

Hemlock woolly adelgid (*Adelges tsugae*) infestation affects water and carbon relations of eastern hemlock (*Tsuga canadensis*) and Carolina hemlock (*Tsuga caroliniana*)

Jean-Christophe Domec^{1,2}, Laura N. Rivera², John S. King², Ilona Peszlen³, Fred Hain⁴, Benjamin Smith² and John Frampton²

¹University of Bordeaux, Bordeaux Sciences Agro UMR INRA-TCEM 1220, 33195, Gradignan, France; ²Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC 27695, USA; ³Department of Forest Biomaterials, North Carolina State University, Raleigh, NC 27695, USA; ⁴Department of Entomology, North Carolina State University, Raleigh, NC 27695, USA

Author for correspondence:

Jean-Christophe Domec

Tel: +1 919-687-7639

E-mail: jdomec@ncsu.edu

Received: 13 September 2012

Accepted: 7 March 2013

New Phytologist (2013) **199**: 452–463

doi: 10.1111/nph.12263

Key words: carbon isotope, hydraulic conductivity, mortality, soil–plant–atmosphere model, stomatal conductance, traumatic resin canals, water potential, wood anatomy.

Summary

- Hemlock woolly adelgid (HWA) is an exotic insect pest causing severe decimation of native hemlock trees. Extensive research has been conducted on the ecological impacts of HWA, but the exact physiological mechanisms that cause mortality are not known.
- Water relations, anatomy and gas exchange measurements were assessed on healthy and infested eastern (*Tsuga canadensis*) and Carolina (*Tsuga caroliniana*) hemlock trees. These data were then used in a mechanistic model to test whether the physiological responses to HWA infestation were sufficiently significant to induce changes in whole-plant water use and carbon uptake.
- The results indicated coordinated responses of functional traits governing water relations in infested relative to healthy trees. In response to HWA, leaf water potential, carbon isotope ratios, plant hydraulic properties and stomatal conductance were affected, inducing a reduction in tree water use by > 40% and gross primary productivity by 25%. Anatomical changes also appeared, including the activation of traumatic cells.
- HWA infestation had a direct effect on plant water relations. Despite some leaf compensatory mechanisms, such as an increase in leaf hydraulic conductance and nitrogen content, tree water use and carbon assimilation were diminished significantly in infested trees, which could contribute to tree mortality.

Introduction

Hemlock trees dominate almost 1 million hectares of forest throughout eastern North America from the southern Appalachians to southern Canada and west to the central Lake States. These forests are on the verge of disappearing because of the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, a significant ecological pest that was introduced from Japan (c. 1955) to the eastern USA (Kizlinski *et al.*, 2002; Havill *et al.*, 2006). In Asia, HWA does not cause severe destruction of its hosts because of native host resistance (McClure, 1996; Havill *et al.*, 2006). In the eastern USA, HWA harms native hemlocks as it extracts through its stylet the carbohydrates from the xylem ray parenchyma cells at the base of needles from the current year's growth (Young *et al.*, 1995). The tree responds by stopping growth and by dropping needles (McClure, 1991). After the current year's needles have been shed from the tree, HWA moves to the next most recent growth. Since its introduction, HWA has caused the severe decline of eastern hemlock, *Tsuga canadensis* (L.) Carr.,

and Carolina hemlock, *Tsuga caroliniana* Engelm., populations (Evans & Gregoire, 2007; Krapfl *et al.*, 2011). The average rate of HWA spread over the past decade has been c. 25 km per year and, at this time, cannot be managed in forests. HWA-induced mortality can be explosive, exceeding 80% within 4 yr of infestation (Orwig *et al.*, 2002; Small *et al.*, 2005). The exact mechanism of mortality in hemlock trees is not known, but one hypothesis postulates that an excessive amount of photosynthate from the tree is extracted by infestation (Broeckling & Salom, 2003), leading to a more general mechanism applicable to all tree species that involves the cessation of metabolism or failure to defend against further biotic attack because of a negative carbon budget (McDowell, 2011).

An alternative hypothesis, put forth for the first time here, is that the trees may be responding to the feeding of HWA by a reaction similar to that of Fraser fir (*Abies fraseri*, Pursh.) to infestation by balsam woolly adelgid (BWA; *Adelges picea* Ratz; Balch *et al.*, 1964). BWA feeds predominantly on the trunk, and infested trees respond rapidly by releasing phenolic compounds

that induce the formation of abnormal xylem, which consists of shorter tracheids, thicker cell walls and smaller lumen (Puritch, 1977). Such abnormal wood formation induces a reduction in xylem permeability (Puritch, 1971; Spicer & Gartner, 1998), affecting whole-plant water acquisition and growth (Arthur & Hain, 1986; Hollingsworth *et al.*, 1991). In Fraser fir, trees also succumb to death within 4 yr (Hollingsworth & Hain, 1991). Given the similarity of time infested, mortality and the proportion of the tree infested (throughout the entire needle-bearing fraction), it is hypothesized that eastern and Carolina hemlocks infested with HWA are exhibiting a reaction similar to that experienced by Fraser fir in response to BWA. We therefore also expect that such a response to infestation is likely to induce changes in stem anatomical properties and, in turn, the ability to move water.

At the whole-tree level, the developmental shift caused by a decrease in leaf area to sapwood area ratio following defoliation from HWA is expected to increase the overall tree hydraulic conductance per unit leaf area (K_{tree} ; Pataki *et al.*, 1998). However, although many studies have examined the relationship between K_{tree} and water loss, K_{tree} remains a complicated factor because it is dependent on the sapwood to leaf area ratio as well as on the root, stem and leaf conductivities. As HWA only feeds on needles, leaf water transport capacity, quantified in terms of leaf hydraulic conductance (K_{leaf}), should be primarily impaired and, together with the decline in leaf area, should be expected to act as one of the main components influencing K_{tree} . Consistent with earlier investigations documenting the coordination of stomatal conductance (g_s) with K_{tree} (Cochard *et al.*, 2002; Meinzer *et al.*, 2004), K_{leaf} and its relationship to stomatal control have been investigated recently, revealing that, in conifers, g_s is very sensitive to K_{leaf} within and among species (Woodruff *et al.*, 2007; Domec *et al.*, 2009a; Johnson *et al.*, 2009). However, little is known about the suites of functional traits involved in limiting the dynamic coordination between K_{tree} , K_{leaf} , g_s and leaf water potential (Ψ_L) in infested trees. A broader understanding of the patterns of stomatal regulation of Ψ_L and transpiration in HWA-infested trees requires a knowledge of the hydraulic architecture of the branches to which the leaves are attached and of the leaves themselves. In addition to direct hydraulic measurement, the use of stable carbon isotope ratios ($\delta^{13}\text{C}$) as an index of gas exchange can be useful to investigate the effect of HWA on growth. Tree ring $\delta^{13}\text{C}$ is particularly relevant because it provides a whole-crown-weighted record of gas exchange that can be discretized to individual years, allowing analyses of the response of individual trees to known years of insect attack (McDowell *et al.*, 2010).

The objective of this study was to ascertain whether the water relations of eastern and Carolina hemlock infested with HWA are being affected in a manner similar to that of BWA-infested Fraser fir. Our first hypothesis was that, in eastern and Carolina hemlocks, HWA infestation impairs both leaf water transport capacity and xylem hydraulic characteristics through the production of abnormal xylem cells. Our second hypothesis was that these effects reduce the water transport capacity at the whole-tree level and influence Ψ_L and g_s . Our third hypothesis was that, when the physiological measurements from single trees are scaled

up, HWA infestation influences whole-stand water use and contributes to a significant reduction in carbon uptake. In order to test these hypotheses, anatomical and physiological measurements related to leaf and stem water transport were measured in healthy and infested hemlocks. These measurements were then used in a process-based model to test whether the physiological responses to HWA infestation were sufficiently significant to induce a change in plant water use and thus plant carbon assimilation at the tree and stand level.

Materials and Methods

Study site and experimental design

The study site was located on the campus of Montreat College in the Southern Appalachian Mountains (35°38'44.62"N, 82°18'14.35"W; elevation, 797 m). The mean annual temperature is 13.3°C and the average annual precipitation is 1218 mm (Black Mountain Digital Media, Black Mountain, NC, USA; <http://www.blackmtdigitalmedia.com/weather.html>). This site was chosen because of the close proximity of mature healthy and infested trees. The field sample size consisted of 15 trees of roughly the same size (*c.* 40 yr old; diameter at breast height, 0.51 ± 0.05 m) and randomly distributed within the plot. Nine trees (four healthy and five infested) were eastern hemlock and six trees (three healthy and three infested) were Carolina hemlock. HWA had been present at the site for 4 yr at the start of the study, but some of the trees (healthy) were being treated with foliar- and soil-injected imidacloprid (Bayer Corp., Kansas City, MO, USA) to limit infestation. The healthy trees had minimal to no needle loss as a result of adelgid infestation. All the non-treated trees were, however, heavily infested, and had an estimated 20–60% needle loss in Carolina hemlock and >60% needle loss in eastern hemlock. Most untreated trees continued to decline over the course of this study, and two of the infested eastern hemlock died before the end of this study. For both species, we visually classified the branch health from 1 to 5, with 1 being completely healthy (no needle loss) and 5 being almost entirely defoliated.

Plant water potential and stomatal conductance

Diurnal changes in leaf water potential (measured every 2 h from 05:30 to 19:30 h) were determined on three separate branches sampled from the upper crown of each tree using a pressure chamber (PMS Instrument, Albany, OR, USA). To encompass a large range of soil moisture, as seen from the decline in predawn water potentials (Ψ_{PD}) which fell from -0.3 MPa in spring to -1.2 MPa in summer, measurements were made from early June to late August of 2007 and 2008. In 2008, on the same days on which the leaf water potentials were measured, the stomatal conductance (g_s) was measured on five branches per treatment between 11:00 and 13:00 h using a steady-state porometer mounted with a narrow aperture chamber (Model Li-1600-01; Li-Cor Inc., Lincoln, NE, USA). Water potentials and g_s were measured on branches classified as 1 for the healthy trees

(all branches were classified as 1 on these trees) and 3–4 on infested trees (> 50% needle loss).

Carbon isotope composition

Branch wood tissue samples were collected in August 2007 and January 2008 for carbon isotope analysis of the branch and leaf tissues. On the infested trees, three branch and three leaf tissues were selected from branches classified as 3–4. In August 2007, six branches were sampled from each treatment and, in January 2008, four branches from the infested trees (classified as 3–4) and two branches from the healthy trees were collected. Each branch sample from both collection times was immediately separated into year of growth (current, second year, third year and fourth year). The second year's growth was selected for analysis because it was the most recently completed growth season. The oven-dried samples (80°C) were separated into branch and leaf tissue, and then ground to a fine powder with a mortar and pestle, after being frozen with liquid nitrogen. Wood powder was washed with a mixture of toluene–ethanol to remove extractives, which also removed nonstructural carbohydrates, ensuring that the carbon isotope composition reflected entirely the carbon assimilated in the corresponding year. Plant material was analyzed for C and N concentration and ^{13}C natural abundance ($\delta^{13}\text{C}$; Cornell University Stable Isotope Laboratory, Ithaca, NY, USA). The reported $\delta^{13}\text{C}$ values were based on the PeeDee Belemnite standard (Craig, 1957). A reduction (more negative values) in $\delta^{13}\text{C}$ is assumed to reflect improved water use efficiency (WUE) over the period of time over which carbon was assimilated (Ehleringer, 1993).

Hydraulic conductivity

For both species, hydraulic measurements were performed on samples cut in January 2008. Samples included three branches from infested trees (50–70% needle loss, scale of 3–4) and three branches from healthy trees (0% needle loss, scale of 1). One of the infested eastern hemlocks used for the January sample died over the winter, so that, during the July sampling, it was replaced with another infested tree.

Samples (whole branches) were immediately frozen and transported to the laboratory at North Carolina State University. Samples were stored in a chest freezer for 2 months before analysis with a hydraulic conductance flow meter (HCFM-XP; Dynamax Inc., Houston, TX, USA) applied in a quasi-steady-state mode, that is, under conditions in which flow and applied pressure were approximately constant with time (Tyree *et al.*, 1993). Preliminary results showed that freezing the samples did not affect the hydraulic conductivity measurements, as also shown previously in two other conifer species (Reid *et al.*, 2003; Rhea, 2010). The HCFM allows the perfusion of water into the base of a branch whilst measuring the flow rate of water into that branch. The whole-branch hydraulic conductance can then be calculated from the applied pressure and flow rate. Analysis began by attaching the whole-branch sample (stem and needles), freshly cut, to the HCFM at approximately the level of 4 yr of growth. This

branch length was chosen because these trees did not retain their needles after the third year. This measurement was recorded as the hydraulic conductance of the whole branch including the stem and leaves ($k_{\text{stem-leaf}}$, $\text{mmol s}^{-1} \text{MPa}^{-1}$). A final conductance measurement after removing all the needles (k_{stem}) was used to calculate the leaf hydraulic conductance K_{leaf} ($\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$) as:

$$K_{\text{leaf}} = 1 / ((1/k_{\text{stem-leaf}} - 1/k_{\text{stem}}) \times A_L) \quad \text{Eqn 1}$$

(A_L , leaf area).

After achieving stable readings with the HCFM, the distal part of the stem was cut off. The remaining 10–12 cm of basal segment of the stem, left connected to the pressure coupling, was perfused again to determine the specific hydraulic conductivity of stem xylem (k_s , $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$):

$$k_s = (k_{\text{segment}} \times L) / A_S \quad \text{Eqn 2}$$

(k_{segment} ($\text{kg s}^{-1} \text{MPa}^{-1}$), hydraulic conductance of the stem (branch xylem); L , length of the segment; A_S , cross-sectional sapwood area). Further, the leaf specific conductivity (LSC, $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$), which represents the efficacy of a branch in conducting water on a leaf area basis, was determined as:

$$\text{LSC} = k_s \times A_S / A_L \quad \text{Eqn 3}$$

Leaf area was determined by first scanning a subsample of fresh needles (Epson Perfection V700 PHOTO; Epson America, Inc., Long Beach, CA, USA) and by measuring their leaf area with Image-J software (National Institutes of Health, Bethesda, MD, USA). Then, the scanned needles and the remaining needles of each sample were weighed, and the total leaf area was estimated on the basis of the specific leaf area (SLA, in $\text{m}^2 \text{kg}^{-1}$) of the scanned needles.

Wood anatomy

Wood anatomy was observed on samples used for hydraulics. Samples were microtomed (Model 57951; Spencer Lens Co., Buffalo, NY, USA) to 30- μm cross-sections and stained in a solution of 0.1% safranin-O. Analysis of the cross-sections was conducted under light microscopy mounted with a digital camera. Samples were photographed and analyzed with Image-Pro software (Media Cybernetics, Inc., Bethesda, MD, USA). Early- and latewood proportions, together with ray counts, were assessed at $\times 100$ magnification on all of the growth rings present in a sample. When false rings within the abnormal wood were present, they were treated as separate rings on which early- and latewood proportions were determined. Tracheid cell wall thickness and lumen diameter were measured using $\times 200$ magnification. For each sample separately, two radial files of tracheids from pith to bark were randomly selected and all the tracheids in each file were measured. One of the two radii measured was located in the area opposite the greatest amount of compression wood, which corresponds to the trunk section that was subjected to mechanical

stress and that occurred in both healthy and infested trees (not to be confused with abnormal wood). The other radius measured was perpendicular to the first radius, thus minimizing the amount of compression wood in the measurements. The tracheid diameter distributions were also estimated for the calculation of the mean hydraulic diameter ($D_h = \Sigma d^5 / \Sigma d^4$, where d is the individual conduit diameter; Kolb & Sperry, 1999). Inside tracheid diameters were calculated by measuring the lengths of lines drawn across the lumen. Hacke *et al.* (2001) demonstrated that water-filled conduits experience large tensile hoop stresses that could lead to cell collapse under drought conditions. The basis for this relationship is that the double cell wall shared by adjacent cells behaves in a manner similar to a long plate of width b (cell diameter) and thickness t (taken as the distance across the double cell wall between two adjoining tracheid lumen), and this plate will buckle under a force proportional to (t/b) . Therefore, after the determination of the mean hydraulic diameter for a section, we made measurements of (t/b) and expressed it as $(t/b)^2$, so that the results were more directly comparable with previously published data, and could be used as an anatomical indicator of xylem water stress resistance (Hacke *et al.*, 2001; Domec *et al.*, 2009b). About 100–140 tracheids were used to measure t and b .

Modeling

To predict the effect of HWA infestation on plant water and carbon exchange, we used the soil–plant–atmosphere (SPA) model which is designed to represent processes common to vascular plants (Williams *et al.*, 1996, 2001; Hill *et al.*, 2008). The scale of parameterization (leaf level) and prediction (canopy level) of the SPA model have been designed to allow the model to provide a tool for the scaling up of leaf-level processes to canopy and landscape scales. SPA is a process-based model that simulates ecosystem photosynthesis and tree water use at fine temporal and spatial scales (30-min time step, multiple canopy and soil layers). In the model, the maximum flux rate of water through vegetation is determined by the difference between the soil and leaf water potential, and is controlled by the tree hydraulic conductance (K_{tree}). Leaf to air energy, water and CO₂ exchange consists of a coupled photosynthesis model (Farquhar model) with a stomatal conductance model. Allometric (leaf area index, tree size), hydraulic (LSC, K_{tree} , minimum leaf potential) and leaf nitrogen content used to drive the model were measured directly and are presented throughout the results (Supporting information Table S1). We estimated K_{tree} from the relationship between the rates of single-leaf transpiration (E , mmol m⁻² s⁻¹) and soil–leaf water potential difference (Loustau *et al.*, 1998). The soil water potential (Ψ_{soil}) was considered to be very close to Ψ_{PD} in the leaves (Breda *et al.*, 2006), and thus K_{tree} was taken as:

$$K_{tree} = E / (\Psi_{PD} - \Psi_{leaf}) \quad \text{Eqn 4}$$

Using the ohm (electrical resistance) analogy applied to a hydraulic circuit, and because K_{tree} is equivalent to $K_{root-leaf}$ the

root to stem hydraulic conductance component of the model ($K_{root-stem}$) was calculated as: $K_{root-stem} = (K_{tree} \times K_{stem-leaf}) / (K_{stem-leaf} - K_{tree})$, where $K_{stem-leaf}$ equals $k_{stem-leaf} / A_L$. The model also requires the root total biomass, which can be calculated from the ratio of total root biomass to the projected leaf area (Table S1). Modeled E saturates when root biomass is high, as K_{tree} becomes more limiting than the soil to root conductance. For both species, a ratio of total root biomass to projected leaf area of 5.1 was chosen because it was sufficient to saturate E , and to come within 7–10% of measured E under high Ψ_{PD} (late spring transpiration data).

Leaf photosynthetic parameters, such as the carboxylation rate of RuBisCO (V_{cmax} , mmol m⁻² s⁻¹) and photosynthetic electron transport (J_{max} , mmol m⁻² s⁻¹), are known to be tightly related to leaf nitrogen content (Field & Mooney, 1986; Dang *et al.*, 1997; Reich *et al.*, 1997a,b). For both species, V_{cmax} values were determined using the equation introduced by Niinemets & Tenhunen (1997) describing the theoretical anatomical and biochemical interactions that may be sources of variation in V_{cmax} :

$$V_{cmax} = 6.25 V_{cr} N_m F_R / SLA \quad \text{Eqn 5}$$

(6.25, ratio of the weight of RuBisCO to the weight of nitrogen in RuBisCO; V_{cr} , specific activity of RuBisCO, which is assumed to be only a function of temperature and is considered to be constant for C3 plants (20.7 $\mu\text{mol}_{CO_2} \text{g}^{-1} \text{RuBisCO} \text{s}^{-1}$ at 25°C; Harley *et al.*, 1992; Niinemets & Tenhunen, 1998); N_m (g g⁻¹), mass of nitrogen in the leaf per total dry mass of leaf; F_R , proportion of leaf nitrogen that is invested in RuBisCO, which has been shown not to vary between species and was taken as 0.15 (Niinemets & Tenhunen, 1998)). Similarly, J_{max} may be expressed as:

$$J_{max} = 8.06 J_{mc} N_m F_B / SLA \quad \text{Eqn 6}$$

(8.06, converts an investment of 1 g of nitrogen in bioenergetics into mmoles of cytochrome *f*; J_{mc} , capacity of electron transport per unit of cytochrome *f*, again considered to be constant among C3 species (156 $\mu\text{mol electrons} \mu\text{mol Cyt}_f^{-1} \text{s}^{-1}$ at 25°C; Nolan & Smillie, 1976); F_B (taken as 0.12; Niinemets & Tenhunen, 1997), fraction of leaf nitrogen in bioenergetics, reflecting the changes in the contents of the primary proteins determining the overall rate of electron transport (Evans & Seemann, 1989)).

We forced the SPA model with soil, meteorological and radiation data collected in 2005 by Black Mountain Digital Media (station Black Mountain 0.1 WSW, NC-BC 31) and available through the CRONOS (Climate Retrieval and Observations Network of the Southeast) database at <http://nc-climate.ncsu.edu/cronos>. This year was chosen as it represented, for this region, an average year in terms of mean annual temperature (13.5°C) and precipitation (1150 mm), and also allowed us to compare SPA outputs with direct measurements of tree water use (Daley *et al.*, 2007; Ford & Vose, 2007) and gross primary productivity (GPP) (ecoregion 66 in Coops *et al.*, 2009). WUE was calculated for each species for winter and summer sampling intervals by dividing GPP by tree water use (T). These WUE values

provide an independent assessment of model validation, as $\delta^{13}\text{C}$ data were not used to calibrate the model.

Statistical analysis

The mixed model procedure (PROC MIXED) in SAS version 9.1 was used for all statistical analyses (SAS, Cary, NC, USA). Species, time and their interaction were employed as sources of variation for data from healthy and infested samples, with species as a fixed effect. A separate ANOVA was executed for vigor class (healthy vs infested), time and their interaction for data from each species. Conductivity and stomatal conductance data were analyzed with the fixed effects of species, vigor class and their interaction as sources of variation. Both sets of carbon isotope data were also analyzed with the fixed effects of vigor class and their interaction as sources of variation.

Results

Water potential

In Carolina hemlock, June predawn water potential (Ψ_{PD}) was -0.30 MPa lower in infested trees than in healthy trees. In eastern hemlock, June Ψ_{PD} never dropped below -0.35 MPa and was no different ($P=0.33$) between healthy and infested trees (Fig. 1a). In Carolina hemlock, diurnal patterns of midday leaf water potential (Ψ_{MD}) in June indicated that the healthy trees reached a more negative water potential earlier during the course of the day than did the infested trees (Fig. 1a), and were able to recover to predawn levels earlier than the infested trees. In June, the Ψ_{MD} value of infested trees of eastern hemlock was -0.45 MPa less negative than that of healthy trees ($P=0.03$). The diurnal change in leaf water status in August followed the same pattern as in June, except that Ψ_{PD} and early-morning water potentials (until 09:00 h) were more negative. In August, Ψ_{PD} of infested trees dropped below -1.0 MPa, and values of infested trees were 0.61 and 0.70 MPa lower than the values of healthy trees for eastern and Carolina hemlock, respectively ($P<0.02$; Fig. 1b). In both June and August, Ψ_{PD} values of infested Carolina hemlock were 0.30 MPa lower ($P<0.001$) than that of infested eastern hemlock. In August, there was no difference in Ψ_{MD} between healthy and infested trees in either species ($P=0.17$ and $P=0.33$ for eastern and Carolina hemlock, respectively). Across the growing season, the water potential gradients ($\Delta\Psi$) from soil to shoot were 43% ($P=0.01$) and 49% ($P=0.01$) lower in infested eastern hemlock and Carolina hemlock trees, respectively.

Carbon isotopic composition, nitrogen content and SLA

For both species, the foliar carbon isotope ratios ($\delta^{13}\text{C}$) from summer sampling (August) were significantly less negative for infested than for healthy trees (Fig. 1b; Table 1), indicative of an increase in WUE. For winter sampling (January), there were significant differences in $\delta^{13}\text{C}$ between healthy and infested trees for Carolina hemlock only (Table 1). In addition, isotopic fractionation resulted in some quantitative differences in $\delta^{13}\text{C}$

between organs, with the wood signal being less negative than leaves. However, between healthy and infested trees and between species, the $\delta^{13}\text{C}$ data of leaves were fully consistent with the $\delta^{13}\text{C}$ data of wood (Table 1).

SLA was different between healthy and infested eastern hemlock trees ($P=0.01$, Table 2). Leaf nitrogen per gram of dry matter and per unit leaf area increased in infested eastern hemlock trees (Table 2), indicating that, in this species, the photosynthetic capacity may have increased with HWA attacks. Both infested and healthy branches appeared to share a common relationship between leaf nitrogen and $\delta^{13}\text{C}$ (Fig. 2). Foliar $\delta^{13}\text{C}$ values, an integrated measure of intrinsic WUE, became less negative with increasing N content (Fig. 2).

Hydraulic parameters and stomatal conductance

For a given diameter and sapwood area (A_s), branches from infested trees had half as much leaf area (A_L) as branches from healthy trees (Table 2). Infestation of branch segments with HWA reduced significantly the xylem hydraulic conductivity (k_s) by 50% ($P=0.02$) and 28% ($P=0.04$) in eastern and Carolina hemlock, respectively. The sharp decrease in $A_L : A_s$ in infested trees was offset by the decrease in k_s of branch xylem, and thus LSC of infested trees increased by $45\text{--}48\%$ ($P<0.03$). In Carolina hemlock, the increase in tree hydraulic conductance (K_{tree}) was the consequence of the large increase ($P=0.05$) in leaf hydraulic conductance (K_{leaf} ; Table 2). Although K_{leaf} was only significantly different between healthy and infested Carolina hemlock trees, in both species, it accounted for almost one-third of the whole-tree hydraulic resistance ($1/K_{\text{tree}}$). The proportion of tree hydraulic resistance located in the leaves decreased significantly ($P<0.04$) in both species from $>30\%$ in healthy trees to $<20\%$ in infested trees (Table 2). Because the hydraulic resistance located in the stem represented $14\text{--}19\%$ of $1/K_{\text{tree}}$ (Table 2), the root to stem component, which corresponded to the tree hydraulic resistance minus the resistances in leaf and stem, comprised $48\text{--}55\%$ of $1/K_{\text{tree}}$ in the healthy trees and $66\text{--}70\%$ in the infested trees (Table S1).

Differences in stomatal conductance (g_s) were significant for species and vigor classes (Table 2). Eastern hemlock trees had higher g_s ($P=0.01$) than Carolina hemlock trees. In both species, HWA infestation decreased g_s by 40% ($P<0.01$ for both species).

Wood anatomy

Anatomical analyses of samples taken from healthy branches of both species showed normal hemlock characteristics, such as distinct rings with abrupt changes in earlywood to latewood structure, with no constitutive xylem ducts and no axial parenchyma cells (Fig. 3a,c). Anatomical results of infested branches were consistent with the hypothesis of abnormal wood formation. The infested branches had a large number of false rings and a smaller percentage of earlywood than did the healthy branches (Table 3; Fig. 3b,d), making it appear that the trees repeatedly slowed down growth sufficiently during the growing season to form

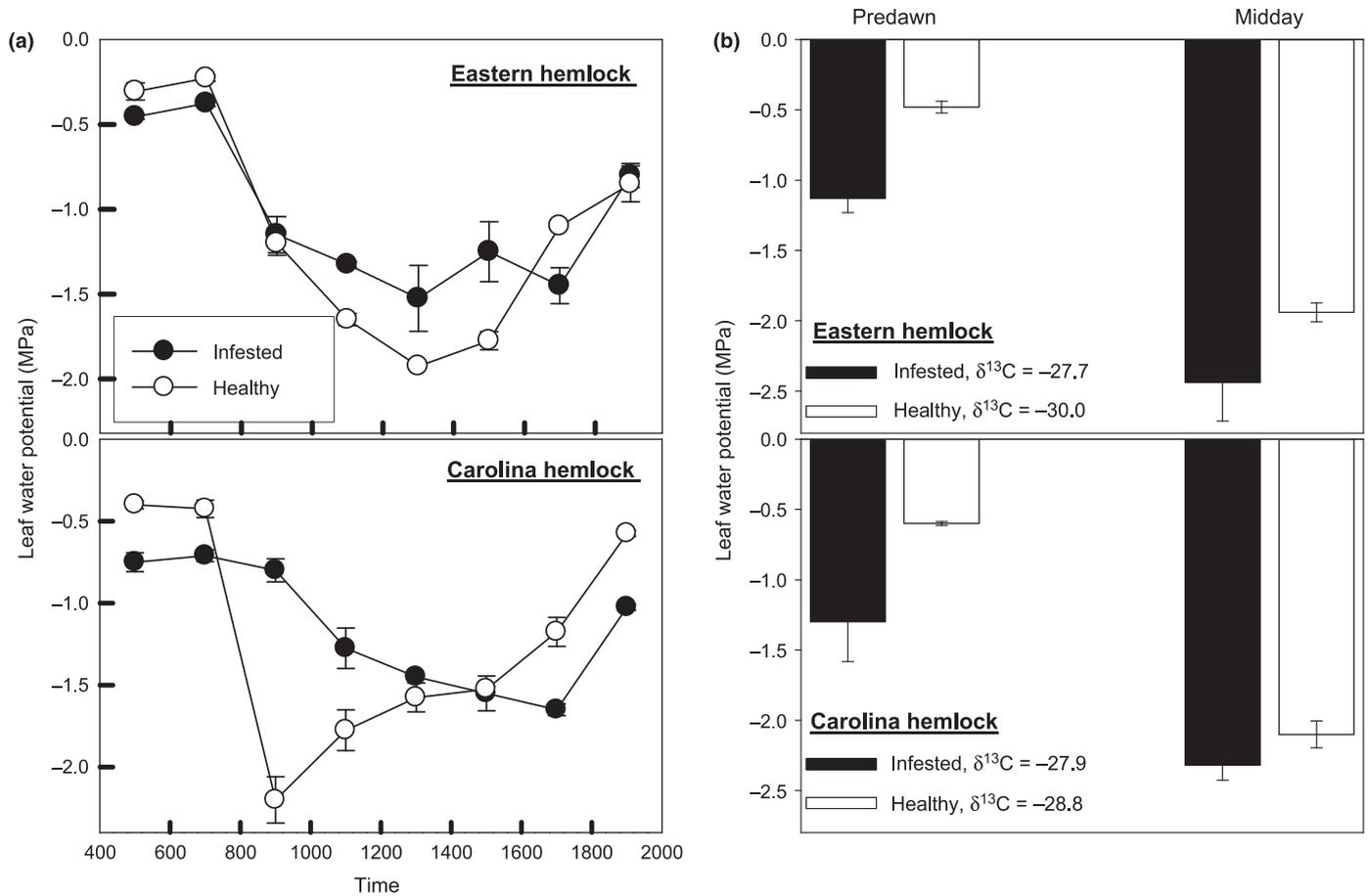


Fig. 1 Diurnal variation in leaf water potentials measured in June during the peak of the growing season (a), and predawn and midday leaf water potentials and leaf carbon isotope discrimination ($\delta^{13}\text{C}$) measured in August during the most drought-prone period (b) in healthy and infested eastern and Carolina hemlock trees by the hemlock woolly adelgid. Error bars represent standard errors (SE).

Table 1 Effect of the hemlock woolly adelgid on stem (wood) and leaf carbon isotope ratios ($\delta^{13}\text{C}$) in eastern hemlock (*Tsuga canadensis*) and Carolina hemlock (*Tsuga caroliniana*) trees

	Stem		Foliage	
	Summer	Winter	Summer	Winter
Eastern hemlock				
Healthy	$-28.86 \pm 0.13\text{a}$	$-28.64 \pm 0.83\text{a}$	$-29.97 \pm 0.16\text{A}$	$-29.64 \pm 0.45\text{A}$
Infested	$-26.90 \pm 0.34\text{b}$	$-26.71 \pm 0.30\text{a}$	$-28.22 \pm 0.18\text{B}$	$-27.34 \pm 0.24\text{B}$
Carolina hemlock				
Healthy	$-28.19 \pm 0.13\text{a}$	$-26.19 \pm 0.08\text{a}$	$-28.76 \pm 0.08\text{A}$	$-27.37 \pm 0.10\text{A}$
Infested	$-27.13 \pm 0.29\text{b}$	$-25.74 \pm 0.03\text{b}$	$-27.95 \pm 0.24\text{B}$	$-27.75 \pm 0.36\text{A}$

Means with different letters are significantly different at the $P \leq 0.05$ level.

some latewood and marginal parenchyma cells (Fig. 3d,e; Table 3). Although the overall mean tracheid diameter was affected by HWA in Carolina hemlock, the lumen tracheid hydraulic diameter decreased significantly in both species ($P < 0.02$). Longitudinal analysis of the infested branches showed that, in both species, there was a decreasing trend in the number of longitudinal ray parenchyma (Table 3). However, this was accompanied by a distortion of the grain with the appearance of large abnormal, unidentified parenchyma cells, which, in eastern hemlock, surrounded abnormal (traumatic) resin canals (Fig. 3e).

These cells contained reddish-brown starch deposits that completely occluded the lumina of axial parenchyma. Although wood anatomy revealed a decrease in mean hydraulic lumen diameter in infested branches (Table 3), there was no difference in $(t/b)^2$ ($P > 0.42$), an indicator of drought stress resistance.

Modeling of tree water use

When scaled to the stand level, measured changes in tree physiology, combined with climate drivers, had strong effects on tree

Table 2 Impacts of hemlock woolly adelgid (HWA) on branch morphological and hydraulic characteristics of eastern hemlock (*Tsuga canadensis*) and Carolina hemlock (*Tsuga caroliniana*) trees

	Eastern hemlock		Carolina hemlock	
	Healthy	Infested	Healthy	Infested
N (%)	1.16 ± 0.14a	1.42 ± 0.07b	0.90 ± 0.11a	0.81 ± 0.07a
SLA (m ² kg ⁻¹)	3.28 ± 0.13a	3.40 ± 0.07b	2.54 ± 0.14a	2.50 ± 0.09a
A _L : A _S (m ² cm ⁻²)	0.26 ± 0.10a	0.11 ± 0.02b	0.31 ± 0.08a	0.15 ± 0.04b
k _s (kg m ⁻¹ s ⁻¹ MPa ⁻¹)	0.72 ± 0.07a	0.36 ± 0.16bc	0.58 ± 0.03b	0.45 ± 0.01ac
K _{leaf} (mmol m ⁻² s ⁻¹ MPa ⁻¹)	10.8 ± 4.7a	19.9 ± 3.9a	5.2 ± 1.4a	13.0 ± 2.8b
LSC (10 ⁻³ kg ⁻¹ m ⁻¹ s ⁻¹ MPa ⁻¹)	0.21 ± 0.08ab	0.31 ± 0.09ab	0.11 ± 0.01b	0.16 ± 0.04a
K _{tree} (mmol m ⁻² s ⁻¹ MPa ⁻¹)	3.3 ± 0.5a	3.5 ± 0.2a	1.7 ± 0.2b	2.1 ± 0.2c
Leaf hydraulic resistance (%)	31 ± 2a	18 ± 3b	33 ± 2b	16 ± 2c
Stem hydraulic resistance (%)	14 ± 2a	17 ± 1b	19 ± 2b	15 ± 3ab
g _s (mmol m ⁻² s ⁻¹)	297 ± 30a	179 ± 22b	126 ± 9c	72 ± 11d

N, leaf nitrogen content as a percentage of leaf dry mass; SLA, specific leaf area; A_L : A_S, leaf area to sapwood area ratio; k_s, xylem hydraulic specific conductivity; K_{leaf} and K_{tree}, leaf and whole-tree hydraulic conductance, respectively; LSC, leaf specific conductivity; g_s, stomatal conductance. One-sided (projected) leaf area values were used to calculate SLA, A_L : A_S, K_{leaf}, LSC, K_{tree} and g_s. Also given are the hydraulic resistance located in the leaf and in the stem (branch xylem) as a percentage of whole-tree hydraulic resistance (1/K_{tree}). Means in the same row with different letters are significantly different at the P ≤ 0.05 level.

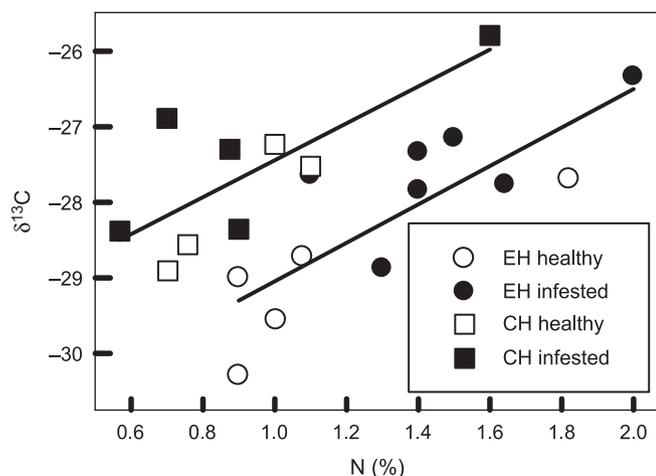


Fig. 2 Linear relationships between leaf nitrogen content (N) and leaf ¹³C natural abundance ($\delta^{13}\text{C}$) in healthy and infested eastern hemlock (EH) and Carolina hemlock (CH) trees ($r^2 = 0.66$ and 0.57 in EH and CH, respectively). Slopes of both linear regression trendlines ($P < 0.05$) varied between 2.45 and 2.54, and were not significantly different from each other ($P = 0.29$). Nonlinear models were also tested, but did not lead to significant regression coefficients. See Table 2 for treatment-assigned differences within each species.

water use (T), GPP and WUE. Our model predicted that a pure stand of eastern hemlock and Carolina hemlock would transpire 608 and 401 mm yr⁻¹, respectively, with 45% being transpired in the winter and spring (Fig. 4a,b). HWA infestation is predicted to have a stronger effect on Carolina hemlock than on eastern hemlock, with reductions in T of 41% and 34%, respectively. In eastern hemlock stands, the SPA model predicted that HWA infestation would reduce annual stand-level transpiration by 38% in the winter (November–April) and by 45% in the summer (May–October). In Carolina hemlock stands, HWA infestation would reduce annual stand-level T by 60% in winter and by 52% in summer. The yearly GPP rates were 1800 and 1380 gC m⁻² yr⁻¹ for healthy and infested eastern hemlock trees,

respectively, and 1600 and 835 gC m⁻² yr⁻¹ (gC = grams of carbon) for healthy and infested Carolina hemlock trees, respectively. HWA infestation was also predicted to have a stronger effect on GPP in Carolina hemlock, with a reduction of 48%, compared with a reduction of 24% in eastern hemlock (Fig. 4c,d). However, because of the stronger effect of HWA on T than on GPP in eastern hemlock, and the opposite in Carolina hemlock, WUE increased in eastern hemlock and decreased in Carolina hemlock following infestation (Fig. 5).

Discussion

Consistent with our first hypothesis, HWA infestation impaired xylem hydraulic characteristics as a result of the production of abnormal xylem cells. However, contrary to our second hypothesis, the negative effect of HWA infestation on sapwood hydraulic conductivity (k_s) did not impair whole-tree hydraulic conductance (K_{tree}), and thus cannot be the direct cause of tree mortality. The increase in K_{tree} was the consequence of the expected decline in leaf to sapwood area ratio (A_L : A_S) and of the unexpected increase in leaf hydraulic conductance (K_{leaf}). In terms of resistance to water flow, HWA decreased by half the contribution of the leaves (1/K_{leaf}) to whole-tree resistance (1/K_{tree}; Table 2). During the spring season, this increase in K_{tree} may have rapidly depleted water in the root zone, thereby increasing the resistance to water uptake at the soil–root interface, as seen by the more negative predawn water potentials (Ψ_{PD}) and the increase in the hydraulic resistance of the root to stem compartment. This root hydraulic dysfunction probably increased drought stress in infested trees during subsequent reductions in water availability, leading to reduced minimum leaf water potentials (Ψ_{leaf}) and stomatal conductance (g_s ; Fig. S1). The increase in carbon isotope ratios ($\delta^{13}\text{C}$) for the infested trees also suggest that the trees were experiencing drought-like water stress (Farquhar *et al.*, 1982; Zhang *et al.*, 1997).

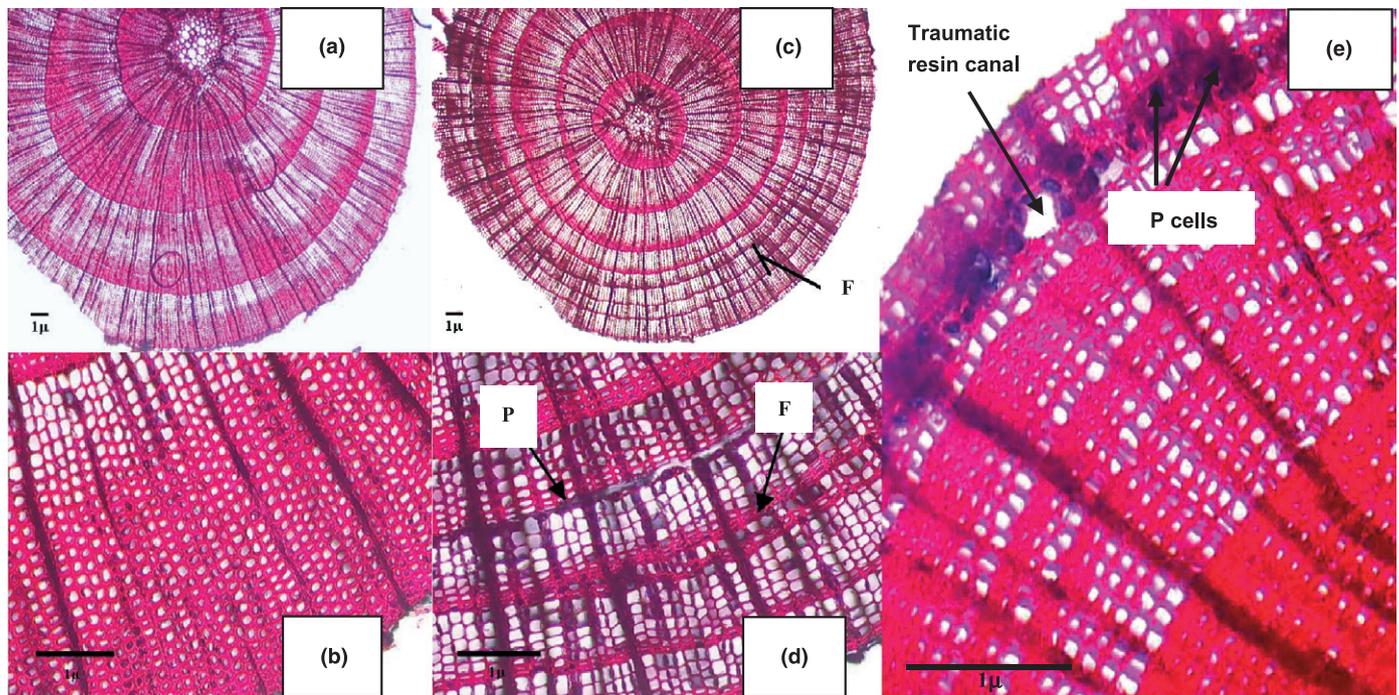


Fig. 3 Light microscope images showing the development of abnormal wood production in branch xylem of eastern hemlock infested with hemlock woolly adelgid (HWA). (a, b) Healthy eastern hemlock showing normal hemlock characteristics, such as distinct rings with abrupt changes in earlywood to latewood structure and very rare longitudinal ray parenchyma. (c, d) Infested eastern hemlock branches showing abnormal wood production characterized by the development of multiple false rings (F) and an increase in parenchyma cells (P). Microphotograph (e) represents an infested eastern hemlock branch section showing the presence of traumatic resin canals and P cells that are specialized for the synthesis and storage of phenolic compounds.

Table 3 Effect of hemlock woolly adelgid (HWA) infestation on branch xylem anatomical characteristics of eastern hemlock and Carolina hemlock trees

	Ring width (cm)	Total number of rings	Ray number	False rings (%)	Earlywood (%)	<i>b</i> (μm)	<i>D_h</i> (μm)	(<i>t/b</i>) ²
Eastern hemlock								
Healthy	0.22 ± 0.03a	3.5 ± 0.6a	68.2 ± 3.5a	0a	75 ± 2a	20 ± 2a	14 ± 1a	0.27 ± 0.01a
Infested	0.15 ± 0.02b	7.2 ± 1.0b	50.5 ± 3.3b	63 ± 9b	66 ± 5b	21 ± 1a	12 ± 1b	0.25 ± 0.02a
Carolina hemlock								
Healthy	0.23 ± 0.02a	3.7 ± 1.1a	51.2 ± 3.3b	12 ± 8a	78 ± 1a	23 ± 2a	19 ± 1c	0.23 ± 0.01a
Infested	0.14 ± 0.03b	6.4 ± 0.9b	47.7 ± 3.3b	75 ± 11b	60 ± 5b	16 ± 1b	13 ± 1ab	0.26 ± 0.03a

b, mean tracheid diameter; *D_h*, mean lumen hydraulic diameter. Total number of rings includes false rings, and ray number corresponds to the total number of radial ray parenchyma per cross-section. The cell wall to tracheid diameter ratio (*t/b*)² is an indicator of xylem water stress resistance. Means in the same column with different letters are significantly different at the *P* ≤ 0.05 level.

The δ¹³C tissue values of both species infested by HWA were less negative than those of healthy trees, and so infestation increased WUE, as demonstrated by the larger reduction in whole-tree water use relative to GPP (Fig. 5). In eastern hemlock, increased WUE was also documented by the significant increase in foliar nitrogen accompanying HWA infestation (Table 1; Fig. 2). Because the majority of leaf nitrogen is bound in photosynthetic enzymes, this implies that photosynthetic capacity may also have increased following infestation. The robust correlations between leaf δ¹³C and foliar nitrogen implied that high leaf nitrogen contents correspond to increased photosynthetic capacities and decreased internal leaf CO₂ concentrations.

Although HWA infestation did not decrease *K_{tree}*, in accordance with our second hypothesis, Ψ_L and *g_s* were influenced. In general there is a strong relation between *g_s* and the aboveground

efficiency of the plant water transport system (Meinzer, 2002; Domec *et al.*, 2010), and yet high *K_{tree}* in the infested trees was not supported by the development of higher *g_s*. By combining Darcy's law and the Penman–Monteith equation, water relations within the tree can be connected to each other according to Ohm's law analog (Hubbard *et al.*, 2001):

$$g_s = A_s/A_L k_s (\Delta\Psi/H)c/D \text{ or } g_s = \text{LSC} (\Delta\Psi/H)c/D \text{ or } g_s = K_{\text{tree}} \Delta\Psi c/D \quad \text{Eqn 7}$$

(*k_s*, xylem specific conductivity; LSC, leaf specific conductivity; ΔΨ/*H*, water potential gradient; *D*, time-averaged vapor pressure deficit of air; *c*, coefficient representing air and water thermodynamic properties). Under the same evaporative demand, Eqn 7 predicts that, within a species, as *K_{tree}* increases with HWA

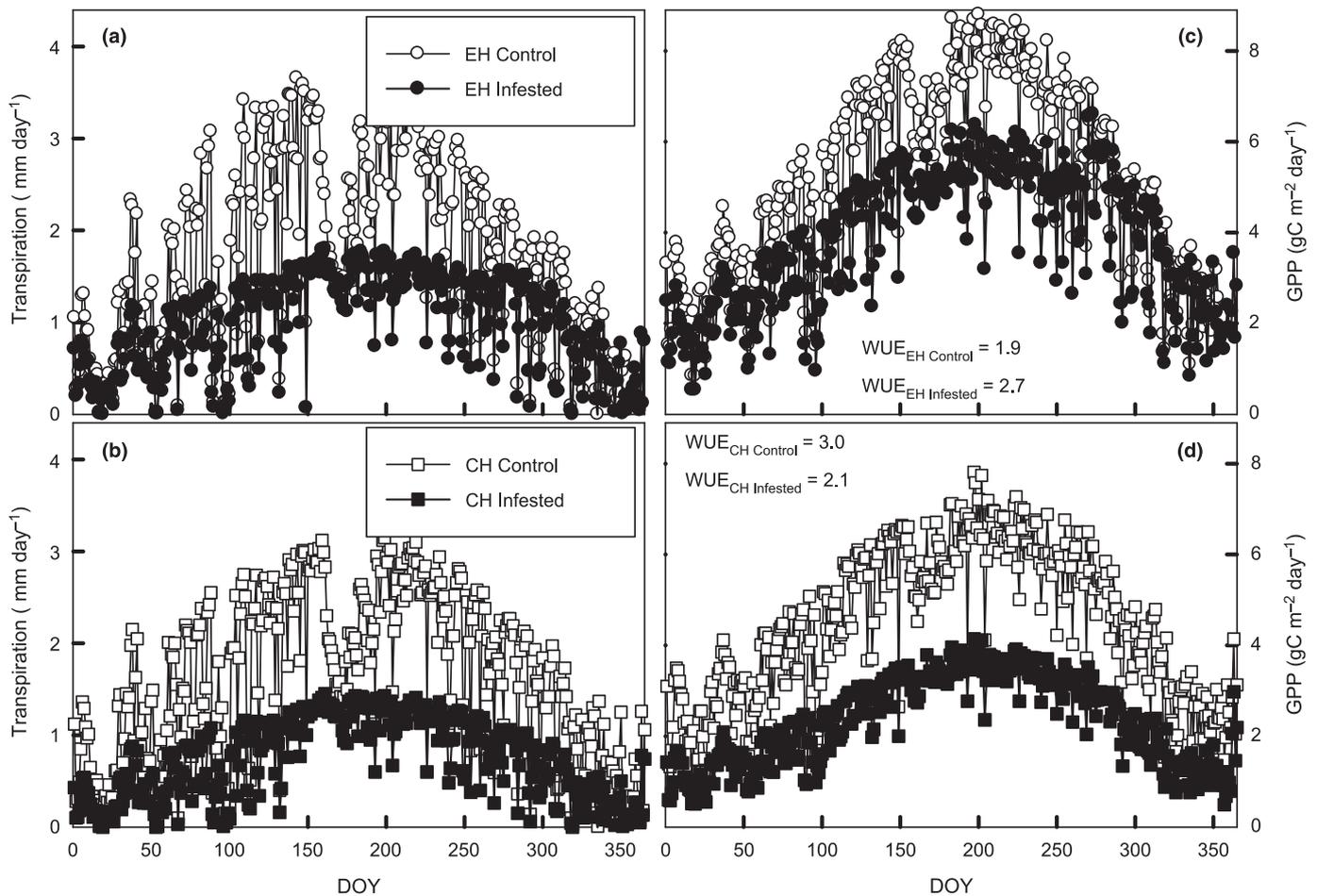


Fig. 4 Modeled (soil-plant-atmosphere, SPA) tree transpiration (T) (a, b) and gross primary productivity (GPP) (c, d) for both hemlock species (EH, eastern hemlock; CH, Carolina hemlock) under control or infested conditions. In (a), the yearly T rates were 608 and 401 mm yr⁻¹ for control and infested EH trees, respectively, and, in (b), 400 and 265 mm yr⁻¹ for control and infested CH trees, respectively. In (c), the yearly GPP rates were 1800 and 1380 gC yr⁻¹ for control and infested EH trees, respectively, and, in (d), 1600 and 835 gC yr⁻¹ for control and infested CH trees, respectively. Values of whole-stand annual water use efficiency (WUE) are also given.

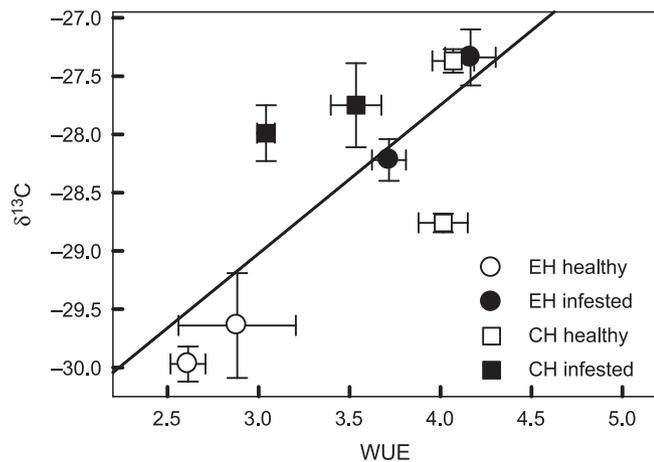


Fig. 5 The relationship between the average water use efficiency (WUE) of the two species (EH, eastern hemlock; CH, Carolina hemlock) and the mean ¹³C natural abundance (δ¹³C) of leaves from winter and growing season periods. The line represents the fitted linear regression line ($R^2 = 0.57$, $P = 0.011$). Error bars represent standard errors (SE).

infestation, so should g_s , unless $\Delta\Psi$ compensates (Fig. S1). High K_{tree} in the infested trees was not supported by the development of higher g_s , because $\Delta\Psi$ declined significantly, largely as a result of the lower Ψ_{PD} (Fig. 1). Predawn water potential measurements are assumed to be an indicator of available water in the soil (Ritchie & Hinckley, 1975). The more negative Ψ_{PD} of infested trees from this study indicated that infestation must have caused a form of resistance in the roots, because these trees were growing on the same site as the healthy trees. A recent study by Nuckolls *et al.* (2009) found that the very fine root biomass of eastern hemlock is indeed reduced when infested with HWA. It has been shown that, if roots are compromised at the soil–root interface, soil–root resistance will increase and the ability of the tree to take up water from the soil will be diminished (Barataud *et al.*, 1995; Sperry *et al.*, 1998). During a drought event, infested trees would be further compromised because of this added resistance. This increase in root resistance was also seen in the decrease in the percentage of resistance represented by the leaves of the infested trees (Table 2), and by the concurrent increase in the root to stem hydraulic resistance from *c.* 50% of tree resistance in the healthy

trees to almost 70% in the infested trees. The increase in root to stem resistance was probably also the consequence of embolism-induced loss of root xylem conductivity, because roots have been shown to be very sensitive to declining xylem water potentials (Domec *et al.*, 2009a,b). Although the Ψ_{PD} values of infested trees were not sufficiently negative to cause root death, they were sufficiently low to induce large rates of root embolism.

Our modeling results were consistent with our third hypothesis and illustrated a reduction in whole-stand water use and carbon uptake, which could lead to mortality through the failure to defend against further biotic attack because of a negative carbon budget. Although we did not have estimates of stand-level T and GPP specifically for the site studied, direct estimates of transpiration for eastern hemlock trees of similar sizes were comparable with our predictions (600–700 mm yr⁻¹; Vose & Swank, 1994; Daley *et al.*, 2007), as were modeling estimates of GPP for the region (1600–1800 gC m⁻² yr⁻¹; Coops *et al.*, 2009). The lower rates of carbon assimilation and growth in the infested trees explained the abnormal wood production, composed of narrow growth rings with smaller hydraulic tracheid diameters and thus with reduced k_s . On attack by HWA, other distinct anatomical changes appeared, including the activation of parenchyma cells and the formation of traumatic resin canals in the xylem (Hudgins *et al.*, 2004; Gonda-King *et al.*, 2012). Eastern and Carolina hemlock may be reacting to infestation in a manner similar to the response of Fraser fir to infestation by BWA. Balch *et al.* (1964) found that BWA induced hormonal changes in the xylem of infested firs by injecting saliva into the tree for feeding. The saliva stimulated the tree to exhibit the characteristics of abnormal wood that resembled compression wood. Perhaps in a similar hormonal reaction to the saliva of HWA, infested eastern and Carolina hemlock trees produce more latewood in the form of false rings, more longitudinal parenchyma and, in eastern hemlock, traumatic resin canals (Fig. S1). Expansion of these parenchyma cells might be a secondary response induced by growth substances liberated from wounded dying cells (Radville *et al.*, 2011), or might result from changes in turgor pressure caused by an accumulation of osmotically active substances (Hudgins *et al.*, 2004). Inducible secondary xylem parenchyma may provide greater resistance capacity as parenchyma cells are involved in the synthesis and storage of phenolics and resin. These parenchyma cells are not part of the normal developmental program of the xylem and resemble the polyphenolic parenchyma cells, which, in some conifers, are seen after massive fungal infestation (Krekling *et al.*, 2000; Krokene *et al.*, 2008). Although we did not closely examine these putative polyphenolic parenchyma cells, we suggest that they are produced in both hemlock species following HWA infestation.

By feeding on starch reserves, HWA creates a sink for photosynthates produced by the leaves (Nuckolls *et al.*, 2009) and, in the case of eastern hemlock, trees compensate by increasing the capacity for photosynthesis, as seen by the increase in leaf nitrogen. However, this compensation was insufficient to limit the decline in carbon uptake (Fig. 4). The direct effect of reduced GPP has a carry-over effect on carbohydrate reserves, which has important implications for the resilience of already infested

trees (Sala *et al.*, 2010; McDowell, 2011). Extended periods with limited photosynthesis may cause mortality because, although carbon allocation to wood growth may be reduced without death, carbon allocation to maintain respiration (Ryan *et al.*, 1995; Bosc *et al.*, 2003) must continue at some basal level, or mortality is inevitable. Assuming that the autotrophic respiration (R_a) of the healthy trees represented 47% of GPP (Waring & Running, 1998), and that this fraction can be applied to the infested trees as R_a is mainly a function of living tissue biomass, it can be calculated that respiration would represent 64% and 91% of GPP in infested eastern and Carolina hemlock, respectively. After taking into account the reduction in leaf area in infested trees, and by assuming that leaf maintenance R_a of hemlock trees corresponds to 18% of total R_a (Waring *et al.*, 1998), respiration would still represent a calculated 60% and 86% of GPP in infested eastern and Carolina hemlock, respectively. For eastern hemlock, the exact respiration cost in infested trees may in fact lie between these numbers as the effect of the decline in leaf area on the respiration cost would be offset by the increase in respiration rates per leaf area because of higher leaf nitrogen content (Reich *et al.*, 2008). The higher proportion of respiration cost would induce a sharp decrease in net carbon productivity (GPP – R_a) which would lower carbon reserves, especially in Carolina hemlock trees. As a consequence, carbon allocation to defense compounds against new insect attack would also be reduced, thus further impairing the resistance of infested trees to subsequent attacks (Coley *et al.*, 1985; McDowell *et al.*, 2007). In addition, insufficient carbohydrate reserves will limit the production of new leaves, and therefore will further reduce GPP and the synthesis of defense compounds (Waring & Pitman, 1985). We therefore hypothesize that, once trees become infested, carbon limitation remains a predisposing mechanism of further vulnerability to HWA attack, and would worsen under water stress (Fig. S1).

A recent modeling study estimated that 40 000 km² of hemlock forest became infested between 1951 and 2008, with an average of 1520 km² yr⁻¹ for the last 20 yr (Fitzpatrick *et al.*, 2012). At that rate, our model predicts that a reduction of 23% in GPP would represent a loss of 632 500 tC yr⁻¹ (tC = tonnes of carbon). Knowing that the total GPP of eastern forests represents *c.* 1 giga tC yr⁻¹ (Xiao *et al.*, 2010), HWA infestation would reduce overall carbon uptake by < 1‰. Overall, we conclude that, at the local scale, HWA can have a large effect on water balance (Ford & Vose, 2007; Guswa & Spence, 2012) and GPP, but that, at the regional scale, HWA infestation is unlikely to have a significant impact on water and carbon fluxes, as hemlock forests represent a small fraction of eastern forests.

Acknowledgements

We would like to acknowledge Dr Brian Joyce and Montreat College, NC, USA for providing the study site, and we thank Professor David D. Ackerly for valuable insights and improvements to the manuscript. Support for the work of J.S.K., J.-C.D. and L.N.R. was provided by Grant no. 08-JV-11330147-038 from the USDA Forest Service (EFETAC), by the NC

Agricultural Research Service via the Christmas Tree Genetics Program and by a research donation from Bayer Corporation. Additional support to J.-C.D. was provided by the DOE – BER Terrestrial Ecosystem Sciences program (11-DE-SC-0006700 – ER65189).

References

- Arthur FH, Hain FP. 1986. Water potential of Fraser fir infested with balsam woolly adelgid (*Homoptera: Adelgidae*). *Environmental Entomology* 15: 911–913.
- Balch RE, Clark J, Bonca JM. 1964. Hormonal action in production of tumours and compression wood by an aphid. *Nature* 202: 721–722.
- Barataud F, Moyne C, Breda N, Granier A. 1995. Soil water dynamics in an oak stand. *Plant and Soil* 172: 29–43.
- Bosc A, de Grandcourt A, Loustau D. 2003. Variability of stem and branch maintenance respiration in a *Pinus pinaster* tree. *Tree Physiology* 23: 227–236.
- Breda N, Huc R, Granier A, Dreyer E. 2006. Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. *Annals of Forest Science* 63: 625–644.
- Broeckling CD, Salom SM. 2003. Volatile emissions of eastern hemlock, *Tsuga canadensis*, and the influence of hemlock woolly adelgid. *Phytochemistry* 62: 175–180.
- Cochard H, Coll L, Le Roux X, Améglio T. 2002. Unraveling the effects of plant hydraulics on stomatal closure during water stress in walnut. *Plant Physiology* 128: 282–290.
- Coley PD, Bryant JP, Chapin FS III. 1985. Resource availability and plant anti-herbivore defense. *Science* 230: 895–899.
- Coops NC, Ferster CJ, Waring RH, Nightingale J. 2009. Comparison of three models for predicting gross primary production across and within forested ecoregions in the contiguous United States. *Remote Sensing of Environment* 113: 680–690.
- Craig H. 1957. Isotopic standards for carbon and oxygen and correlation factors for mass spectrometric analysis of carbon dioxide. *Geochimica et Cosmochimica Acta* 12: 133–149.
- Daley MJ, Phillips NG, Pettijohn JC, Hadley JC. 2007. Water use by eastern hemlock (*Tsuga Canadensis*) and black birch (*Betula lenta*): implications of effects of the hemlock woolly adelgid. *Canadian Journal of Forest Research* 37: 2031–2040.
- Dang QL, Margolis HA, Sy M, Coyea MR, Collatz GJ, Walthall CL. 1997. Profiles of photosynthetically active radiation, nitrogen and photosynthetic capacity in the boreal forest: implications for scaling from leaf to canopy. *Journal of Geophysical Research Atmosphere* 102: 28845–28859.
- Domec J-C, King JS, Noormets A, Treasure EA, Gavazzi MJ, Sun G, McNulty SG. 2010. Hydraulic redistribution of soil water by roots affects whole stand evapotranspiration and net ecosystem carbon exchange. *New Phytologist* 187: 171–183.
- Domec J-C, Noormets A, King JS, Sun G, McNulty SG, Gavazzi M, Boggs J, Treasure E. 2009a. Decoupling the influence of leaf and root hydraulic conductances on stomatal conductance and its sensitivity to vapor pressure deficit as soil dries in a drained loblolly pine plantation. *Plant, Cell & Environment* 32: 980–991.
- Domec J-C, Warren JM, Meinzer FC, Lachenbruch B. 2009b. Safety factors for xylem failure by implosion and air-seeding within roots, trunks and branches of young and old conifer trees. *IAWA Journal* 30: 101–120.
- Ehleringer JR. 1993. Carbon and water relations in desert plants: an isotopic perspective. In: Ehleringer JR, Hall AE, Farquhar GD, eds. *Stable isotopes and plant carbon–water relations*. San Diego, CA, USA: Academic Press, 155–172.
- Evans AM, Gregoire TG. 2007. Geographically variable model of hemlock woolly adelgid spread. *Biological Invasions* 9: 369–382.
- Evans JR, Seemann JR. 1989. The allocation of protein nitrogen in the photosynthetic apparatus: costs, consequences, and control. In: Briggs WR, ed. *Photosynthesis*. Plant biology 8. New York, NY, USA: Alan R. Liss Inc., 183–205.
- Farquhar GD, O'Leary MH, Berry JA. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology* 9: 121–137.
- Field C, Mooney HA. 1986. The photosynthesis–nitrogen relationship in wild plants. In: Givnish TJ, ed. *On the economy and form of plant function*. Cambridge, UK: Cambridge University Press, 25–55.
- Fitzpatrick MC, Preisser EL, Porter A, Elkinton JS, Ellison AM. 2012. Modeling range dynamics in heterogeneous landscapes: invasion of the hemlock woolly adelgid in eastern North America. *Ecological Applications* 22: 472–486.
- Ford CR, Vose JM. 2007. *Tsuga canadensis* (L.) Carr. mortality will impact hydrological processes in southern Appalachian forest ecosystems. *Ecological Applications* 17: 1156–1167.
- Gonda-King L, Radville L, Preisser E. 2012. False ring formation in eastern hemlock branches: impacts of hemlock woolly adelgid and elongate hemlock scale. *Environmental Entomology* 41: 523–531.
- Guswa AJ, Spence CM. 2012. Effect of throughfall variability on recharge: application to hemlock and deciduous forests in western Massachusetts. *Ecohydrology* 5: 563–574.
- Hacke UG, Sperry JS, Pockman WT, Davis SD, McCulloh KA. 2001. Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* 126: 457–461.
- Harley PC, Thomas RB, Reynolds JF, Strain BR. 1992. Modelling photosynthesis of cotton in elevated CO₂. *Plant, Cell & Environment* 15: 271–282.
- Havill NP, Montgomery ME, Yu G, Shiyaek S, Caccone A. 2006. Mitochondrial DNA from hemlock woolly adelgid (*Hemiptera: Adelgidae*) suggests cryptic speciation and pinpoints the source of the introduction to eastern North America. *Annals of the Entomological Society of America* 99: 195–203.
- Hill TC, Williams M, Moncrieff JB. 2008. Modelling feedbacks between a boreal forest and the planetary boundary layer. *Journal of Geophysical Research* 113: D15122.
- Hollingsworth RG, Blum U, Hain FP. 1991. The effect of adelgid-altered wood on sapwood conductance of Fraser fir Christmas trees. *IAWA Bulletin New Series* 12: 235–239.
- Hollingsworth RG, Hain FP. 1991. Balsam woolly adelgid (*Homoptera: Adelgidae*) and spruce-fir decline in the southern Appalachians: assessing pest relevance in a damaged ecosystem. *Florida Entomologist* 74: 179–187.
- Hubbard RM, Ryan MG, Stiller V, Sperry JS. 2001. Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. *Plant, Cell & Environment* 24: 113–121.
- Hudgins JW, Christiansen E, Franceschi VR. 2004. Induction of anatomically based defense responses in stems of diverse conifers by methyl jasmonate: a phylogenetic perspective. *Tree Physiology* 24: 251–264.
- Johnson DM, McCulloh KA, Woodruff DR, Meinzer FC. 2009. Leaf hydraulic conductance, measured *in situ*, declines and recovers daily: leaf hydraulics, water potential and gas exchange in four temperate and three tropical tree species. *Tree Physiology* 29: 879–887.
- Kizlinski ML, Orwig DA, Cobb RC, Foster DR. 2002. Direct and indirect ecosystem consequences of an invasive pest on forests dominated by eastern hemlock. *Journal of Biogeography* 29: 1489–1503.
- Kolb KJ, Sperry JS. 1999. Transport constraints on water use by the Great Basin shrub, *Artemisia tridentata*. *Plant, Cell & Environment* 22: 925–935.
- Krapf KJ, Holzmueller EJ, Jenkins MA. 2011. Early impacts of hemlock woolly adelgid in *Tsuga canadensis* forest communities of the southern Appalachian Mountains. *The Journal of the Torrey Botanical Society* 138: 93–106.
- Krekling T, Franceschi VR, Berryman AA, Christiansen E. 2000. The structure and development of polyphenolic parenchyma cells in Norway spruce (*Picea abies*) bark. *Flora* 195: 354–369.
- Krokene P, Nagy NE, Krekling T. 2008. Traumatic resin ducts and polyphenolic parenchyma cells in conifers. In: Schaller A, ed. *Induced plant resistance to herbivory*. Dordrecht, Netherlands: Springer, 147–169.
- Loustau D, Domec J-C, Bosc A. 1998. Interpreting the variations in xylem sap flux density within the trunk of maritime pine (*Pinus pinaster* Ait.): application of a model for calculating water flows at tree and stand levels. *Annales des Sciences Forestières* 55: 29–46.
- McClure MS. 1991. Density-dependent feedback and population cycles in *Adelges tsugae* (*Homoptera: Adelgidae*) on *Tsuga canadensis*. *Environmental Entomology* 20: 258–264.

- McClure MS. 1996. Biology of *Adelges tsugae* and its potential for spread in the northeastern United States. In: Salom SM, Tigner TC, Reardon RC, eds. *Proceedings of the first hemlock woolly adelgid review*. Forest Health Technology Enterprise Team 96-10. Morgantown, WV, USA: USDA Forest Service, 9–15.
- McDowell NG. 2011. Mechanisms linking drought, hydraulics, carbon metabolism, and vegetation mortality. *Plant Physiology* 155: 1051–1059.
- McDowell NG, Adams HD, Bailey JD, Kolb TE. 2007. The role of stand density on growth efficiency, leaf area index and resin flow in southwestern ponderosa pine forests. *Canadian Journal of Forest Research* 37: 343–355.
- McDowell NG, Allen CD, Marshall L. 2010. Growth, carbon-isotope discrimination, and drought-associated mortality across a *Pinus ponderosa* elevational transect. *Global Change Biology* 16: 399–415.
- Meinzer FC. 2002. Co-ordination of liquid and vapor phase water transport properties in plants. *Plant, Cell & Environment* 25: 265–274.
- Meinzer FC, Woodruff DR, Shaw DC. 2004. Integrated responses of hydraulic architecture, water and carbon relations of western hemlock to dwarf mistletoe infection. *Plant, Cell & Environment* 27: 937–946.
- Niinemets U, Tenhunen JD. 1997. A model separating leaf structural and physiological effects on carbon gain along light gradients for the shade-tolerant species *Acer saccharum*. *Plant, Cell & Environment* 20: 845–866.
- Niinemets U, Tenhunen JD. 1998. An analysis of light effects on foliar morphology, physiology, and light interception in temperate deciduous woody species of contrasting shade tolerance. *Tree Physiology* 18: 681–696.
- Nolan WG, Smillie RM. 1976. Multi-temperature effects on Hill reaction activity of barley chloroplasts. *Biochimica Biophysica Acta* 440: 461–475.
- Nuckolls AE, Wurzbarger N, Ford CR, Hendrick RL, Vose JM, Kloeppel BD. 2009. Hemlock declines rapidly with hemlock woolly adelgid infestation: impacts on the carbon cycle of southern Appalachian forests. *Ecosystems* 12: 179–190.
- Orwig DA, Foster DR, Mausel DL. 2002. Landscape patterns of hemlock decline in New England due to the introduced hemlock woolly adelgid. *Journal of Biogeography* 29: 1475–1487.
- Pataki DE, Oren R, Phillips N. 1998. Responses of sap flux and stomatal conductance of *Pinus taeda* L. trees to stepwise reductions in leaf area. *Journal of Experimental Botany* 49: 871–878.
- Puritch GS. 1971. Water permeability of the wood of Grand fir (*Abies grandis* (Doug.) Lindl.) in relation to infestation by the balsam woolly aphid, *Adelges piceae* (Ratz.). *Journal of Experimental Botany* 22: 936–945.
- Puritch GS. 1977. Distribution and phenolic composition of sapwood and heartwood in *Abies grandis* and the effects of the balsam woolly aphid. *Canadian Journal of Forest Research* 7: 54–62.
- Radville L, Chaves A, Preisser EL. 2011. Variation in plant defense against invasive herbivores: evidence for a hypersensitive response in eastern hemlocks (*Tsuga canadensis*). *Journal of Chemical Ecology* 37: 592–597.
- Reich PB, Grigal DF, Aber JD, Gower ST. 1997b. Nitrogen mineralization and productivity in 50 hardwood and conifer stands on diverse soils. *Ecology* 78: 335–347.
- Reich PB, Tjoelker MG, Pregitzer KS, Wright IJ, Oleksyn J, Machado J-L. 2008. Scaling of respiration to nitrogen in leaves, stems and roots of higher land plants. *Ecology Letters* 11: 793–801.
- Reich PB, Walters MB, Ellsworth DS. 1997a. From tropics to tundra: global convergence in plant functioning. *Proceeding of the National Academy of Sciences, USA* 94: 13730–13734.
- Reid DEB, Silins U, Lieffers VJ. 2003. Stem sapwood permeability in relation to crown dominance and site quality in self-thinning fire origin lodgepole pine stands. *Tree Physiology* 23: 833–840.
- Rhea LK. 2010. *Implications of elevated atmospheric carbon dioxide and tropospheric ozone for water use in stands of trembling aspen and paper birch*. PhD thesis, North Carolina State University, Raleigh, NC, USA.
- Ritchie GA, Hinckley TM. 1975. The pressure chamber as an instrument for ecological research. *Advances in Ecological Research* 9: 165–254.
- Ryan MG, Gower ST, Hubbard RM, Waring RH, Gholz HL, Cropper WP, Running SW. 1995. Woody tissue maintenance respiration of four conifers in contrasting climates. *Oecologia* 101: 133–140.
- Sala A, Piper F, Hoch G. 2010. Physiological mechanisms of drought-induced tree mortality are far from being resolved. *New Phytologist* 186: 274–281.
- Small MJ, Small CJ, Dreyer GD. 2005. Changes in a hemlock dominated forest following woolly adelgid infestation in southern New England. *Journal of the Torrey Botanical Society* 132: 458–470.
- Sperry JS, Adler FR, Campbell GS, Comstock JP. 1998. Limitation of plant water use by rhizosphere and xylem conductance: results from a model. *Plant, Cell & Environment* 21: 347–359.
- Spicer R, Gartner BL. 1998. Hydraulic properties of Douglas-fir branches and branch halves with reference to compression wood. *Tree Physiology* 18: 777–784.
- Tyree MT, Sinclair B, Lu P, Granier A. 1993. Whole shoot hydraulic resistance in *Quercus* species measured with a new high pressure flowmeter. *Annales des Sciences Forestieres* 50: 417–423.
- Vose JM, Swank WT. 1994. Effects of long-term drought on the hydrology and growth of a white pine plantation in the southern Appalachians. *Forest Ecology and Management* 64: 25–39.
- Waring RH, Landsberg JJ, Williams M. 1998. Net primary production of forests: a constant fraction of gross primary production? *Tree Physiology* 18: 129–134.
- Waring RH, Pitman GB. 1985. Modifying lodgepole pine stands to change susceptibility to mountain pine-beetle attack. *Ecology* 66: 889–897.
- Waring RH, Running S. 1998. *Forest ecosystems, analysis at multiple scales*. San Diego, CA, USA: Academic Press.
- Williams M, Bond BJ, Ryan MG. 2001. Evaluating different soil and plant hydraulic constraints on tree function using a model and sap flow data from ponderosa pine. *Plant, Cell & Environment* 24: 679–690.
- Williams M, Rastetter EB, Fernandes DN, Goulden ML, Wofsy SC, Shaver GR, Melillo JM, Munger JW, Fan S-M, Nadelhoffer KJ. 1996. Modelling the soil–plant–atmosphere continuum in a *Quercus–Acer* stand at Harvard Forest: the regulation of stomatal conductance by light, nitrogen and soil/plant hydraulic properties. *Plant, Cell & Environment* 19: 911–927.
- Woodruff DR, McCulloh KA, Warren JM, Meinzer FC, Lachenbruch B. 2007. Impacts of tree height on leaf hydraulic architecture and stomatal control in Douglas-fir. *Plant, Cell & Environment* 30: 559–569.
- Xiao J, Zhuang Q, Baldocchi DD, Law BE, Richardson AD, Chen J, Oren R, Starr G, Noormets A, Ma S *et al.* 2010. A continuous measure of gross primary production for the conterminous United States derived from MODIS and AmeriFlux data. *Remote Sensing of Environment* 114: 576–591.
- Young RF, Shields KS, Berlyn GP. 1995. Hemlock woolly adelgid (*Homoptera: Adelgidae*): stylet bundle insertion and feeding sites. *Annals of the Entomological Society of America* 88: 827–835.
- Zhang JW, Feng Z, Cregg BM, Schumann CM. 1997. Carbon isotopic composition, gas exchange, and growth of three populations of ponderosa pine differing in drought tolerance. *Tree Physiology* 17: 461–466.

Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 The hypothesized conceptual model describing the interactions between structural and physiological changes following hemlock woolly adelgid (HWA) infestation in hemlock trees.

Table S1 Parameter values and variables used in the soil–plant–atmosphere model to provide stand-level water use and carbon exchange estimates of the healthy and infested (HWA) eastern and Carolina hemlock trees

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.