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Failure under stress: the effect of the exotic herbivore *Adelges tsugae* on biomechanics of *Tsuga canadensis*

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• *Background and Aims* Exotic herbivores that lack a coevolutionary history with their host plants can benefit from poorly adapted host defences, potentially leading to rapid population growth of the herbivore and severe damage to its plant hosts. The hemlock woolly adelgid (*Adelges tsugae*) is an exotic hemipteran that feeds on the long-lived conifer eastern hemlock (*Tsuga canadensis*), causing rapid mortality of infested trees. While the mechanism of this mortality is unknown, evidence indicates that *A. tsugae* feeding causes a hypersensitive response and alters wood anatomy. This study investigated the effect of *A. tsugae* feeding on biomechanical properties at different spatial scales: needles, twigs and branches.
• *Methods* Uninfested and *A. tsugae*-infested samples were collected from a common garden experiment as well as from naturally infested urban and rural field sites. Tension and flexure mechanical tests were used to quantify biomechanical properties of the different tissues. In tissues that showed a significant effect of herbivory, the potential contributions of lignin and tissue density on the results were quantified.
• *Key Results Adelges tsugae* infestation decreased the abscission strength, but not flexibility, of needles. *A. tsugae* feeding also decreased mechanical strength and flexibility in currently attacked twigs, but this effect disappeared in older, previously attacked branches. Lignin and twig tissue density contributed to differences in mechanical strength but were not affected by insect treatment.
• *Conclusions* Decreased strength and flexibility in twigs, along with decreased needle strength, suggest that infested trees experience resource stress. Altered growth patterns and cell wall chemistry probably contribute to these mechanical effects. Consistent site effects emphasize the role of environmental variation in mechanical traits. The mechanical changes measured here may increase susceptibility to abiotic physical stressors in hemlocks colonized by *A. tsugae*. Thus, the interaction between herbivore and physical stresses is probably accelerating the decline of eastern hemlock, as HWA continues to expand its range.

Key words: Biomechanics, *Tsuga canadensis*, eastern hemlock, *Adelges tsugae*, HWA, herbivory, invasive, wood density, lignin, tensile strength, flexural strength.

INTRODUCTION

Plants and insect herbivores often have a long history of co-evolution. Over time, insects develop the ability to efficiently remove nutrients from plant tissues, and the plants in turn develop toxins and other deterrents to help protect against herbivore damage (Strauss and Agrawal, 1999; Gatehouse, 2002; Xiang and Chen, 2004; Núñez-Farfán, 2007). Relationships between specialist herbivores and their host plants can lead to complex coevolutionary arms races (Agrawal, 2000; Ruuhola *et al.*, 2001). However, in the case of exotic species, this system of coadaptation is circumvented (Gandhi and Herms, 2010). An exotic herbivorous insect may encounter naive plant species, allowing the insect to rapidly increase its population and become invasive. In such a system the host plants may experience severe damage and population decline (reviewed in Gandhi and Herms, 2010).

Furthermore, the type of insect feeding can affect the severity of damage sustained by the plant (*sensu* Walling, 2000; also see Preisser and Bastow, 2006). Leaf-chewing herbivores can cause extensive cell damage and induce defensive responses, yet their effects on plant fitness are often limited (Karban and Baldwin, 1997; Orians *et al.*, 2011). Compared with leaf-chewing herbivores, piercing–sucking herbivores often have larger effects on plant fitness (Meyer, 1993; Zvereva *et al.*, 2010). These insects target phloem or parenchyma cells that are critical to nutrient transport and long-term storage (van Bel, 1990) and, by avoiding large-scale rupture, can prevent rapid plant defensive signalling (Walling, 2008; Heidel and Baldwin, 2004; Kaloshian and Walling, 2005). Thus, high densities of an invasive piercing–sucking specialist herbivore that targets stem tissues should therefore present a worst-case herbivory scenario, as the insects thrive even on highly stressed plants (Koricheva and Larsson, 1998).

The hemlock woolly adelgid (*Adelges tsugae*, HWA) is an exotic hemipteran that presents one example of this narrative. HWA is decimating stands of eastern hemlock (*Tsuga canadensis*) in its invaded range, the hemlock forests of the eastern USA.

Resistance to HWA is very low in *T. canadensis* (McClure, 1995), and as a consequence entire stands are dying back and new hemlock recruitment in infested stands is minimal (Orwig *et al.*, 2002; Preisser *et al.,* 2011). Upon infestation, HWA rapidly decreases eastern hemlock growth (McClure, 1991), alters foliar nitrogen content (Miller-Pierce *et al.*, 2010; Gómez *et al.*, 2012) and induces a hypersensitive response (Radville *et al.*, 2011) and increased needle loss (Stadler, 2005).

One proposed mechanism for the severe impact of HWA on eastern hemlock is that a cumulative hypersensitive response across thousands of attacked sites induces a general stress response, leading to altered wood quality (Radville *et al.*, 2011). Evidence for phytotoxicity is partially supported by elevated peroxide levels (indicative of a hypersensitive response), found in both the infested and distal needles of HWA-infested trees (Radville *et al.*, 2011). This hypersensitive response appears to lead to changes in wood anatomy, in that branches of HWA-infested trees form more false rings [an abnormal band of thick-walled latewood cells that reduce water transport (Bolton and Petty, 1978)] than branches of uninfested trees (Gonda-King *et al.*, 2012; Domec *et al.*, 2013). Similarly, infestation of *Abies balsamea* by balsam woolly adelgid (*Adelges piceae*) has been shown to cause abnormal xylem resembling compression wood (which is characterized by thickened cell walls, lignification, a decrease in water conductivity and increased wood brittleness) (Balch *et al.*, 1964). Additionally, in low-vigour (Skene, 1972) and HWA-infested (Walker, 2012) eastern hemlock, latewood tracheid cell walls become thinner and the cell diameter decreases, likely with biomechanical consequences such as weakened branches. It is also possible that HWA induces lignification (Davin and Lewis, 2005; Liu *et al.*, 2007), which could increase wood brittleness.

We hypothesize that HWA feeding alters the mechanical properties of stems, including enhanced lignification and the thinning of xylem cell walls. Because the damage can lead to osmotic stress, we expect this to lead to premature needle abscission and increased branch and twig brittleness. In this study we used biomechanical measurements to test this potential mechanism. We studied the biomechanics of HWA-infested trees by measuring tissue responses to mechanical loading, under the hypothesis that HWA feeding causes weaker and less flexible responses to mechanical forces in the needles, twigs and branches.

To quantify the effects of HWA on eastern hemlock biomechanics under a wide range of infestation histories, we used branches from trees at multiple sites. We selected a forested site and an urban site to include hemlocks grown in different abiotic environments. Additionally, to control for within-site variation and the history of infestation among trees, we sampled from a common garden experiment.

MATERIALS AND METHODS

Study sites

We chose three study locations based on infestation history, environmental conditions and site types, taking account of greater levels of environmental heterogeneity and allowing extrapolation to hemlock stands in nature. Shade conditions in particular can alter plant structure by affecting the balance between resource allocation to light capture and biomechanical support (Givnish, 1995). In 2011 and 2012, we sampled from two naturally infested sites, a rural and an urban site. The rural site, located at Mt Tom State Reservation in Holyoke, MA, is a shaded forest stand typical of hemlock habitat, with a well-established history of HWA infestation (Orwig *et al.*, 2002; Preisser *et al.*, 2008, 2011) and a high density of downed foliage in the infested regions (C. Orians, personal observation). The urban site, a sunny field at Tufts University in Medford, MA, has a mix of ornamental trees. Additionally, we sampled from a common garden experiment established in 2007 (uninfested hemlock saplings from Peltham, MA) at an experimental farm at the University of Rhode Island (Kingston, RI). These seedlings were then inoculated with either HWA or control foliage following standard protocols (Butin *et al.*, 2007) over the course of four growing seasons. Trees were caged in individual PVC enclosures covered by mosquito netting to prevent cross-contamination between treatments. Further details of the establishment and maintenance of the common garden are described elsewhere (Miller-Pierce *et al.*, 2010).

Sample collection

We collected samples of the following three tissue types at 1–1·5 m height from each tree: branches (5 mm diameter, ∼3–4 years old), and the youngest twigs (1 mm diameter, 10 months old) and their corresponding needles. We assessed branch age by leaf scar nodes. Table 1 summarizes the type and number of samples collected.

For all twig and needle samples, we randomly sampled one large branch per cardinal direction on each tree, and randomly selected a three-branch subset. Twig segments from these branches were sorted into the following categories: uninfested (no HWA present), low infestation (0·01–6 HWA cm^{-1}) and moderate infestation (6·01–23 HWA cm^{-1}). In order to focus on the localized HWA effect, we sampled only needles with HWA feeding at the base. We therefore allocated all moderately infested segments to needle testing, and randomly subsampled the remaining segments for twig and needle testing. For needles, upon collection all samples were wrapped in damp paper towels in sealed plastic bags, refrigerated (4 °C) and tested within 2 days of collection. We stored all prepared twig samples at room temperature in sealed plastic bags and tested these within 5 days of initial collection.

Whereas HWA feeds actively on the youngest twig class, the insects do not feed directly on older branches. For branches from the rural and urban sites, samples were categorized as infested if any HWA were present on the youngest foliage distal to the sample branch, or as uninfested in the complete absence of HWA. We selected all branches for linear growth and a few secondary branches. Following sample collection, we pruned all secondary branches and stored the branch segments in damp paper towels in sealed plastic bags at 4 °C. Branches were tested within 2 days of sampling.

Mechanical testing

We assessed the strength and flexibility of all samples. Strength was assessed by measuring the stress (load applied per unit area), while flexibility was determined by measuring

TABLE 1. *Collection methods for all hemlock samples by measurement and site*

Measurement	Site	Number of trees	Samples per tree	n	Unit of replication	Tree age
Needle tension	Rural	10	5 A, 5 U	10 A, 10 U	Branch	Mature
	Urban	3	4 A, 4 U	3 A, 3 U		Mature
	Common garden	n/a				
Twig tension	Rural	10	3 A, 3 U	29 A, 30 U	Branch	Mature
	Urban	63 A, 3 U	13 A, 17 U			Mature
	Common garden	6 A, 8 U	3	12 A, 16 U		Sapling
Branch flexure	Rural	9	2 A, 2 U	9 A, 9 U	Tree	Mature
	Urban	14	1 A	14 A		Mature
	Common garden	6 A, 8 U	2	6 A, 8 U		Sapling

Sites were as follows: rural, Mount Tom State Reservation, Holyoke MA, 42·268775, −72·614609; urban, Tufts University Campus, Medford MA, 42·409421, −71·120662; common garden, East Farm at University of Rhode Island, Kingston RI, 41·47315, −71·510388.
A, *A. tsugae*-inf ested; U, uninfested, *n*, number of observations per site, infestation category and tissue type.
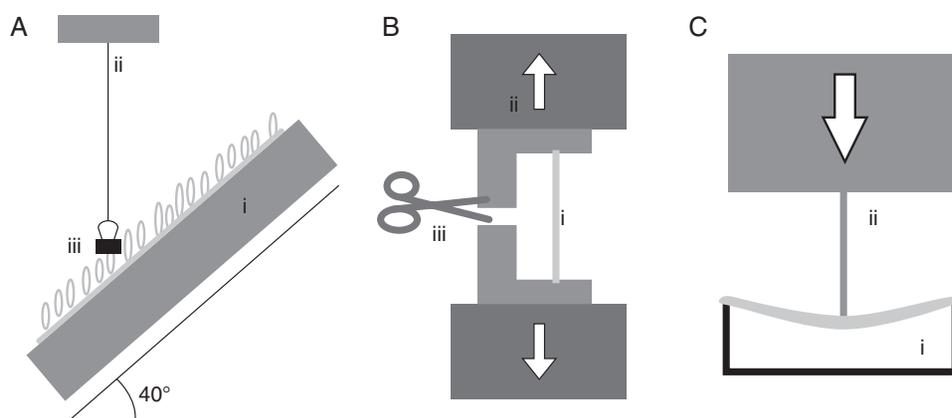
FIG. 1. Methods used for mechanical testing of small specimens from *Tsuga canadensis*. (A) Needle tension. (i) The twig segment is secured in a press vice lengthwise, at 40 °. (ii) A binder clip is attached to the load cell arm by fine motor wire. (iii) Each needle is clipped at its midpoint and stress is applied until abscission. (B) Twig tension. (i) The twig segment is glued into a card frame support. (ii) The frame is loaded into pneumatic clamps. (iii) The frame is cut immediately prior to application of stress. (C) Branch flexure. (i) The branch is loaded onto a two-point support. (ii) Stress is applied to the centre of the branch using a load cell arm.

tissue strain (a measure of relative axial deformation). Strain describes the flexibility of a tissue. We measured stress and strain under deformation and failure, to quantify mechanical responses to HWA. Under small strains plant tissues behave elastically, such that all deformation is reversible, and stress is proportional to strain. This stress:strain ratio (i.e. Young's modulus) describes a structural property of the material. The stress required to initiate permanent deformation (i.e. yield strength) marks the point where the plant material begins to behave plastically.

Mechanical failure, or breaking, of samples can occur in multiple ways. Under tensile stress, which we applied to needle and twig samples, fracture occurs due to crack initiation and propagation. Under bending, which we applied to branch samples, the sample experiences three modes of stress: tensile stress, compressive stress and shear stress. We minimized shear stresses through our choice of sample dimensions (see the Branches section), such that sample failure would be expected to occur primarily due to a combination of compressive stress on the concave side and tensile stress on the convex side of the branch, causing bending. Eventually, flexure causes failure due to excessive plastic deformation and buckling of the light hemlock wood, beyond the yield strength of the sample; a similar result has been seen in *Salix* (van Casteren *et al.*, 2011).

In cases of sample fracture, we quantified maximum stress and the corresponding strain prior to specimen failure (Fig. 2). We used an Instron universal testing machine (UTM, Model 3366) for mechanical testing, and performed data collection and processing with Bluehill software (Instron).

Needles. While previous studies of plant–herbivore systems have examined leaf toughness as work-to-shear (Read *et al.*, 2009), needle abscission is the prevalent herbivore effect in the HWA-hemlock system. Consequently, we focused on the force and displacement at which abscission occurred. We only tested five 10-month-old needles from field sites, from a single twig segment per tree, unless otherwise omitted (e.g. due to failure during loading) (Table 1), for the mechanical properties listed above. We averaged the multiple measurements per twig segment to calculate one replicate value per tree. Needle samples were standardized by cutting 3-cm-long segments, and from these selecting five central needles oriented 40 ° from the stem. We secured twigs in a metal press vice along their length (Fig. 1A), oriented at a 40 ° angle. We attached a small (1 g) binder clip to the load cell arm by a fine motor wire to facilitate loading of single needle samples (modified from Thurber *et al.*, 2010) (Fig. 1A). Each tested needle was secured at its midpoint by the clip and stressed with a 10 N load cell at
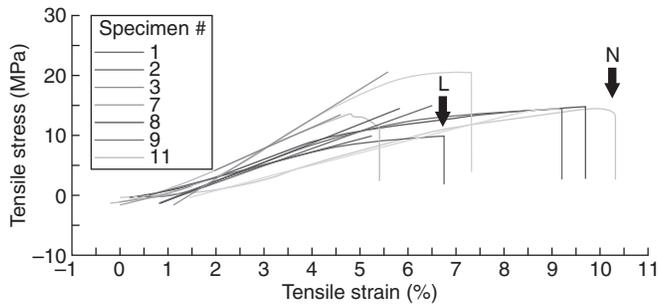
Fig. 2. Sample output from tensile test of hemlock twigs, depicting the stress–strain curve. All samples were loaded at the same rate, and load (represented as MPa, *y*-axis) as well as sample extension (*x*-axis) were measured. L, sample typical of low HWA infestation, with low stress and strain at failure; N, sample typical of uninfested, with higher stress and strain at failure.

0.2 mm min^{-1}. For low- and moderate-infestation sampling, we tested only needles with HWA attached at the base. We tested tension on needles to quantify the strength necessary for needle abscission (maximum stress) relative to average cross-sectional petiole area. The strain at maximum stress (displacement at the time of abscission) is a measure of flexibility.

Twigs. In order to correlate current insect feeding with mechanical properties, we quantified tension in randomly selected 10-month-old twigs. From each branch sampled (see the Sample collection section), only one twig per large branch was analysed. Twig diameter, length and HWA density of each sample were measured. Twigs were stripped of their needles and glued with cyanoacrylate adhesive into a two-layered card frame support with a 15 mm span (modified from DesRochers *et al.*, 2009) (Fig. 1B). The frame was loaded into the Instron UTM tension setup with pneumatic clamps, and the support was cut to allow tensile loading of the twig. Tension was applied using a 100 N load cell at 0.2 mm min^{-1}. We quantified maximum stress (force required to fracture the twig) as a measurement of strength relative to twig cross-sectional area, and strain at maximum stress (vertical displacement at the time of breakage) as a measure of flexibility. The non-linear stress–strain response of twigs under tension precluded us from examining yield stress.

Following mechanical testing of twigs, we quantified lignin concentration (Bonello and Blodgett, 2003) and tissue density (oven-dry mass per fresh volume) of twig and branch samples using standard methods. In small wood specimens, dry tissue density and cell wall chemistry (as lignin, cellulose and hemicellulose contents) are often better predictors of mechanical behaviour than histological traits (Gibson, 2012; Winandy and Rowell, 2005). For instance, lignification of secondary cell walls increases the stiffness and strength of woody tissues (Gibson, 2012).

Branches. We collected 40-month-old branches previously exposed to HWA over multiple growing seasons to study the cumulative effects of insect feeding on biomechanics over time. We randomly selected two branches per tree, one each from the east and west sides. From the urban site, we were only able to sample from the south side of each tree. All trees at the urban site were heavily infested with HWA, such that no comparison could be made between infested and uninfested branches. We measured branch diameter and length for all samples. We tested all branches on the Instron UTM three-point flexure setup, with a 100 N load cell (Fig. 1C). When possible, branches were loaded topside down to mimic downward bending in the field. We oriented them with a support span of $8.5-10$ cm (at least 15 times the branch diameter to minimize shear stresses), and applied the central load at 5 mm min^{-1}. Branches that twisted or slipped during the run were omitted from the analyses. Branch measurements were averaged to calculate one value per tree. Since the most ecologically relevant factor in large branch stresses is irreversible deformation, we focused on yield strength (stress required to cause plastic deformation, MPa) as a measure of strength, and modulus of elasticity (initial rate of increase of stress per strain, MPa) as a measure of flexibility.

Statistical analysis

We inspected all data for normality (Shapiro–Wilk test) and homoscedasticity (Bartlett's test) prior to analysis. To meet assumptions of normality, twig tissue density was square root transformed, twig strain was log transformed, and needle stress and strain were inverse square root transformed. To account for variation in insect infestation density, infestation level was included [i.e. none, low ($0.01-6$ HWA cm^{-1}) or moderate ($6.01-23$ HWA cm^{-1})] as a factor in twig and needle analysis. In twigs, we analysed the effect of tissue density and lignin concentration on stress and strain by linear regression. The effects of insect level on tissue density and lignin concentration were assessed by the *t*-test. For each tissue type, we used a two-way multivariate analysis of variance (MANOVA) to find mechanical response variables impacted by site and insect level. We then ran ANOVAs on any response variables that were significant under MANOVA. For significant ANOVAs with more than two insect levels or a significant interaction, we ran *post hoc* Fisher's LSD tests. For twig strain, our sample sizes were unequal across insect levels. Because Fisher's LSD is sensitive to this, we validated the results of Fisher's LSD tests with unpaired *t*-tests within each site, Bonferroni-corrected for multiple comparisons. We performed all statistical analyses in R (R Development Core Team, 2011).

RESULTS

Needles

We measured a significant effect of HWA infestation level and site on the mechanical traits tested in needles (two-way MANOVA; Table 2A). Subsequent ANOVA analysis for maximum stress revealed that insect infestation significantly reduced the strength required to cause needle abscission (Table 2A). However, the effect of HWA on needle strength was lost in *post hoc* Fisher's LSD analysis (Fig. 3A, B). Neither site nor insect presence significantly impacted needle flexibility measured as strain at maximum stress (Fig. 3C, D) (ANOVA; Table 2A).

Twigs

We observed significant effects of insect infestation level and site on biomechanical properties (two-way MANOVA;

TABLE 2. *Results of hemlock biomechanical analyses by site and HWA infestation*

Model	Source	d.f.	*F*-ratio	*P*-value	Significance
(A) Needles					
MANOVA	Insect	2	3·20	0·017	*
	Site	1	8·24	0·001	**
	Insect × site	1	0·76	0·477	
	Residual	39			
ANOVA, transformed stress	Insect	2	6·30	0·004	**
	Site	1	16·7	$2·14 \times 10^{-4}$	***
	Insect × site	1	1·13	0·294	
	Residual	39			
ANOVA, transformed strain	Insect	2	0·654	0·525	
	Site	1	0·001	0·982	
	Insect × site	1	0·270	0·606	
	Residual	39			
(B) Twigs					
MANOVA	Insect	2	9·85	$1·00 \times 10^{-4}$	***
	Site	4	18·2	$5·69 \times 10^{-13}$	***
	Insect × site	4	2·11	0·080	†
	Residual	112			
ANOVA, stress	Insect	1	13·4	$3·00 \times 10^{-4}$	***
	Site	2	11·6	$2·66 \times 10^{-5}$	***
	Insect × site	2	0·279	0·757	
	Residual	112			
ANOVA, log strain	Insect	1	8·34	$4·70 \times 10^{-3}$	**
	Site	2	29·5	$5·08 \times 10^{11}$	***
	Insect × site	2	4·25	0·017	*
	Residual	112			
(C) Branches					
MANOVA	Insect	1	1·10	0·341	
	Site	1	73·6	$6·76 \times 10^{-16}$	***
	Insect × site	1	0·020	0·980	
	Residual	53			
ANOVA, stress	Insect	1	1·91	0·173	
	Site	1	104	$4·04 \times 10^{-14}$	***
	Insect × site	1	0·013	0·909	
	Residual	53			
ANOVA, modulus	Insect	1	0·937	0·338	
	Site	1	90·8	$4·43 \times 10^{-13}$	***
	Insect × site	1	0·015	0·902	
	Residual	53			

    MANOVAs of mechanical traits are reported for each tissue type tested (needles, twigs, branches). For significant MANOVAs, ANOVAs by trait (stress, strain or modulus) are reported.

    Significance codes: $*P < 0·05$, $**P < 0·01$, $***P < 0·001$, $†P < 0·1$.

Table 2B). Further analysis indicated that infested twigs were consistently weaker (by 25 % on average) under tensile stress than uninfested twigs across all three sites (two-way ANOVA; Table 2B, Fig. 4A–C). We observed a disordinal interaction between infestation and site for twig brittleness measured as strain (two-way ANOVA; Table 2B, Fig. 4D–F). *Post hoc* analysis by Fisher's LSD and Bonferroni-corrected unpaired *t*-tests indicated that while infested twigs were brittler at the urban site, HWA infestation had no significant effect at the rural or common garden sites (Fig. 4D–F).

We also found that lignin content and tissue density predicted maximum tensile stress across treatments, but not tensile strain. There was a significant positive relationship between maximum stress and lignin concentration (linear regression: $R^2 = 0·145$, $P < 0·001$; Fig. 5A) and tissue density (linear regression: $R^2 = 0·207$, $P = 6·77 \times 10^{-7}$; Fig. 6A) across all samples. Tensile strain appeared to be insensitive to lignin concentration (linear regression: $R^2 = 0·0002$, $P = 0·901$; Fig. 5B) and to tissue density (linear regression: $R^2 = 0·029$, $P = 0·078$;

Fig. 6B). Lignin and density, however, did not differ between the two insect treatments (Table 3) and thus do not explain the effects of HWA on branch mechanics.

Branches

Previously infested branches did not differ significantly in any of the biomechanical traits measured between insect infestation levels (two-way MANOVA; Table 2C). However, both flexural yield stress and flexibility (Young's modulus) differed by site (two-way ANOVA, Table 2C, Fig. 7). Our findings at the urban field site were consistent with the mechanics of infested branches at the rural site; mean yield stress and mean modulus of infested branches were 41·2 and 1852 MPa, respectively.

DISCUSSION

Our results indicate that HWA feeding alters some of the biomechanical properties of eastern hemlock tissues, with evidence
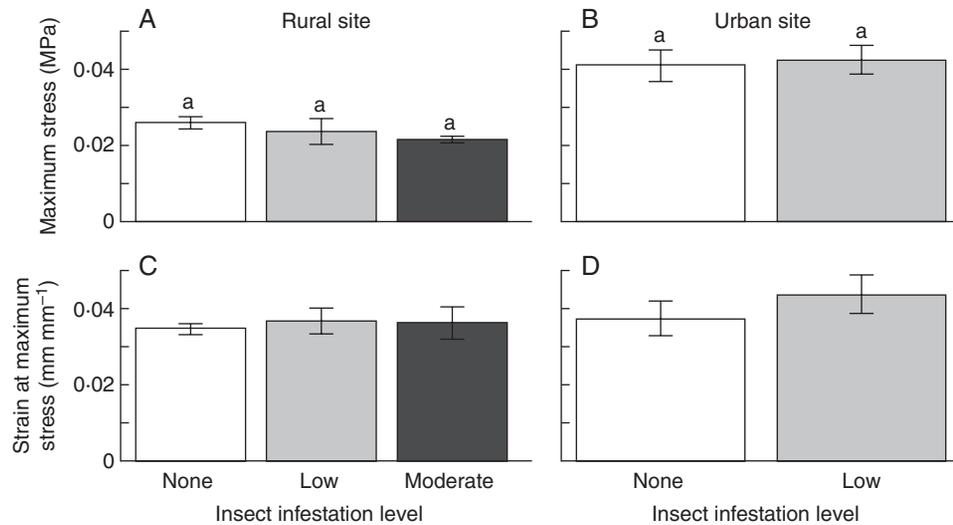
FIG. 3. Effect of HWA infestation level and site on abscission biomechanics of hemlock needles shown as maximum stress and as strain at maximum stress (mean $\pm$ s.e.). None, segments of 0 insects cm^{-1}; Low, 0·0–6 insects cm^{-1}; Moderate, 6·01–23 insects cm^{-1}. Different letters in (A–B) indicate significant differences between insect levels within site, by *post hoc* Fischer's LSD test at $\alpha = 0.05$.
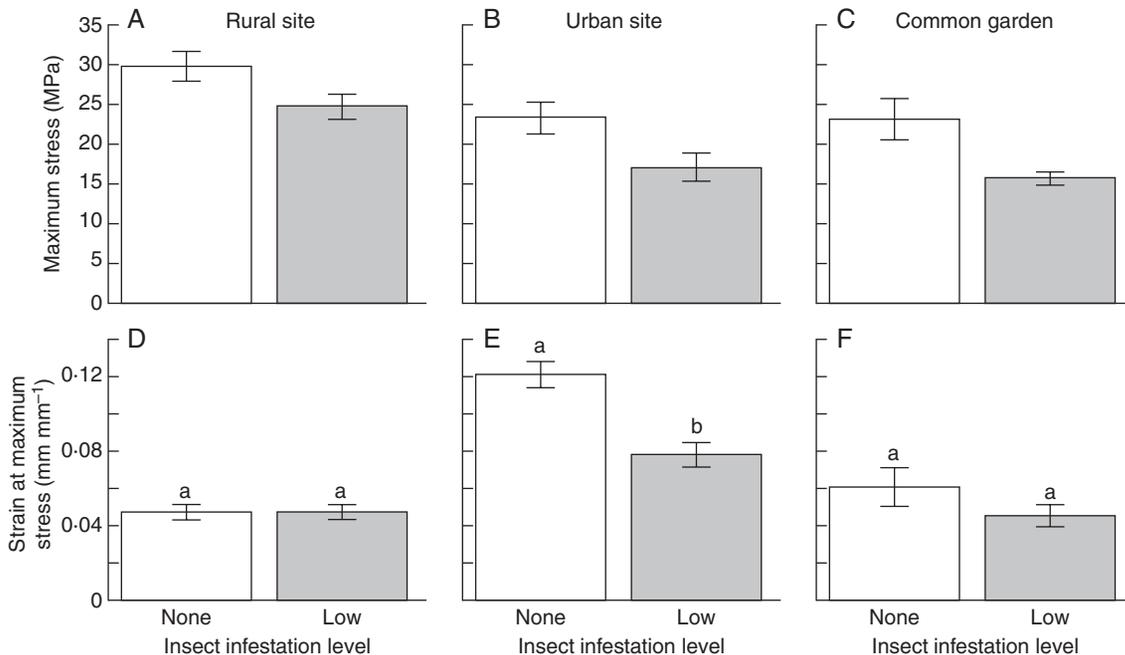
FIG. 4. Effect of HWA infestation level and site on tensile biomechanics of 10-month-old hemlock twigs shown as maximum stress and as strain at maximum stress (mean $\pm$ s.e.). None, segments of 0 insects cm^{-1}; Low, 0·01–6 insects cm^{-1}. Different letters in panels (D–F) indicate significant differences between insect levels within site, confirmed by both *post hoc* Fischer's LSD test and Bonferroni-corrected unpaired *t*-tests at $\alpha = 0.05$.

of decreased flexibility and strength in HWA-infested trees. The effects of HWA on hemlock biomechanics were strongest in currently infested twigs and were measurable in the strength of attached needles. HWA feeding had no apparent lasting impacts on the mechanical properties of previously infested branches or on the flexibility of currently infested needles. The changes to the twigs and needles suggest that a decrease in mechanical resistance of attacked trees could contribute to the

mortality of infested trees over time (McClure, 1991; Stadler, 2005). As strength and, at some sites, flexibility, decreased in infested twigs, and abscission strength decreased in infested needles, these trees could become more sensitive to abiotic mechanical stressors such as strong winds, ice storms or snow pack.

While the negative effects of invasive insects on native plants are well documented, the mechanisms behind these effects are
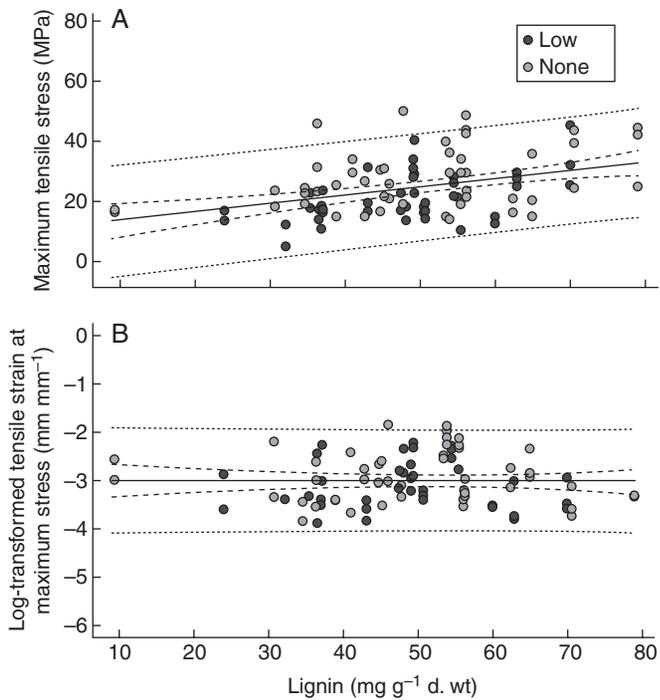
FIG. 5. Linear regression of tensile mechanics against lignin concentration in hemlock twigs. Symbol colour indicates HWA infestation level. Solid line is the best fit line, dashed lines show the confidence interval and dotted lines show the prediction interval. For stress (A), $R^2 = 0.145$, $P < 0.001$. For log-transformed strain (B), $R^2 = 2.00 \times 10^{-4}$, $P = 0.901$.
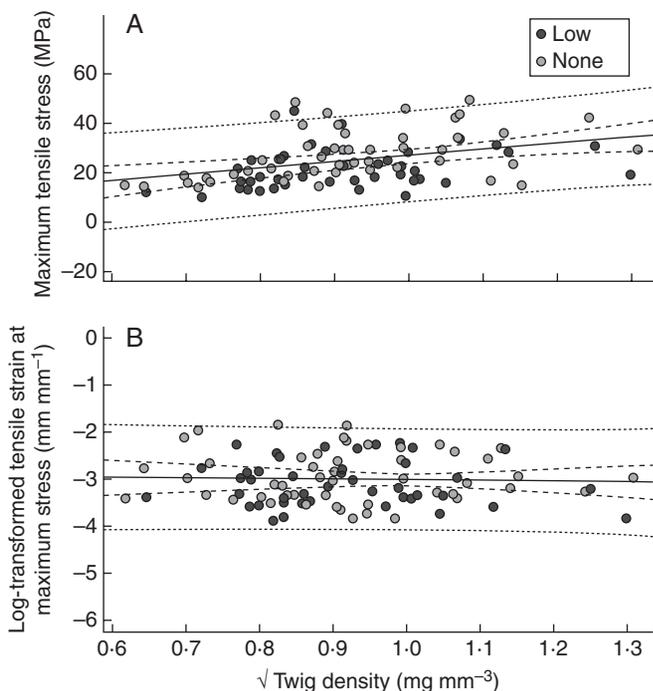
FIG. 6. Linear regression of tensile mechanics against square root transformed tissue density in hemlock twigs. Symbol colour indicates HWA infestation level. Solid line is the best fit line, dashed lines show the confidence interval and dotted lines show the prediction interval. For stress (A), $R^2 = 0.207$, $P = 6.77 \times 10^{-7}$. For log-transformed strain (B), $R^2 = 0.029$, $P = 0.078$.

often poorly understood. When herbivores cause complete defoliation, the lack of plant defence is clear. In the case of non-defoliating invasive insects with devastating effects, the reasons can be elusive. It appears that HWA induces a hypersensitive response that changes wood properties (Gonda-King *et al.*, 2012), which may ultimately affect needle and twig retention. This is the first study to demonstrate that feeding by an invasive herbivore alters the mechanical properties of needles and twigs.

Herbivores and pathogens are known to affect needle longevity; defoliating insects increase longevity in *Pinus* due to reduced needle cast in response to slow growth (Kurkela *et al.*, 2005; Drenkhan *et al.*, 2006), while fungal infection decreases needle longevity in *Pseudotsuga* (Hansen *et al.*, 2000) and *Larix* (Krause and Raffa, 1992). We hypothesized that a decrease in needle attachment strength and flexibility in the abscission zone causes the increased rate of needle loss observed in HWA-infested hemlocks (Orwig *et al.*, 2002; Stadler *et al.*, 2005). This is supported by our observation that needle abscission strength was reduced by HWA feeding. However, HWA had no effect on the flexibility of the needle abscission layer. Perhaps continuous HWA feeding induces localized branch drought stress (Domec *et al.*, 2013), such that decreased turgor pressure in twigs causes premature needle abscission, as seen in some *Picea* trees (Maier-Maerker and Koch, 1995). This induced abscission is likely detrimental to HWA, as needles proximate to the feeding site provide a key nutrient source to the stem-feeding HWA via xylem storage cells (Young *et al.*, 1995) that are laterally connected to the phloem (van Bel, 1990). Our results also indicate that the increased needle loss observed in infested hemlock stands (Stadler *et al.*, 2005) may begin soon after infestation along the young, currently infested twigs.

The changes in woody tissues were most pronounced following recent infestation. In young twigs, plausible explanations for the biomechanical changes observed here include abnormal xylem formation or a change in resource allocation priorities. Previous research indicates that HWA alters xylem anatomy by inducing false ring formation in young branches (Gonda-King *et al.*, 2012), which can result in thickened xylem walls in the false rings (Bolton and Petty, 1978). If cells in these false rings have thickened walls resembling those of adelgid-infested *A. balsamea* and of compression wood, an increase in lignin and in twig brittleness is expected (Balch *et al.*, 1964). However, in our study twig lignification was not predictive of brittleness or impacted by HWA infestation. It has been suggested that the brittleness associated with elevated lignin is actually due to low relative cellulose content rather than lignin content *per se* (Johnson *et al.*, 2006). Consequently, we cannot exclude low cellulose content in the false rings as a potential cause of twig brittleness.

Since lignin did not predict mechanical responses to infestation, we suggest two additional, non-mutually exclusive explanations. First, the loss of tissues resulting from HWA attack, along with induced changes in resource allocation, could affect the tree's ability to allocate resources to structural reinforcement in the infested twigs and to new growth. Specifically, the removal of nutrient stores from the xylem ray parenchyma by HWA feeding may decrease local nutrient availability (Shigo, 1989 as cited in Oten, 2011; but see Gómez *et al.*, 2012). These cells store nutrients for release during periods of rapid growth, as

TABLE 3. *Results of tissue composition analyses in hemlock twigs by site and HWA infestation level*

Variable	Source	d.f.	*F*-ratio	*P*-value	Significance
Lignin concentration (mg g^{-1})	Insect	1	0·211	0·649	
	Site	2	6·46	0·004	**
	Insect × site	2	0·751	0·480	
	Residual	33			
Square root of density (mg mm^{-3})	Insect	2	0·034	0·855	
	Site	1	14·0	$4·20 \times 10^{-6}$	***
	Insect × site	2	0·086	0·918	
	Residual	103			

ANOVAs by variable (lignin concentration, tissue density) are reported. Both variables are measured for oven-dry samples
Significance codes: **$P < 0.01$, ***$P < 0.001$.
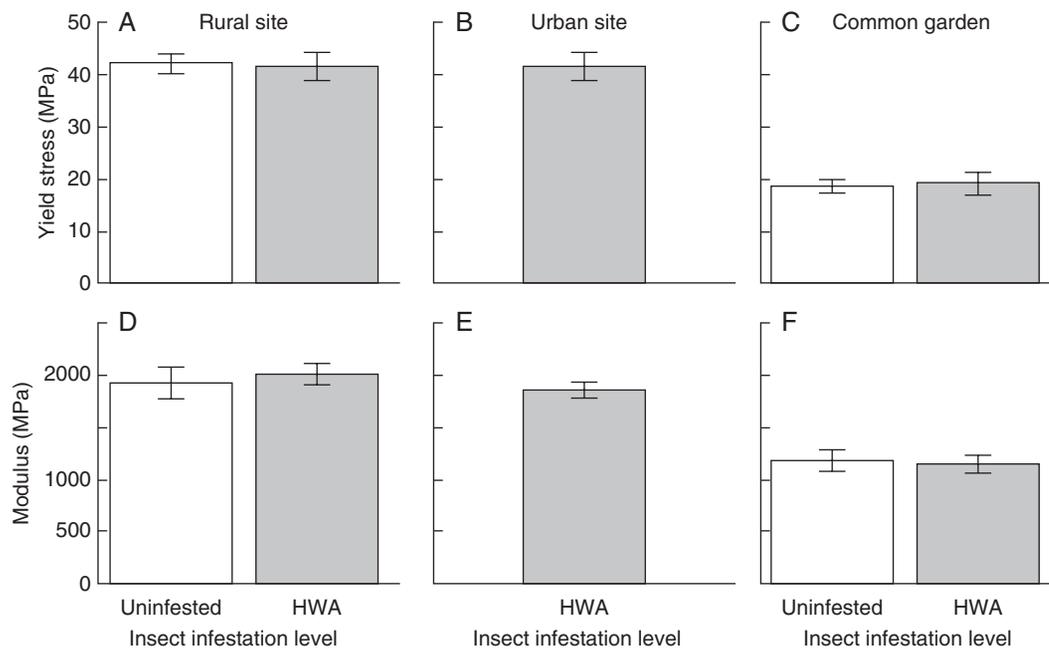
FIG. 7. Flexural biomechanics of hemlock branches by site and HWA presence shown as yield stress and Young's modulus. Values are means ± s.e. No significant effect of insect presence was found.

lipids and starch granules (Pallardy, 2008; Begum *et al.*, 2010). If HWA feeding depletes xylem ray parenchyma cells of glucose and other carbohydrates, the building blocks of cellulosic polymers will be depleted, likely leading to cell wall weakening (Pallardy, 2008), explaining the observed increase in brittleness and weakness in young HWA-infested twigs. Second, an excess of nitrogen localized to sites of HWA feeding, as is observed in naturally infested trees (Stadler *et al.* 2005) and in experimentally infested trees (Gómez *et al.*, 2012; N. Soltis unpubl. res.), may decrease mechanical reinforcement. Under high-nutrient conditions, tissue density, strength and stiffness decrease in some plant groups (Craine *et al.*, 2001; Lamberti-Raverot and Puijalon, 2012). This may be due to cell wall hydrolysis for accelerated growth and development (Labavitch, 1981), including amino acid synthesis. Further experiments are needed to connect the biomechanics of herbivore-attacked twigs to chemical composition, in terms of nutrient availability and cell wall components.

Interestingly, twig brittleness under HWA attack was affected by an interaction with site, due to the weak HWA effect at the rural and common garden sites. Twigs from trees at the urban site, with intermediate infestation densities and low health [as assessed by minimal new growth foliage, dry and dull mature needles and bare twigs (N. Soltis, personal observation)], were more brittle when HWA was feeding on them. It may be that recent years of HWA infestation were most severe at the urban site, and a decline in tree health has led to both increased branch brittleness and decreased HWA density. A milder infestation history at the rural site and a briefer infestation history at the common garden may have prevented a measurable increase in brittleness due to HWA infestation. We find it noteworthy that the common garden trees still exhibited the same pattern as urban site trees, despite the briefer infestation.

HWA feeding did not significantly affect the mechanics of 3- to 4-year-old branches. For small branches, however, brittleness may be best characterized by mechanics at yield relative to mechanics at fracture (Beismann *et al.*, 2000). Future experiments examining fracture in *T. canadensis* branches under flexure stress may clarify whether the mechanical effects of

HWA persist over multiple years. Alternatively, large branches, free of direct HWA feeding, may recover in subsequent growth seasons to structurally resemble unattacked branches. After HWA ceases feeding on the storage cells within a branch, these cells are released from localized nutrient stress, and growing cells may develop normally with no subsequent reduction of strength, diminishing the effect of one layer of low-quality cells. Thus, the mechanical effects of HWA herbivory are limited to the currently attacked tissues.

Overall, twig breakage and needle loss due to physical factors may reduce plant fitness due to both biomass and meristem loss. Our results may explain the more frequent needle (Stadler *et al.*, 2005) and twig (Nuckolls *et al.*, 2009) litterfall patterns observed in infested stands. By our measurements, young hemlock twigs break under 16–40 N of force. In the field, tree branches can accumulate up to 2 g cm^{-1} snow and 4 g cm^{-1} of ice under heavy precipitation (Cannell and Morgan, 1989), which along the length of our twig segments would amount to 0·4 N snow or 0·8 N ice. Ice buildup alone is therefore not expected to cause breakage in hemlock twigs.

While little is known about twig-level wind stresses in the field, the force of wind on large branches is substantial. At wind speeds of 25 m s^{-1} (a strong gale), drag forces on tree branches can range from 20 to 80 N, while at 14 m s^{-1} (a strong breeze) drag forces reach 10–40 N on large (1·2 m) branches (Butler *et al.*, 2012). Furthermore, in softwood trees wind loads in excess of 20 m s^{-1} cause mechanical instability (Spatz and Bruechert, 2000). While the forces experienced by small twigs will be much less, high winds or the combination of wind and ice loading may cause young branch breakage in hemlocks. Further, chronic stresses caused by recurring wind events also contribute to mechanical failure in trees, and may be damaging to hemlock twigs (Mitchell, 2013).

In addition, the changes in branch biomechanics may have implications beyond the risk of damage in the wake of physical stressors. In addition to preventing breakage, biomechanical traits may serve to deter herbivores and protect against feeding damage. Structurally reinforced tissues, such as toughened leaves, experience less herbivory (Coley, 1983). Chemicals for structural reinforcement may also protect plants from feeding damage; cell walls with high cellulose or lignin content are less digestible to chewing insect herbivores (Clissold *et al.*, 2004). HWA may avoid highly cellulosic and lignified tissues. Through cell wall fortification, plants increase mechanical barriers to prevent insect access to food sources, especially phloem-feeding aphids (Divol *et al.*, 2007). Dense tissues may also increase tissue longevity and nutrient conservation, which could benefit HWA. In sum, biomechanical changes induced by HWA may increase *T. canadensis* susceptibility to further herbivory and accelerate decline. Cumulative effects of structural damage, increased branch breaking and foliage loss may alter the litter composition and nutrient cycling of infested stands, exacerbating the ecological impact of HWA on eastern forests.

ACKNOWLEDGEMENTS

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