3B-E).

Discussion

Although both insects have piercing-sucking mouth parts, infestation by *F. externa* and *A. tsugae* had markedly different effects on wood formation. Branches from *A. tsugae*-infested trees had a greater number of false rings than branches from uninfested trees (Fig. 3). Branches infested with *A. tsugae* had 50% more false rings than control branches and 30% more false rings than *F. externa*-infested trees. In contrast, infestation by *F. externa* did not significantly increase false ring formation. Despite the difference in false ring formation, there were no between-treatment differences in annual ring width or earlywood and latewood production.

The presence of false rings in all three treatments suggests that environmental factors can influence false

Table 2. ANOVA table for the treatment effects on branch size (Panel A) and age (Panel B)

Test	Exact F	Numerator DF	Denominator DF	Prob>F
A. Branch radius				
Treatment	0.287	2	15	0.755
Location	0.026	1	15	0.874
Treatment*location	0.181	2	15	0.836
B. Annual growth rings				
Treatment	0.099	2	15	0.906
Location	3.039	1	15	0.102
Treatment*location	0.735	2	15	0.496

Table 3. rm-ANOVA table for the effects of treatment, location, and time on false ring density (Panel A), earlywood width (Panel B), latewood width (Panel C), radius width (Panel D), and earlywood: latewood ratio (Panel E)

Test	Exact F	NumDF	DenDF	Prob>F
A. False Ring Density				
Treatment	7.125	2	15	0.007
Location	3.430	1	15	0.084
Treatment*Location	0.892	2	15	0.431
Time	0.276	1	15	0.607
Time*treatment	0.129	2	15	0.880
Time*location	0.040	1	15	0.844
Time*treatment*location	0.042	2	15	0.959
B. Earlywood width				
Treatment	0.208	2	15	0.815
Location	1.443	1	15	0.248
Treatment*location	1.391	2	15	0.279
Time	1.488	1	15	0.241
Time*treatment	0.025	2	15	0.976
Time*location	0.603	1	15	0.450
Time*treatment*location	0.472	2	15	0.633
C. Latewood width				
Treatment	0.692	2	15	0.516
Location	0.049	1	15	0.828
Treatment*location	1.132	2	15	0.348
Time	2.317	1	15	0.149
Time*treatment	0.279	2	15	0.760
Time*location	0.787	1	15	0.389
Time*treatment*location	1.639	2	15	0.227
D. Annual ring width				
Treatment	0.596	2	15	0.564
Location	0.364	1	15	0.556
Treatment*location	0.206	2	15	0.816
Time	1.894	1	15	0.189
Time*treatment	0.151	2	15	0.861
Time*location	1.286	1	15	0.275
Time*treatment*location	1.482	2	15	0.259
E. Earlywood: latewood ratio				
Treatment	0.125	2	15	0.884
Location	1.267	1	15	0.278
Treatment*location	2.086	2	15	0.159
Time	0.154	1	15	0.700
Time*treatment	0.419	2	15	0.665
Time*location	0.074	1	15	0.789
Time*treatment*location	0.199	2	15	0.822

ring formation in eastern hemlock. Dry conditions and drought stress have been associated with the formation of false rings in several other conifer species

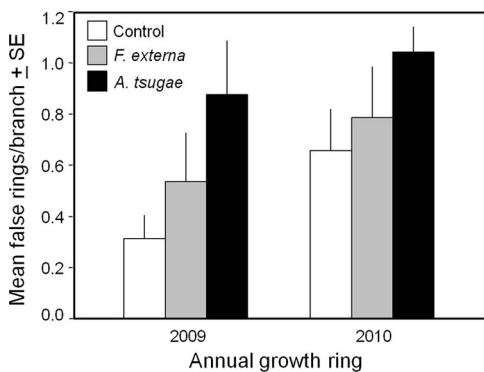


Fig. 3. Mean \pm 1 SE false rings per treatment in each of the 2009 and 2010 annual growth rings. Branches from trees in the *A. tsugae*-only treatment had significantly more false rings than branches from trees in either the *F. externa*-only or control treatments.

(Wimmer et al. 2000, Copenheaver et al. 2006, Hoffer and Tardif 2009). Although our experiment was not set up to test this hypothesis, our results nonetheless suggest that eastern hemlock may respond similarly. The highest densities of false rings occurred in summer 2010, a period that was much warmer and drier than summer 2009 (NOAA 2011). In 2009, Kingston, RI experienced its third wettest summer (43.1 cm of rainfall) since 1895, with an average temperature of 20.5°C and 7 d in which the temperature exceeded 30°C. In contrast, summer 2010 had one-third less rainfall (28.2 cm) but was the hottest summer since 1895, with an average temperature of 22.4°C and 17 d over 30°C (NOAA 2011). Although it cannot be tested, the substantial climatic differences between the two summers provide a plausible explanation for the overall increase in the number of false rings in 2010.

Although environmental parameters undoubtedly are important, *A. tsugae*-infested branches still contained significantly more false rings than branches from either *F. externa* or control trees. This finding corroborates observations made on mature eastern and Carolina hemlock (Walker-Lane 2009). Walker-Lane (2009) noted an association between false ring formation and *A. tsugae* infestation, but was unable to determine whether this association was correlative or causative. By experimentally infesting trees with *A. tsugae* and *F. externa* and observing the resulting changes in wood anatomy, our work confirms that *A. tsugae* infestation is responsible for an increase in false ring formation.

Perhaps the most likely explanation for our findings is that the increased number of false rings in *A. tsugae*-infested branches is a consequence of plant hypersensitivity, a defense mechanism against sessile herbivores and pathogens (Fernandes 1990). The hypersensitive response induces cell death by increasing the reactive oxygen species (Heath 2000), which isolates the herbivore or pathogen and prevents it from establishing a suitable nutritional site (Wong and Berryman 1977, Fernandes 1990, Bonello et al. 2006). At low herbivore densities, a successful hypersensitive response can increase plant resistance; if herbivores survive and increase to high densities, however, the response could become lethal to the host plant (Bi and Felton 1995, Bonello et al. 2006). Radville et al. (2011) demonstrated *A. tsugae* infestation stimulated both a localized and systemic hypersensitive response in needles adjacent to *A. tsugae* settlement, whereas *F. externa* infestation only induced a localized response. In North American fir trees, the formation of rotholz rings is associated with the hosts' hypersensitive response to feeding by the balsam woolly adelgid (Arthur and Hain 1985). In such cases, the increased production of nonconductive rotholz rings may serve to isolate the wounded tissue where the balsam woolly adelgid feeds. Although this is an effective defense at low densities, high adelgid densities cause the stem to become nonconductive and can kill the tree (Arthur and Hain 1985). Although the preceding applies specifically to rotholz and it is unknown whether false rings are related to rotholz formation, false rings also

are known to be indicative of drought stress, and recent research suggests *A. tsugae*-infested hemlocks have lower transpiration rates and increases in $\delta^{13}\text{C}$, both symptoms of drought stress (Walker-Lane 2009). In addition, Gómez et al. (2012) showed that *A. tsugae*-infested trees contained higher levels of proline, an amino acid indicative of water stress, than uninfested control trees. In such a scenario, feeding by *A. tsugae* would cause a systemic hypersensitive response that alters wood anatomy, disrupting water transport, and increasing water stress. It is also possible that *A. tsugae* infestation may directly (i.e., without the involvement of plant hypersensitivity) increase water stress in eastern hemlocks. If *A. tsugae* infestation intensifies the degree of water stress experienced by hemlocks, the resulting drought-like conditions may increase false ring formation.

The most likely explanation for the fact that *F. externa* did not induce false ring formation involves this species' feeding location. Although *A. tsugae* feeds at the base of the needle petiole on xylem ray parenchyma cells (Young et al. 1995), *F. externa* feeds directly on the underside of the needle on mesophyll cells (McClure 1980). Although located in close physical proximity to each other, *F. externa* is thus a foliar feeder, whereas *A. tsugae* is a stem feeder. Although defensive responses to *F. externa* could be confined to foliar tissue, similar responses to *A. tsugae* feeding would be much more likely to affect cambial growth (and thus water transport). This argument is compatible with the idea that, by virtue of its role as a stem feeder, *A. tsugae* induces a systemic hypersensitive response in cambial tissue that ultimately affects false ring formation and water transport. The decreased response of the plant to *F. externa* feeding also could be because of species-specific differences in *A. tsugae* and *F. externa* densities. *Adelges tsugae* has two generations per year in New England, whereas *F. externa* only has one (McClure 1978). It should be noted, however, that whole-tree *A. tsugae* densities were consistently lower than *F. externa* densities for the duration of the study (Fig. 1), a fact that suggests even low *A. tsugae* densities induce a greater degree of false ring formation than higher *F. externa* densities. Although unproven, it also has been suggested that a component of *A. tsugae* saliva is 'toxic' (Young et al. 1995) and that it injects chemicals during feeding that adversely affect plant health (Preisser and Elkinton 2008). If true, this toxic substance could also help explain why *A. tsugae* induces false ring formation and *F. externa* does not.

Despite finding differences in the number of false rings, we did not find differences in other variables. This fact may be because of our relatively low degree of within-treatment replication; although we began our experiment with a higher degree of replication, sapling mortality and cross-contamination over the 4-yr course of the experiment reduced our numbers. Another issue concerns microclimate: although all of our hemlocks were enclosed in fine-mesh mosquito netting that acted as shade cloth, it is nonetheless likely that they experienced hotter and drier condi-

tions than hemlocks growing in the shaded forest understory. Although our decision to grow the trees at East Farm was motivated by the need for deer fencing and accessibility to a water source (trees were watered in the summer of 2007 to reduce transplant-related mortality), our findings highlight the need for similar experiments under closed-canopy conditions.

The occurrence of false rings in *A. tsugae*-infested trees may provide insight into the detrimental effect of *A. tsugae* on tree health. Regardless of the mechanism behind the false ring formation, their presence restricts water flow. False rings are anatomically similar to compression wood in that they both have thick-walled xylem cells that increase resistance to the flow of water through xylem tissue (Bolton and Petty 1978). Compression wood conducts water less efficiently than does normal wood (Spicer and Gartner 1998), and there is also evidence that insect-induced false rings impede water transport. Mitchell (1967) found that trees infested by the balsam woolly adelgid absorbed and transported less dye (a proxy for water) than uninfested subalpine and grand fir trees. Rotholz rings appeared to inhibit dye transport and infested trees had half as many conducting tree rings (Mitchell 1967). Puritch (1971) showed that balsam woolly adelgid interfered with the water conduction in grand fir, evident in the reduced permeability of sapwood in balsam woolly adelgid-infested trees. Because balsam woolly adelgid and its hosts are closely related to *A. tsugae* and eastern hemlock, it seems reasonable to assume that false rings formed in *A. tsugae*-infested eastern hemlock will correlate with changes in water transport efficiency (an idea first suggested by Walker-Lane 2009). If the false rings produced in *A. tsugae*-infested trees are indicative of water stress, this may explain why *A. tsugae* has such a severe impact on tree mortality.

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