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Original Article

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

Tsuga canadensis

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Running title: The exotic herbivore *Adelges tsugae* alters biomechanics of *Tsuga canadensis*

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ABSTRACT

- *Background and Aims:* Exotic herbivores that lack a co-evolutionary history with their host plants can sometimes benefit from poorly-adapted host defenses, potentially leading to rapid population growth of the herbivore and severe damage to their preferred plant hosts. The hemlock woolly adelgid (*Adelges tsugae,* 'HWA') is an exotic hemipteran which feeds on the long-lived conifer eastern hemlock (*Tsuga canadensis*), causing rapid mortality of infested trees. While the mechanism of this mortality is still unknown, evidence indicates that HWA feeding causes a hypersensitive response and alters wood anatomy. This could affect the integrity of tissues locally and systemically. Here, we investigate the effect of HWA feeding on tree biomechanical properties at different spatial scales: needles, twigs, and branches.

- *Methods:* We collected uninfested and HWA-infested samples from a common garden experiment as well as from naturally-infested urban and rural field sites. We used tension and flexure mechanical tests to quantify biomechanical properties of the different tissues. In tissues that showed a significant effect of herbivory, we quantified the potential contribution of lignin and tissue density on the results.

- *Key Results:* HWA infestation decreased the abscission strength, but not flexibility, of needles. HWA 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. We discuss possible contributors to these mechanical effects, including altered growth patterns and cell wall chemistry. Consistent site effects emphasize the role of environmental variation in mechanical traits. The mechanical

1 changes measured here may increase susceptibility to abiotic physical stressors in HWA-

2 colonized hemlocks.

3 **Key words:** *biomechanics, Tsuga canadensis, Adelges tsugae, herbivory, invasive*

INTRODUCTION

Plants and insect herbivores often have a long history of coevolution. 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 (Gatehouse, 2002; Xiang and Chen, 2004; Strauss, 1999; Núñez-Farfán, 2007). Relationships between specialist herbivores and their host plants can lead to complex coevolutionary arms races (Agrawal, 2000; Ruuhola, 2001). However, in the case of exotic species, this system of coadaptation is circumvented (Gandhi and Herms, 2010). An exotic herbivorous insect may encounter naïve 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 to leaf-chewing herbivores, piercing-sucking herbivores often have larger effects on plant fitness (Meyer, 1993; Zvereva, Lanta, and Kozlov 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 signaling (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).

4

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 United States. 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; Gomez *et al.*, 2012), and induces a hypersensitive response (HR) (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 systemic HR 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 an HR) found in both the infested and distal needles of HWA-infested trees (Radville *et al.*, 2011). This HR 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-vigor (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 in 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, allowing for greater levels of environmental heterogeneity and for 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,

is 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 to 1.5 meter height from

each tree: branches (5 mm diameter, approximately three to four years old), and the youngest

twigs (1 mm diameter, ten 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 per cm), and moderate infestation (6.01 - 23 HWA per cm). In order to focus on

the localized HWA effect, we sampled only needles with HWA feeding at the base. As such, we

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 two

days of collection. We stored all prepared twig samples at room temperature in sealed plastic

bags, and tested these within five days of initial collection.

While HWA feed 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 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 $4^{o}C$. Branches were tested within two

days of sampling.

Mechanical testing

We assessed strength and flexibility of all samples. Strength was assessed by measuring

the stress (load applied per unit area), while flexibility was determined by measuring 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 *branches*), such that sample failure occurs

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 is also 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 (Figure 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

We only tested five ten-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 40º from the stem. We secured

twigs in a metal press vise along their length (Figure 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) (Figure 1A). Each tested needle was

secured at its midpoint by the clip, and stressed with a 10N load cell at 0.2 mm/min. 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.

1

2 *Twigs*

3   In order to correlate current insect feeding with mechanical properties, we quantified

4 tension in randomly selected ten-month-old twigs. From each branch sampled (see *sample*

5 *collection*), only one twig per large branch was analyzed. Twig diameter, length, and HWA

6 density of each sample was measured. Twigs were stripped of their needles and glued with

7 cyanoacrylate adhesive into a two-layered card frame support with 15 mm span (modified from

8 DesRochers *et al*., 2009) (Figure 1B). The frame was loaded into the Instron UTM tension setup

9 with pneumatic clamps, and the support was cut to allow tensile loading of the twig. Tension was

10 applied using a 100 N load cell at 0.2 mm min^{-1}. We quantified maximum stress (force required

11 to fracture twig) as a measurement of strength relative to twig cross-sectional area, and strain at

12 maximum stress (vertical displacement at the time of breakage) as a measure of flexibility. The

13 nonlinear stress-strain response of twigs under tension precluded us from examining yield stress.

14   Following mechanical testing of twigs, we quantified lignin concentation (Bonello and

15 Blodgett 2003) and tissue density (oven-dry mass per fresh volume) of twig and branch samples

16 using standard methods. In small wood specimens, dry tissue density and cell wall chemistry (as

17 lignin, cellulose, and hemicellulose content) are often better predictors of mechanical behavior

18 than histological traits (Gibson 2012; Winandy and Rowell 2005). For instance, lignification of

19 secondary cell walls increases stiffness and strength of woody tissues (Gibson 2012).

20

21 *Branches*

22   We collected 40-month-old branches previously exposed to HWA over multiple growing

23 seasons to study the cumulative effects of insect feeding on biomechanics over time. We

1 randomly selected two branches per tree, one each from the east and west sides. From the urban

2 site, we were only able to sample from the south side of each tree. All trees at the urban site were

3 heavily infested with HWA, such that no comparison could be made between infested and

4 uninfested branches. We measured branch diameter and length for all samples. We tested all

5 branches on the Instron UTM 3-point flexure setup, with a 100 N load cell (Figure 1C). When

6 possible, branches were loaded topside-down to mimic downward bending in the field. We

7 oriented them with a support span of 8.5-10 cm (at least 15 times the branch diameter to

8 minimize shear stresses), and applied the central load at 5 mm/min. Branches that twisted or

9 slipped during the run were omitted from the analyses. Branch measurements were averaged to

10 calculate one value per tree. Since the most ecologically relevant factor in large branch stresses is

11 irreversible deformation, we focused on yield strength (stress required to cause plastic

12 deformation, MPa) as a measurement of strength, and modulus of elasticity (initial rate of

13 increase of stress per strain, MPa) as a measure of flexibility.

14

15 *Statistical analysis*

16   We inspected all data for normality (Shapiro-Wilk test) and homoscedasticity (Bartlett's

17 test) prior to analysis. To meet assumptions of normality, twig tissue density was square-root

18 transformed, twig strain was log transformed, and needle stress and strain were inverse square-

19 root transformed. To account for variation in insect infestation density, infestation level was

20 included [i.e. none, low (0.01-6 HWA/cm), or moderate (6.01-23 HWA/cm)] as a factor in twig

21 and needle analysis. In twigs, we analyzed the effect of tissue density and lignin concentration on

22 stress and strain by linear regression. The effects of insect level on tissue density and lignin

23 concentration were assessed by t-test. For each tissue type, we used a 2-way multivariate analysis

1   of variance (MANOVA) to find mechanical response variables impacted by site and insect level.

2   We then ran ANOVAs on any response variables that were signficant under MANOVA. For

3   significant ANOVAs with more than 2 insect levels, or a significant interaction, we ran post-hoc

4   Fisher's LSD tests. For twig strain, our sample sizes were unequal across insect levels. Because

5   Fisher's LSD is sensitive to this, we validated the results of Fisher's LSD tests with unpaired t-

6   tests within each site, Bonferroni-corrected for multiple comparisons. We performed all

7   statistical analyses in R (R development core team, 2011).

8

9   RESULTS

10 *Needles*

11   We measured a significant effect of HWA infestation level and site on the mechanical

12 traits tested in needles (2-way MANOVA: Table 2A). Subsequent ANOVA analysis for

13 maximum stress revealed that insect infestation significantly reduced the strength required to

14 cause needle abscission (Table 2A). However, the effect of HWA on needle strength was lost in

15 post-hoc Fisher's LSD analysis (Figure 3A, B). Neither site nor insect presence significantly

16 impacted needle flexibility (Figure 3C, D) (ANOVA: Table 2A).

17 *Twigs*

18   We observed significant effects of insect infestation level and site on biomechanical

19 properties (2-way MANOVA: Table 2B). Further analysis indicated that infested twigs were

20 consistently weaker (by 25% on average) under tensile stress than uninfested twigs across all

21 three sites (2-way ANOVA: Table 2B, Figure 4 A, B, C). We observed a disordinal interaction

22 between infestation and site for twig brittleness (2-way ANOVA: Table 2B, Figure 4 D,E,F).

23 Post-hoc analysis by Fisher's LSD and Bonferonni-corrected unpaired t-tests indicated that while

1   infested twigs were brittler at the urban site, HWA infestation had no significant effect at the

2   rural or common garden sites (Figure 4 D, E, F).

3   We also found that lignin content and tissue density predicted maximum tensile stress

4   across treatments, but not tensile strain. There was a significant positive relationship between

5   maximum stress and lignin concentration (linear regression: $R^2 = 0.145$, p<0.001, Figure 5A) and

6   tissue density (linear regression: $R^2 = 0.207$, p=6.77E-07, Figure 6A) across all samples. Tensile

7   strain appeared to be insensitive to lignin concentration (linear regression: $R^2 = 0.0002$, p=0.901,

8   Figure 5B) and to tissue density (linear regression: $R^2 = 0.029$, p=0.078, Figure 6B). Lignin and

9   density, however, did not differ between the two insect treatments (t=0.8543, 96 df, p=0.395; t=-

10   0.5664, 105 df, p=0.572, respectively) and thus do not explain the effects of HWA on branch

11   mechanics.

12

13   *Branches*

14   Previously–infested branches did not differ significantly in any of the biomechanical

15   traits measured between insect infestation levels (2-way MANOVA: Table 2C). However, both

16   flexural yield stress and flexibility (Young's modulus) differed by site (2-way ANOVA: Table

17   2C, Figure 7). Our findings at the urban field site were consistent with the rural and common

18   garden sites; mean yield stress and mean modulus of infested branches were 41.2 MPa and 1852

19   MPa, respectively.

20

21   DISCUSSION

22   Our results indicate that HWA feeding alters some of the biomechanical properties of

23   eastern hemlock tissues, with evidence of decreased flexibility and strength in HWA-infested

trees. The effects of HWA on hemlock biomechanics were strongest in currently-infested twigs, and measurable in the strength of attached needles. HWA feeding has 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 (Stadler, 2005; McClure, 1991). 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 often poorly understood. When herbivores cause complete defoliation, the lack of plant defense is clear. In 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) which 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). As such, 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 localized nutrient availability (Shigo, 1989 as cited in Oten, 2011; but see Gomez *et al*., 2012). These cells store nutrients for release during periods of rapid growth, as 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 experimentally-infested trees (N. Soltis unpublished data), 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.

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 from 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, 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 three to four-year-old

1 branches. For small branches, however, brittleness may be best characterized by mechanics at

2 yeild relative to mechanics at fracture (Beismann *et al*., 2000). Future experiments examining

3 fracture in *T. canadensis* branches under flexure stress may clarify whether the mechanical

4 effects of HWA persist over multiple years. Alternatively, large branches, free of direct HWA

5 feeding, may recover in subsequent growth seasons to structurally resemble unattacked branches.

6 After HWA cease feeding on the storage cells within a branch, these cells are released from

7 localized nutrient stress, and growing cells may develop normally with no subsequent reduction

8 of strength – diminishing the effect of one layer of low-quality cells. As such, the mechanical

9 effects of HWA herbivory are limited to the currently-attacked tissues.

10 *Implications*

11 Overall, twig breakage and needle loss due to physical factors may reduce plant fitness

12 due to both biomass and meristem loss. Our results may explain the more frequent needle

13 (Stadler *et al*., 2005) and twig (Nuckolls *et al*., 2009) litterfall patterns observed in infested

14 stands. By our measurements, young hemlock twigs break under 16 – 40 N of force. In the field,

15 tree branches can accumulate up to 2 g/cm snow and 4 g/cm of ice under heavy precipitation

16 (Cannell and Morgan, 1989), which along the length of our twig segments would amount to 0.4

17 N snow or 0.8 N ice. As such, ice buildup alone is not expected to cause breakage in hemlock

18 twigs.

19 While little is known about twig-level wind stresses in the field, the force of wind on

20 large branches is substantial. At wind speeds of 25 m/s (a strong gale), drag forces on tree

21 branches can range from 20 to 80 N, while at 14 m/s (a strong breeze), drag forces reach 10 to 40

22 N on large (1.2 m) branches (Butler et al., 2012). Furthermore, in softwood trees, wind loads in

23 excess of 20 m/s 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.

18

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Figure 1: Depiction of setups for mechanical testing. A: Needle tension. i. Twig segment is secured in a press vise 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 applied until abscission. B: Twig tension. i. Twig segment is glued into card frame support. ii. Frame is loaded into pneumatic clamps. iii. Frame is cut immediately prior to stress application. C: Branch flexure. i. branch is loaded onto 2-point support. ii. Stress is applied to the center of the branch using load cell arm.

Figure 2: Sample output of twig tensile test depicting the stress-strain curve. All samples are loaded at the same rate, and load (represented as MPa, y-axis) as well as sample extension (x-axis) are measured. L: sample typical of low-infestation, with low stress and strain at failure. N: sample typical of uninfested, with higher stress and strain at failure.

Figure 3: Biomechanics of needles by site and insect density, as maximum stress and as strain at maximum stress (mean ± SE). None includes segments of 0 insects/cm, low includes 0.0-6 insects/cm, moderate includes 6.01-23 insects/cm. Different letters in figures A and B indicate significant differences between insect level within site, by post-hoc Fischer's LSD test at α=0.05 level.

Figure 4: Biomechanics of 10-month-old twigs from each site, as maximum stress and as strain at maximum stress (mean ± SE). None includes segments of 0 insects/cm, low includes 0.01 to 6 insects/cm. Different letters in figures D, E, and F indicate significant differences between insect

level within site, confirmed by both post-hoc Fischer's LSD test and bonferroni-corrected

unpaired t-tests at α=0.05 level.

Figure 5: Linear regression of tensile mechanics against lignin content in twigs. Solid best fit

line, dashed confidence interval, and dotted prediction interval. For stress, A, R^2 = 0.145,

$p<0.001$. For log-transformed strain, B, R^2 = 0.0002, p=0.901.

Figure 6: Linear regression of tensile mechanics against square-root transformed tissue density in

twigs. Solid best fit line, dashed confidence interval, and dotted prediction interval. For stress, A,

R^2= 0.207, p=6.77E-07. For log-transformed strain, B, R^2=0.029, p=0.078.

1   Figure 7: Biomechanics of large branches at each site, as yield stress and modulus. Values are

2   means ± SE.

3

1 Table 1: Collection methods for all samples by measurement and site. Sites as follows: rural,

2 Mount Tom State Reservation, Holyoke MA, 42.268775,-72.614609; urban, Tufts University

3 Campus, Medford MA, 42.409421,-71.120662; common garden, East Farm at University of

4 Rhode Island, Kingston RI, 41.47315,-71.510388. 'A' signifies HWA-infested, 'U' signifies

5 uninfested. 'n' signifies number of observations per site, infestation category, and tissue type.

6 'Samples per tree' and 'n' are equivalent for A and U samples unless noted.

7

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

8

Table 2: Results of biomechanics analysis by tissue, site, and insect infestation category.

A. Needles

Model	Source	df	F-ratio	P-value	Signif.
MANOVA	Insect	2	3.197	0.0174	*
	Site	1	8.241	0.0011	**
	Insect x Site	1	0.756	0.4766	
	Residual	39			
ANOVA: stress	Insect	2	6.297	0.0043	**
	Site	1	16.667	0.0002	***
	Insect x Site	1	1.132	0.2939	
	Residual	39			
ANOVA: transformed strain	Insect	2	0.525	0.5254	
	Site	1	0.982	0.9822	
	Insect x Site	1	0.606	0.6061	
	Residual	39			

B. Twigs

Model	Source	df	F-ratio	P-value	Signif.
MANOVA	Insect	2	9.854	1.0000E-04	***
	Site	4	18.217	5.6920E-13	***
	Insect x Site	4	2.114	0.0799	.
	Residual	112			
ANOVA: stress	Insect	1	13.369	3.0000E-04	***
	Site	2	11.591	2.6600E-05	***
	Insect x Site	2	0.279	0.7573	
	Residual	112			
ANOVA: log strain	Insect	1	8.338	4.7000E-03	**
	Site	2	29.509	5.0810E-11	***
	Insect x Site	2	4.254	0.0165	*
	Residual	112			

C. Branches

Model	Source	df	F-ratio	P-value	Signif.
MANOVA	Insect	1	1.100	0.3405	
	Site	1	73.639	6.7620E-16	***

	Insect x Site	1	0.020	0.9804	
	Residual	53			
ANOVA: stress	Insect	1	1.906 104.26	0.1732	
	Site	1	0	4.0370E-14	***
	Insect x Site	1	0.013	0.9096	
	Residual	53			
ANOVA: modulus	Insect	1	0.937	0.3375	
	Site	1	90.792	4.4300E-13	***
	Insect x Site	1	0.015	0.9024	
	Residual	53			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

1

2