Impact of hemlock woolly adelgid (*Adelges tsugae*) infestation on xylem structure and function and leaf physiology in eastern hemlock (*Tsuga canadensis*)

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Abstract. Hemlock woolly adelgid (*Adelges tsugae* Annand) (HWA) is an invasive insect that feeds upon the foliage of eastern hemlock (*Tsuga canadensis* (L.) Carrière) trees, leading to a decline in health and often mortality. The exact mechanism leading to the demise of eastern hemlocks remains uncertain because little is known about how HWA infestation directly alters the host's physiology. To evaluate the physiological responses of eastern hemlock during early infestation of HWA, we measured needle loss, xylem hydraulic conductivity, vulnerability to cavitation, tracheid anatomy, leaf-level gas exchange, leaf water potential and foliar cation and nutrient levels on HWA-infested and noninfested even-aged trees in an experimental garden. HWA infestation resulted in higher xylem hydraulic conductivity correlated with an increase in average tracheid lumen area and no difference in vulnerability to cavitation, indicating that needle loss associated with HWA infestation could not be attributed to reduced xylem transport capacity. HWA-infested trees exhibited higher rates of net photosynthesis and significant changes in foliar nutrient partitioning, but showed no differences in branch increment growth rates compared with noninfested trees. This study suggests that HWA-induced decline in the health of eastern hemlock trees is not initially caused by compromised water relations or needle loss.

Introduction

Hemlock woolly adelgid (HWA) (*Adelges tsugae* Annand) is an invasive insect introduced to the United States from East Asia. First reported in 1951 in the eastern United States (Souto *et al.* 1995), this insect has spread rapidly into the north-eastern United States and has been responsible for the decline and death of many eastern hemlock (*Tsuga canadensis* (L.) Carrière) trees (Orwig *et al.* 2002; Gómez *et al.* 2015). HWA feed on xylem ray parenchyma cells at the base of young needles, ultimately causing loss of vigour, premature needle drop and defoliation (Young *et al.* 1995; Stadler *et al.* 2005). HWA actively feed in the spring to early summer, aestivate during the warmer summer months and resume feeding again in the autumn (Stadler *et al.* 2005). Infestations of HWA can result in the death of mature eastern hemlocks within 4 years (McClure 1991).

Although the effect of HWA infestations on forest health are well documented (Orwig *et al.* 2013), little is known about this invasive insect's impact on physiological processes in eastern

hemlock during early stages of infestation. One possibility for how HWA impacts eastern hemlock is that consuming photosynthates stored in parenchyma cells causes needle loss and an overall reduction in growth and crown vigour (i.e. branch dieback, bud mortality) (McClure 1991; Young *et al.* 1995). A second possibility is that HWA infestation impairs water transport through the formation of abnormal xylem tissue (Domec *et al.* 2013). A common anatomical feature resulting from HWA infestation is the formation of false rings (Gonda-King *et al.* 2012), which are dark-coloured latewood bands of thick-walled tracheids that exhibit a gradual transition to earlywood on either side of the band (Stokes and Smiley 1996). False rings have been linked to decreased xylem hydraulic conductivity (Domec *et al.* 2013; but see Salleo *et al.* 2003) and are formed in some species in response to drought conditions (Hollingsworth and Hain 1992; Cherubini *et al.* 2003; Copenheaver *et al.* 2006). According to research linking the structural properties of tracheids and vulnerability to cavitation

(Mayr and Cochard 2003; Rosner *et al.* 2007), the thin-walled tracheids that form after false rings may increase the vulnerability of infested trees to cavitation (Copenheaver *et al.* 2006). However, the potential connection between vulnerability to cavitation and HWA infestation has not been tested.

Much research has focussed on trees that have been heavily impacted by chronic HWA infestation. Though they are informative in evaluating physiological parameters of heavily diseased trees during the onset of HWA-induced mortality, such results could be attributed to secondary responses to loss of vigour and needle loss, rather than the direct impact of the HWA infestation. In this study, we evaluate the influence of HWA infestation on (1) needle loss; (2) xylem structure and function (i.e. tracheid dimensions, branch increment growth rate, hydraulic conductivity and vulnerability to cavitation); (3) photosynthetic rate and stomatal conductance; and (4) foliar nutrient levels, as nutrient status represents another way that HWA could affect the vigour of eastern hemlock. Using infested and noninfested trees in an experimental garden, we integrate these measurements to explore the direct impact of HWA on young hemlock trees during the early stages of infestation.

Materials and methods

Study site and treatment

Experiments were conducted at an experimental site established by Miller-Pierce *et al.* (2010) to investigate the impact of infestation by HWA on the growth and foliar chemistry of eastern hemlock (*Tsuga canadensis* (L.) Carriè). Details of this experimental site are outlined in Miller-Pierce *et al.* (2010) and Gonda-King *et al.* (2012), and the relevant details are summarised as follows. In 2007, 0.7- to 1-m tall hemlock saplings were collected from Pelham, Massachusetts, a site that was, at the time, on the northern boundary distribution limit for HWA. Observations of both saplings and surrounding trees confirmed that insects were absent. The saplings were transplanted in a rectangular grid, spaced 2 m apart, in an open field located at East Farm, University of Rhode Island, (Kingston, Rhode Island; 41°28′23.5056′′N 71°30′37.53′′W). The mean annual temperature is 10.5°C and the mean annual precipitation is 1343 mm. Within a randomised complete block design, the trees in each row were randomly assigned a treatment. We conducted our tests on HWA-infested and noninfested (control) trees. Yearly inoculations with HWA occurred each spring from 2007 to 2011. To prevent cross-contamination, each tree was covered by a $1 \times 1 \times 2$ m (length × width × height) enclosure consisting of PVC piping and mosquito netting. Analyses of insect densities were conducted in autumn and spring from 2007 to 2010 (Miller-Pierce and Preisser 2012) and again in the summer of 2011 confirmed the treatments. In 2011, control trees had zero adelgids, whereas HWA-infested trees had an average of 2.6 adelgids per cm branch (Soltis and Orians, unpubl. data), which represents a moderate to heavy level of HWA infestation (Paradis 2011).

Collection of plant material from and measurement of six HWA-infested trees and eight control hemlock trees (~10 years old) occurred on the 9 and 16 June 2011 during the feeding cycle of the adelgid. For measurements of xylem structure and function at the site of infestation, two branches were removed from each tree with hand pruners in the early morning. This method of collection was replicated for control trees. Each branch was submerged in water during excision (Wheeler *et al.* 2013). After excision, each branch was immediately recut underwater to remove a 30-cm stem segment. Stem samples were kept underwater during transport to the laboratory and until processing. Branches and needles distal to the cut stem segment were collected, stored in large ziplock bags and kept cool for transport and until processing. To coincide with the duration of treatment, only branches 3–5 years old were used for analyses of hydraulic conductivity, vulnerability to cavitation, needle loss, wood anatomy, and growth rate.

Hydraulic conductivity

Measurements of the hydraulic conductivity of hemlock were conducted by measuring the flow driven by a gravity head through a stem sample to an analytical balance (Sartorius Model CPA225D) (Sperry *et al.* 1988). A perfusion solution of 10 mM KCl in deionised ultrafiltered water (MilliQ UV plus, Millipore) was used. Prior to all measurements, the perfusion solution was refiltered through a 0.2-μm syringe filter (Acrodisc syringe filters, Pall Corporation). From the 30-cm stem segments collected in the field, a section 3–5 years old, 3.25–7.7 mm in diameter and 14.5 cm in length was recut underwater. Prior to measurements, both ends of the segment were shaved with a sharp razor blade. Maximum conductivity (K_{max}) was measured after removal of embolisms by flushing stems with 10 mM KCl at 0.1 MPa for 20 min. Hydraulic conductivity was referenced to the xylem cross-sectional area (xylem-specific conductivity, K_s) or total downstream leaf area (leaf-specific conductivity, LSC) (Ewers and Zimmermann 1984). Xylem area for each branch segment was measured via image analysis (ImageJ Software, National Institutes of Health) of xylem cross-sections from the proximal end of each branch segment, and was calculated by taking the average diameter of two perpendicular cross-sectional axes. There was no significant difference in mean branch diameter between treatments ($P > 0.5$).

Leaf area distal to the measured branch segment was calculated by scanning fresh needles (Model LI-3100 Area Meter, LI-COR). The dry weight (DW) and scanned area (i.e. specific leaf area in $m^2 kg^{-1}$) of a subset of needles was used to estimate the total leaf area distal to the measured stem segment based on the DW of all needles from each sample (Domec *et al.* 2013). The Huber value, which represents the ratio of sapwood area to needle area, was used for calculations of LSC (Tyree and Zimmermann 2013).

Vulnerability to cavitation

The vulnerability of the xylem to cavitation was measured by following the centrifuge technique (Alder *et al.* 1997) with a perfusion solution of 10 mM KCl in deionised ultrafiltered water (MilliQ UV plus, Millipore). Prior to all measurements, the perfusion solution was re-filtered through a 0.2 μm syringe filter (Acrodisc syringe filters, Pall Corporation). Stem segments were prepared and hydraulic conductivity was measured as stated above. All stems were decorticated up to 5 mm at each end to allow for a proper seal during hydraulic conductivity

measurements and flushed with 10 mM KCl at 0.1 MPa for 20 min before spinning in the centrifuge. Hydraulic conductivity was measured after spinning at successively more negative pressures at the centre of rotation (0, –0.5, –1, –3, –5, –7 and –9 MPa; 3 min of rotation time for each pressure) and percent loss of conductivity (PLC) was calculated as $(100 \, (1 - K_{result} \, K_{max}^{-1})$, where K_{result} refers to conductivity following each spin and K_{max} is the maximum conductivity (flushed) measured before centrifugation. For comparisons between treatments, the point at which PLC = 50 and the mean cavitation pressure were used. Mean cavitation pressure was calculated by plotting vulnerability curves as the loss of hydraulic conductivity per unit of xylem pressure change (compared with PLC, which is plotted as the cumulative loss of conductance). The mean of this distribution was calculated based on the midpoint of each pressure change (Sperry and Ikeda 1997).

Needle loss resulting from HWA-infestation

HWA-induced needle loss was estimated by calculating the relative needle biomass, which represents the ratio of branch needle biomass to branch cross-sectional area (Långström *et al.* 2004). The total dry mass of all needles distal from the branch excision point was correlated with the branch cross-sectional area. There was no significant difference in mean branch diameter between treatments ($P > 0.5$) and needles collected for analyses of needle loss represented multiple years of growth. Although the possible impacts of HWA-infestation on branch extension were not evaluated in this study, an analysis of needle density via image analysis (ImageJ Software, National Institutes of Health) on 3-cm-long segments of current-year shoots (i.e. needles formed from budbreak to the June sampling date 2011) showed no difference in needle production between treatments ($P > 0.5$). Based on this analysis of needle production, differences in relative needle biomass between treatments are assumed to be a result of feeding by HWA and induced needle loss in prior growing seasons.

Wood anatomy and growth rate

From 30-cm branch segments collected in the field, a distal portion of the branch segment used for hydraulic measurements was collected and fixed in formalin : acetic acid : ethyl alcohol (1 : 1 : 9) for anatomical measurements. The base of each segment was mounted on a sliding microtome and two 10-µm sections per branch were taken. Sections were placed in 0.1% safranin O stain solution (filtered through a 0.2-µm syringe filter) for 2 min and rinsed in deionised ultrafiltered water for 2 min. Sections were then mounted on glass slides with 50% glycerol to prevent dehydration. Analysis of cross-sections was conducted using fluorescent microscopy at 200× magnification. Samples were photographed in a series of four radial files extending from the bark to the pith. In each sample, one radial file free of compression wood was analysed for tracheid dimensions by use of ImageJ Software (Jansen and Choat 2011; Scholz *et al.* 2013); an average of 958 tracheids per stem were measured. Average tracheid lumen area (A_t) was calculated as the total lumen area in each image divided by the total number of tracheids in each image. Tracheid diameter was calculated to determine the hydraulically weighted mean

diameter (D_h) based on the calculation $D_h = \sum d^5 \div \sum d^4$, where d is the diameter of a conduit (Sperry *et al.* 1994). Based on the Hagen–Poiseuille law, the D_h weights tracheid diameter with the estimated hydraulic conductance of the conduits. Growth rate was calculated by dividing the cross-sectional area of each branch by the number of growth rings excluding rings that were formed before the start of the experimental treatments, resulting in an average branch area increment growth (expressed as $mm^2 \, year^{-1}$) after treatment (Speer 2010).

Photosynthesis and stomatal conductance

Six HWA-infested trees and eight control trees were measured for net photosynthetic rate and stomatal conductance on 16 June 2011 at 0800–1100 hours. Measurements were made on HWA-free current-year needles from randomly chosen, sun-exposed, upper canopy branches using a portable photosynthesis system (Model LI-6400, LI-COR). Use of a Li-Cor Needle Chamber (Model 6400–07, LI-COR) with a clear Propafilm (Innovia Films Inc.) window chamber top allowed for measurements under natural light with ambient air conditions (PAR, 1600 µmol m^{-2} s^{-1}; CO_2, 371 µmol mol^{-1}; average temperature, 31°C). Three measurements per branch were taken. A photograph through the clear chamber top was taken of each set of needles measured, which was used to calculate needle area via image analysis (ImageJ Software, National Institutes of Health). The net rate of photosynthesis was adjusted on the basis of needle area. Needles shaded by the stem or other needles were not included in the calculation of needle area. There was no significant difference in needle overlap (i.e. shading) or needle area in the gas exchange chamber between treatments ($P > 0.5$).

Leaf water potential

Leaf water potential was measured on 9 and 16 June 2011 with a Scholander pressure-bomb (Scholander *et al.* 1965). At predawn and at midday, two 10-cm-long current-growth shoots per tree were collected, sealed in individual ziplock bags with a wet paper towel and covered in a dark container. Samples were immediately transported back to the laboratory for processing. For each sample, the entire 10-cm shoot was placed inside the pressure-bomb chamber for measurement.

Foliar chemical analysis

From branches removed for measurements of hydraulic conductivity and vulnerability to cavitation, needles of mixed age classes were collected and oven-dried at 55°C. Levels of %N in addition to P, K, B, Fe, Na, Ca, Mg, Zn, Mn and Cu were measured using the method of inductively-coupled plasma atomic emission spectrometry performed by the University of Massachusetts Soil and Plant Tissue Testing Laboratory, Amherst, MA, USA.

Statistical analysis

Prior to all analyses, a Levene's test for equality of variances was run. Independent two-sample *t*-tests were used to compare differences in means between HWA-infested and control (HWA-free) trees. All analyses were performed with SPSS statistical software (IBM Corporation).

Results

Hydraulic conductivity and cavitation resistance

We found differences in xylem hydraulic conductivity but not in vulnerability to cavitation. Specifically, there were no differences in the point at which PLC = 50 ($P > 0.1$) or mean cavitation pressure ($P > 0.1$) between the two treatments (Fig. 1). However, we did find significant differences in native K_s and LSC between HWA-infested and control trees (Fig. 2). Specifically, native K_s ($P = 0.03$) and native LSC ($P < 0.001$) were higher in HWA-infested versus control trees. There were no differences between native and flushed K_s, and between native and flushed LSC within treatments ($P > 0.1$). There were no significant correlations between stem age and K_s or LSC within treatments ($P > 0.1$). Between-treatment differences in LSC were largely driven by the smaller leaf area distal to the measured branch segments from the HWA-infested trees (Table 1).

Needle loss, wood anatomy and growth rates

HWA-infested trees had significantly lower relative needle biomass ($42.5 \pm 8.1\,\mathrm{g\,cm^{-2}}$) than control trees ($60.0 \pm 2.7\,\mathrm{g}$ $\mathrm{cm^{-2}}$), which we interpret as evidence of HWA-induced needle loss ($P < 0.05$; Table 1). HWA-infested trees had larger A_t and higher D_h than control trees ($P < 0.05$; Table 1). The treatments did not differ in branch area increment growth rates ($P = 0.34$; Table 1).

Photosynthesis and stomatal conductance

Net photosynthetic rate differed between treatments ($P = 0.038$; Fig. 3a), with higher rates in HWA-infested than in control trees. Although there was a trend for higher rates of stomatal conductance in HWA trees than in control trees, there was no significant difference between treatments ($P = 0.155$; Fig. 3b).

Leaf water potential

Measurements of leaf water potential at predawn and midday showed no significant treatment differences ($P = 0.219$ and $P = 0.181$, respectively). Mean predawn leaf water potentials ($\pm$ s.d.) for HWA-infested trees and control trees were 0.18 ± 0.03 MPa and 0.16 ± 0.04 MPa, respectively. Mean midday leaf water potentials ($\pm$ s.d.) for HWA-infested trees and controls trees were 1.08 ± 0.1 MPa and 1.13 ± 0.09 MPa, respectively. At midday water potentials of around -1.0 MPa, trees are not predicted to experience significant cavitation.
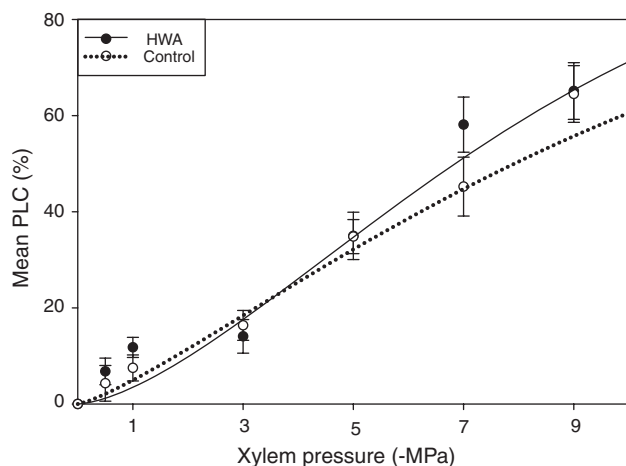

Fig. 1. Treatment differences in the percent loss of conductivity (PLC) under increasing pressure for *T. canadensis*. The data are fitted by least-squares regression with a Weibull function. There are no treatment differences in the point at which PLC = 50 ($P > 0.1$) or mean cavitation pressure ($P > 0.1$). Values are the average PLC $\pm$ s.e. (hemlock woolly adelgid (HWA) $n = 9$; control $n = 12$).
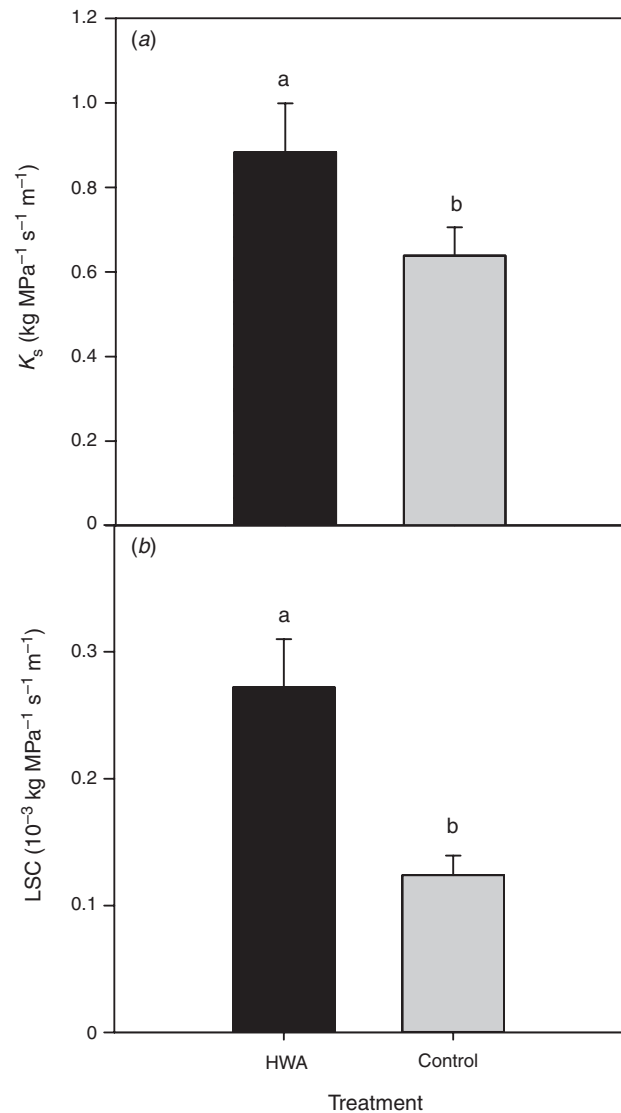

Fig. 2. Comparison of hydraulic conductivity in *T. canadensis* across treatments with the hemlock woolly adelgid (HWA) and the control. Values were adjusted for (a) xylem-specific conductivity (K_s) and for (b) leaf-specific conductivity (LSC). Values are means $\pm$ s.e. (HWA $n = 10$; control $n = 13$). Different lowercase letters are significantly different according to an independent two-sample t-test ($P < 0.05$).

Table 1. Treatment differences in Huber value (sapwood area divided by leaf area), estimated needle loss, wood anatomy and growth rates of *T. Canadensis* infected with hemlock woolly adelgid (HWA) and uninfected controls

Means ($\pm$ s.e.) in the same row with different lowercase letters are significantly different ($P \leq 0.05$; HWA $n = 10$; control $n = 12$). RNB, relative needle biomass (i.e. estimated needle loss calculated as branch needle biomass divided by branch cross-sectional area); A_t, average tracheid lumen area; D_h, hydraulically weighted mean diameter (HWA $n = 10$; control $n = 11$); BAI, branch area increment growth rate (HWA $n = 10$; control $n = 13$)

	HWA	Control
Huber value (10^{-4} cm^2/cm^2)	$3.4 \times 10^{-4} \pm 5.0 \times 10^{-5}$a	$1.9 \times 10^{-4} \pm 1.9 \times 10^{-5}$b
RNB (g cm^{-2})	42.6 ± 8.1a	60.0 ± 2.7b
A_t (μm^2)	135.3 ± 11.1a	96.6 ± 10.2b
D_h (μm)	16.1 ± 0.6a	14.0 ± 0.7b
BAI (mm^2 year^{-1})	8.2 ± 1.2a	7.5 ± 1.1a

Foliar chemistry

An analysis of the total foliar chemistry of hemlock needles found significant differences between treatments (Table 2). Compared with control trees, HWA trees exhibited higher levels of %N in addition to higher levels of P, K, B, Fe and Na. There were no treatment differences in the foliar levels of Ca, Mg, Zn, Mn or Cu.

Discussion

The goal of this study was to shed light on the physiological processes by which HWA affects the health and vigour of young hemlock trees. More specifically, we tested the hypothesis that HWA infestation leads to an impairment in the water transport capacity of the xylem either through alterations in xylem anatomy or increased vulnerability to cavitation. Although we found evidence of significant alterations in xylem anatomy, the higher K_s observed in HWA-infested plants in this study, coupled with the lack of any impact on vulnerability to cavitation, suggests that HWA-induced needle loss at the early stage of infestation was not the result of impaired water supply. Instead, both needle loss and higher K_s contributed to higher water supply capacity on a per-leaf area basis (LSC). This did not, however, translate into higher stomatal conductance or leaf water potential, indicating that branch hydraulic conductivity did not limit stomatal apertures in these plants.

A previous study (Domec *et al.* 2013) also found that HWA infestation led to an increase in water supply capacity on a leaf area basis (LSC). However, the increase in LSC was driven entirely by needle loss; the hydraulic conductivity of the wood (K_s) was lower in the infected plants. In contrast, in our study, HWA infestation led to an increase in A_t and higher K_s. Although we do not know the reason for this difference, the fact that the trees in this study were younger in age (~10 yr old and ~2 m in height versus ~40 years old) and growing in an open garden where resource availability is expected to be high may be relevant. Additionally, differences in temperature and evaporative demand between the two sites (North Carolina
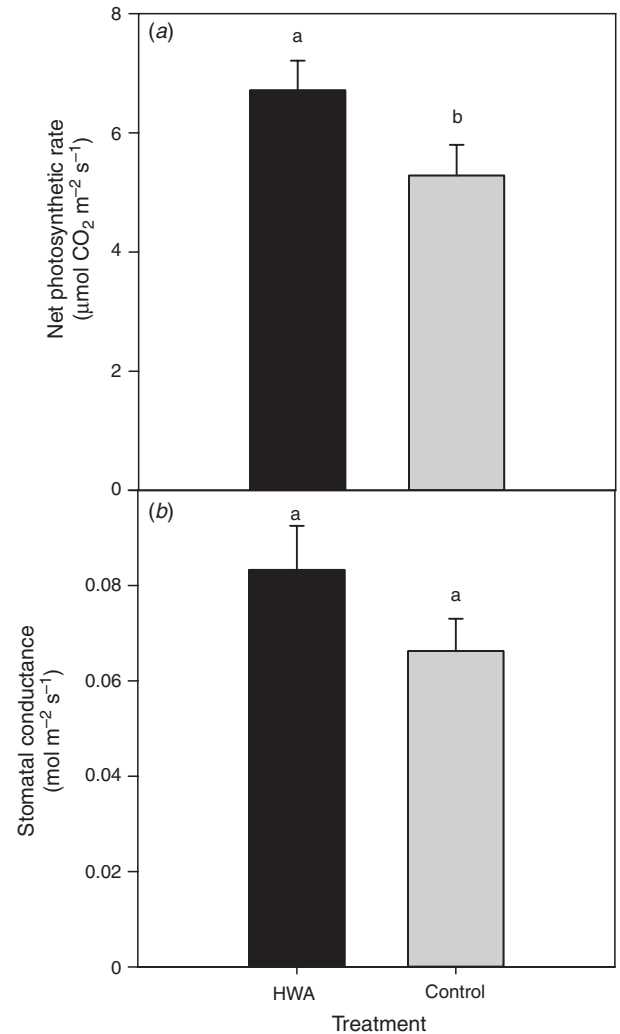

Fig. 3. Treatment differences in *T. canadensis* (*a*) net photosynthetic rate and (*b*) stomatal conductance with values as means $\pm$ s.e. (hemlock woolly adelgid (HWA) $n = 6$; Control $n = 8$). Different lowercase letters are significantly different according to an independent two-sample *t*-test ($P < 0.05$).

versus Rhode Island) could also contribute to dissimilarity in the impact of HWA on xylem structure.

One important component of hemlock's response to infestation appears to be the greater occurrence of false rings, which are known to occur following both artificial and insect-induced defoliation (Salleo *et al.* 2003; Thomas *et al.* 2006; Rossi *et al.* 2009). On the same plants as in this study, Gonda-King *et al.* (2012) calculated that HWA-infested trees have 50% more false rings. Similarly, Domec *et al.* (2013) reported abnormal wood production, including the presence of false rings in HWA-infested eastern hemlock trees growing in North Carolina. Favourable growing conditions such as adequate light and minimum competition for resources from neighbouring plants could have resulted in the observed increases in A_t and D_h as xylogenesis resumes following periods of stress and false ring formation (Wimmer *et al.*

Table 2.   Analysis of treatment differences in foliar chemistry of *T. canadensis* infected with hemlock woolly adelgid (HWA) and uninfected controls

Means ($\pm$ s.e.) in the same row with different lowercase letters are significantly different ($P \leq 0.05$; HWA $n = 12$; control $n = 15$). ppm, parts per million

Element	HWA Mean $\pm$ s.e.	Control Mean $\pm$ s.e.
%N	2.26 $\pm$ 0.08a	1.96 $\pm$ 0.06b
P (ppm)	2642 $\pm$ 103a	2238 $\pm$ 106b
K (ppm)	5189 $\pm$ 276a	4441 $\pm$ 267b
B (ppm)	28.4 $\pm$ 1.8a	21.0 $\pm$ 0.9b
Fe (ppm)	47.6 $\pm$ 2.3a	42.6 $\pm$ 0.9b
Na (ppm)	91.9 $\pm$ 4.9a	75.1 $\pm$ 3.5b
Ca (ppm)	3429 $\pm$ 297a	3477 $\pm$ 293a
Mg (ppm)	1244 $\pm$ 106a	1372 $\pm$ 105a
Zn (ppm)	19.5 $\pm$ 2.1a	17.2 $\pm$ 1.3a
Mn (ppm)	26.6 $\pm$ 3.4a	26.6 $\pm$ 5.5a
Cu (ppm)	3.74 $\pm$ 0.34a	3.55 $\pm$ 0.34a

2000). Although there was no treatment-induced difference in branch area increment growth, our findings are consistent with Salleo *et al.* (2003), who showed that insect-induced defoliation events increased false ring density, which in turn was associated with the production of more wood per year with wider conduits and a higher conductive area. Other studies on false ring formation after artificial or insect-induced defoliation events found no overall change in average tracheid lumen area (Thomas *et al.* 2006; Rossi *et al.* 2009), providing further support for false rings not necessarily leading to lower water transport capacity.

We observed higher photosynthetic rates in trees experiencing early stages of HWA infestation. As trees suffer more chronic infestations of HWA, photosynthesis and conductance rates have been shown to decline (Rubino *et al.* 2015). The higher rates of photosynthesis observed here corresponds well with the higher foliar N content of these plants and could have contributed to their sustained rate of wood production. Previous work showed that the %N levels of HWA-infested foliage were lower in the first year than in noninfested trees (Miller-Pierce *et al.* 2010) but were higher in chronically infested individuals (Stadler *et al.* 2005; Gómez *et al.* 2012; Domec *et al.* 2013). One explanation for this pattern is that HWA-induced increases in both the foliar %N and photosynthetic rates could be a stress response used to compensate for depleted photosynthate stores and loss of foliage (Eyles *et al.* 2011), or changes in sink strength (Domec *et al.* 2013). This is consistent with increased levels of both proline, an amino acid associated with water stress, and glutamine, a product of increased glutamine synthetase activity, which have been observed in HWA-infested trees (Rhodes *et al.* 1999; Miflin and Habash 2002; Gómez *et al.* 2012). We also found significantly higher levels of foliar P, K, B, Fe and Na in trees subjected to HWA infestation, which could be driven by the demand for ions such as P (involved in protein synthesis), K (a key component of stomatal regulation and ATP synthesis via photophosphorylation), Fe (an essential element for chlorophyll development and function) and B

(involved in membrane integrity and function, particularly facilitating transport of K for guard cell regulation) by higher rates of photosynthetic activity (Marschner 1995).

Changes in foliar cations and leaf chemistry could impact the health of hemlock trees in ways that we are only beginning to understand. Gómez *et al.* (2012) proposed that the seasonal feeding habits of HWA trigger intermittent stress events, resulting in elevated foliar nutrient levels, which could, in turn, make individual eastern hemlocks more palatable to HWA. This theory is supported by a survey showing that foliar chemistry (particularly increased N and K) was correlated with the susceptibility of eastern hemlock to HWA (Pontius *et al.* 2006). Recent work by Gonda-King *et al.* (2014) also demonstrates a strong correlation between the physiology of infested hemlocks and the life-stage of the HWA. They found that high leaf N levels were only present during diapause (summer months); after diapause (autumn months), infested hemlocks exhibited decreased water potential and photosynthesis. Lastly, Radville *et al.* (2011) found evidence of a hypersensitive response by detecting elevated levels of hydrogen peroxide in HWA-infested foliage. After also detecting high levels of H_2O_2 in HWA-free needles growing on infested trees, Radville *et al.* (2011) suggested that HWA infestation results in a systemic defence response, providing support for the hypothesis that HWA-induced mortality in eastern hemlocks could be caused by hypersensitive response in foliar tissue.

Our study suggests that changes in xylem properties may not be responsible for the reduction in leaf area that results from early HWA infestation. However, as trees are weakened as a result of prolonged HWA infestation, impaired stem hydraulics may develop as a secondary response. HWAs appear to impact eastern hemlock in a variety of ways. For example, Nuckolls *et al.* (2009) reported that fine root biomass of eastern hemlock decreased following infestation with HWA, which could indicate disruptions in whole-tree budgeting of nonstructural carbohydrates. In fact, Soltis *et al.* (2015) found that HWA infestation altered the nonstructural carbohydrate distributions, resulting in higher starch storage in older branches than in new needles. Regarding the biomechanical properties of the xylem, recent work showed that HWA decreases the strength and flexibility of branches proximal to the infestation site (Soltis *et al.* 2014). Thus the correlation we observed between increased hydraulic conductivity and tracheid lumen area could come at a cost if larger tracheids compromise the biomechanical integrity of the wood. In light of these and previous findings, more work is needed to further understand the complexity of stress responses in eastern hemlock in relation to plant age, environmental conditions, the duration and intensity of infestation, and life-stages of HWA.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

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