



Elevated light levels reduce hemlock woolly adelgid infestation and improve carbon balance of infested eastern hemlock seedlings



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ABSTRACT

The rapid loss of eastern hemlock (*Tsuga canadensis*) due to infestation with hemlock woolly adelgid (*Adelges tsugae*, HWA) has greatly altered structure and function of eastern forests. Numerous control strategies including local pesticide use and biocontrol with predator beetles have been implemented with considerable cost and varying success. Silviculture treatments that increase incident light on surviving hemlock trees to reduce infestation and ameliorate carbon starvation have been proposed as another possible conservation strategy, yet no controlled studies have evaluated the coupled plant-insect responses to increased light. We conducted a nursery experiment on artificially infested eastern hemlock seedlings under varying levels of ambient light, ranging from 0 to 90% shade. We measured HWA infestation (ovisac density), short- and long-term indicators of carbon balance (leaf chlorophyll fluorescence, net photosynthesis, total nonstructural carbohydrate content, and shoot growth), and nutrition (leaf N content). We hypothesized that higher light would result in reduced HWA densities, higher C assimilation rates, and improved tissue non-structural carbohydrate balance; and these effects would ameliorate the effects of infestation on C balance and lead to improved seedling growth. HWA density decreased with increasing light, and was highest in the 90% shade treatments. However, photosystem II efficiency and net assimilation were also lower under the higher light treatment. Despite tradeoffs between reduced infestation and reduced leaf function from higher light, and little variation in sugar content among treatments, both leaf and root starch content and seedling growth were higher under the higher light treatment. Increasing light levels improves long-term carbon balance for hemlock seedlings in the presence of HWA. Although hemlock typically occurs in deeply shaded forests, our results suggest that silvicultural treatments such as forest thinning that increase light exposure may reduce HWA abundance and lead to better tree C balance, and may be an effective component of large-scale conservation and restoration strategies.

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1. Introduction

Invasive species can have substantial impacts on ecosystem structure and function, particularly when foundation species are affected (Ellison et al., 2005; Lovett et al., 2006, 2016). Several recent examples of invasive pests and pathogens in forests demonstrate the effects of defoliation and/or tree mortality on stand structure, community composition, productivity, evapotranspiration, and nitrogen cycling (Lovett et al., 2010; McManamay et al.,

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2011; Flower et al., 2013; Brantley et al., 2013). One of the most severe recent invasive pest outbreaks affects eastern hemlock (*Tsuga canadensis* (L.) Carr.), a foundation species in forests of eastern North America (Ellison et al., 2005; Orwig et al., 2012). The natural range of eastern hemlock extends from northern Georgia and Alabama to southern Canada and westward into the central Great Lakes states (McWilliams and Schmidt, 2000). At present, eastern hemlock trees of all ages and sizes in the eastern and southernmost portion of the range are declining and dying as a result of attack by, and their susceptibility to, the hemlock woolly adelgid (HWA, *Adelges tsugae* Annand) (Orwig et al., 2002; Ford et al., 2012). Decline is progressive and mortality can occur within four years of infestation (Orwig et al., 2002; Ford et al.,

2012). Morbidity and mortality of hemlock has affected riparian forest and stream ecosystem function (Ellison et al., 2005; Ford and Vose, 2007; Ford et al., 2012; Orwig et al., 2012; Webster et al., 2012; Brantley et al., 2013, 2015), demonstrating a need for effective mitigation and restoration strategies that can be implemented quickly and at large scales.

A wide variety of management strategies are being developed and implemented to reduce the impacts of HWA on eastern hemlock, including chemical control (Cowles et al., 2006), biological control (Onken and Reardon, 2011), host gene conservation (Jetton et al., 2013), and enhancement of host resistance (Montgomery et al., 2009). Although chemical control has been used with great success to temporarily protect individual trees, financial costs, logistical constraints of manually treating large areas, and environmental constraints greatly limit its use as a sustainable strategy for protecting natural stands (Vose et al., 2013). Biological control shows some promise as several released predatory species are successfully establishing in eastern forests (Mausel et al., 2010; Hakeem et al., 2013; Vose et al., 2013; Mayfield et al., 2015; Zietlow, unpublished data); but the time required for widespread predator proliferation and dispersal, and timing predator release effectively for outbreak species such as HWA, may limit the effectiveness of biological control as a stand-alone strategy. Coupling these control strategies with forest management practices that improve tree host resistance and increase stand resilience (*sensu* Holling, 1973) may provide a long-term management solution, but direct evidence supporting the efficacy of such treatments is scant.

One possible strategy to reduce the effects of HWA in infested hemlock stands is to increase light availability for surviving eastern hemlock trees through silvicultural treatments such as thinning or selective harvests (Fajvan, 2008). Eastern hemlock is one of the most shade-tolerant tree species in eastern North America and can survive in environments where as much as 99% of incident sunlight is intercepted by the canopy (Bourdeau and Laverick, 1958; Hadley, 2000). However, growth in such environments is slow and individual trees may not reach full height unless openings in the canopy allow enough light into the understory for trees to grow more rapidly (Bourdeau and Laverick, 1958; Spurr and Barnes, 1980; Brisette and Kenefic, 2000). For example, in a long-term study in Maine, eastern hemlock recruitment, regeneration, and growth all responded positively to selective harvesting that created large canopy openings (Brisette and Kenefic, 2000). Increases in ambient light from silvicultural treatments may similarly improve survival and growth of infested hemlock trees by increasing ambient light availability for improved photosynthate production for physiological maintenance, growth, reproduction, and/or defense.

Although the exact mechanism of mortality in eastern hemlock from HWA is still unknown, HWA has been shown to both induce water stress and negatively affect tree C balance (Gomez et al., 2012; Domec et al., 2013; Gonda-King et al., 2014). The hypothesized mechanism with the most observational support is one involving C starvation (McClure, 1991; Young et al., 1995; Broeckling and Salom, 2003; Domec et al., 2013; Gonda-King et al., 2014). As eastern hemlock trees are infested, HWA crawlers (mobile, first instar nymphs) settle at the base of leaves (needles) and insert their stylets into xylem ray parenchyma cells (Young et al., 1995). After insertion, nymphs become sessile and feed on carbohydrates in the xylem ray parenchyma cells until maturity (McClure, 1989). In addition to direct carbohydrate extraction, hydraulic changes from infestation include result in reduced stomatal conductance. This reduction may be due to physical injury from stylet insertion or from chemical cues in response to the

infestation (Radville et al., 2011; Gonda-King et al., 2014). Reduced photosynthesis resulting from these hydraulic changes leads to loss of leaves, further depletion of carbohydrates, and subsequent lack of new shoot growth. Thus, the C starvation hypothesis suggests that these feeding nymphs negatively affect whole-tree C balance both directly, through photosynthate extraction, and indirectly through a series of structural and physiological changes that alter hydraulic function of the tree (Domec et al., 2013; Gonda-King et al., 2014). Creating artificial canopy openings to increase light exposure might improve C balance in infested hemlock by both reducing infestation and reducing light limitation for photosynthesis, thus mitigating the effects of carbohydrate extraction and leaf loss, and reducing the risk of C starvation from HWA infestation.

Increased light may also benefit infested eastern hemlock trees indirectly by reducing HWA infestation severity. Several studies suggest that increased light and temperature can reduce HWA densities (Sussky and Elkinton, 2015; Mayfield and Jetton, 2013; Hickin and Preisser, 2015; Mech, 2015) which benefits eastern hemlock by reducing stored carbon extraction at the leaf base. In prior artificial infestation studies using potted seedlings, HWA adult densities increased with increasing shade (Mayfield and Jetton, 2013), and HWA settlement was higher on second year growth under high- compared to low- shade treatments (Hickin and Preisser, 2015); however, these studies did not address a full range of possible light conditions and did not assess concurrent physiological responses on seedlings. Temperature extremes related to increased exposure from more open canopies may also affect HWA survival. For example, extreme heat events, characterized by abnormally high maximum air temperatures over several days to several weeks negatively affect HWA survival (Sussky and Elkinton, 2015; Mech, 2015). Extreme cold (e.g. $<-30^{\circ}\text{C}$) has also been shown to reduce HWA populations (Parker et al., 1998, 1999; Skinner et al., 2003). Data across the eastern US in a cold event when temperatures ranged from -16 to -31°C , showed HWA mortality ranging from 17 to 100% (McAvoy et al., 2014); however, the progeny generation was recovered at all sites the following spring, indicating HWA populations can recover from extreme cold events (McAvoy et al., 2014).

To examine the effects of light environment on HWA infestation, and hemlock C balance in the presence of HWA, we measured HWA density and several key indicators of C balance following artificial infestation of hemlock seedlings under shade treatments from 0% (full sunlight) to 90% shade over one year. We assessed short-term, semi-integrated measures of leaf physiological function and stress, as well as long-term integrated measures of C balance. Specifically, we measured leaf-level gas exchange and chlorophyll fluorescence; total nonstructural carbohydrates (TNC) (soluble sugar and starch) in leaves, stems, and roots; and seedling growth parameters including diameter and height increment, and shoot growth of infested seedlings. These data will help answer three questions related to environmental effects on the plant-insect system and physiological mechanisms of plant mortality: (1) is HWA infestation affected by ambient light levels?, (2) how do short- and long-term indicators of plant C balance respond to light treatment in the presence of HWA? and (3) how does overall seedling growth respond to light in the presence of HWA? We predicted that increased light would improve plant C balance indirectly by reducing HWA infestation levels, and directly by promoting plant C fixation and storage thus reducing mortality risk from C starvation. Results from this study will contribute to our understanding of the mechanisms of hemlock mortality and may suggest strategies for implementation of large scale silvicultural practices that will contribute to hemlock conservation.

2. Methods

2.1. Study area

The study was conducted on a nursery plot at the North Carolina Department of Agriculture and Consumer Services Mountain Research Station in Waynesville, NC, USA (35°29'16"N, 82°58'01"W). This site is located in the southern Appalachian Mountains and is within the southern range of eastern hemlock. Elevation at the site is ~820 m above sea-level and climate is classified as humid subtropical. Mean annual temperature is 12.8 °C, but the site can experience extreme annual temperature variations from –30 to 37 °C. Mean annual rainfall is 1239 mm and is evenly distributed throughout the year.

2.2. Experimental design and shade treatments

Four rows of five seedling shade tents were established within a rectangular plot (12 m × 11 m) in an open field receiving full sunlight (Fig. 1). The ground was covered with brown polypropylene landscape fabric (Dewitt Company, Sikeston, MO, USA) and covered with a shallow layer (5 cm) of cypress mulch. Shade tents (1 × 1 × 1.3 m) were spaced 1 m apart within rows and 2 m apart between rows. One of the following shade treatments was

randomly assigned to each of the five tents within each row using appropriately rated shade cloth: 0% (no shade cloth), 30%, 50%, 70%, and 90%. The 90% shade treatment was achieved by applying two layers of the 70% shade cloth (reflective “Aluminet®” shade cloth, Ecologic Technologies, Inc., Pasadena, MD, USA).

One hundred four-year-old bare-root eastern hemlock seedlings, that had been previously untreated with pesticides, were purchased (Pikes Peak Nursery, Penn Run, PA, USA) and potted (Fafard® 3B growing mix, Sun Gro Horticulture, Agawam, MA, USA) in 6 L plastic containers in May 2013. Seedlings were treated with 15 g of Multicoat 15–16–17 fertilizer (Haifa Group, Altamont Springs, FL, USA) on the soil surface, and watered with tap water. Five seedlings were added to each shade tent on 12 Jul 2013. Extra cypress mulch was packed around each pot to a depth of 10–15 cm to insulate the roots and soil during the winter. Seedlings were watered regularly (twice weekly for 2–4 h, adjusted as needed to local weather and rainfall conditions) during the spring, summer and fall with a button dripper irrigation system (Drip Depot, Inc., White City, OR) supplying 1.9 L water per hour to the soil surface of each pot. With the exception of momentarily uncovering the tents or removing seedlings to make measurements or infest seedling with adelgids, seedlings remained in the shade tents for the remainder of the study which ended in May 2015.

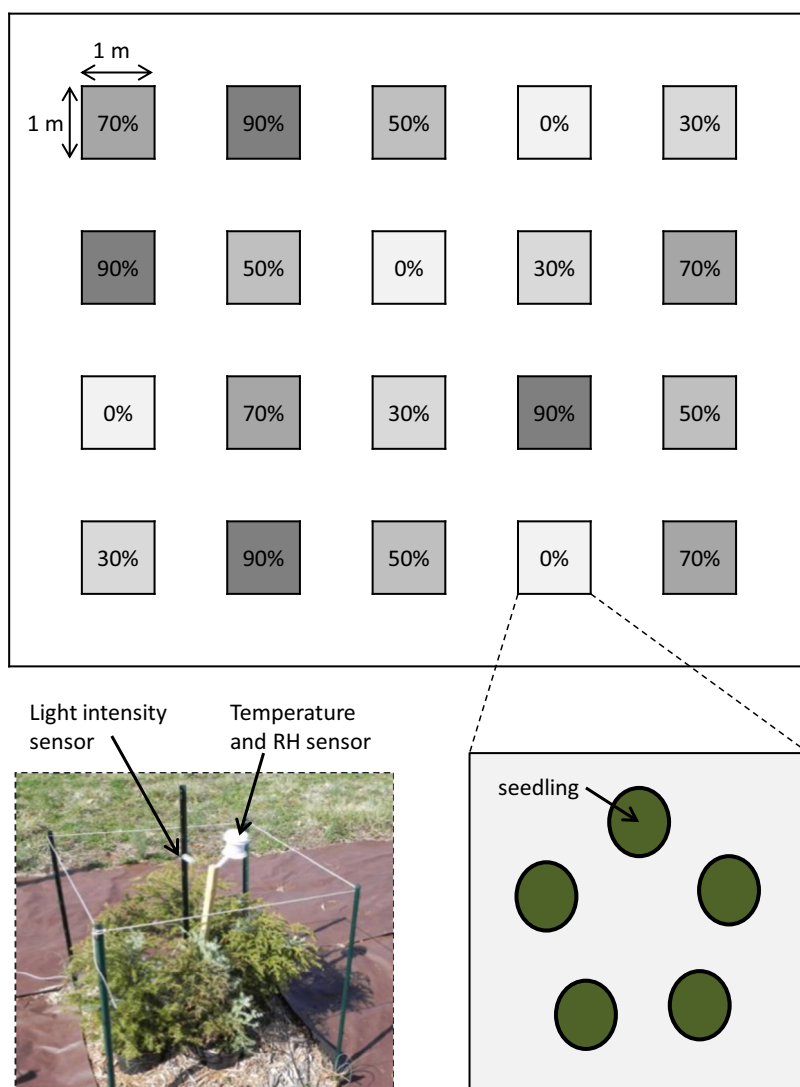


Fig. 1. Layout of artificial shade plots (percent shade indicated within squares) and eastern hemlock seedlings (full light treatment) at Waynesville, NC, 2013–2015.

Air temperature and relative humidity were measured every 15 min in a subset of treatment replicates ($n = 2$ sensors per light treatment), from Mar 2014 through May 2015 using Onset® S-THB-M008 sensors shielded from radiation and mounted 0.6 m above the ground. Spot measurements of photosynthetic photon flux density (PPFD, $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) were measured with a quantum sensor (Li-190R, Li-Cor Biosciences, Lincoln, NE, USA) above and below the shade cloth to validate shade cloth ratings.

2.3. Infestation with HWA

In March 2014, nine months after placement in the shade tents, all 100 seedlings were artificially infested with *A. tsugae* crawlers of the progrediens generation—i.e. the spring generation that remains on the host tree (McClure, 1989)—to simulate timing of the natural HWA life cycle. Eastern hemlock branches infested with *A. tsugae* sistens ovisacs and progrediens eggs were collected along Soak Creek in Rhea County, TN (35.72°, -84.87°). Infested branches were cut into 40 cm-long pieces with cut ends immersed in tap water until used. Shoots were visually distinguished as being either heavily or lightly infested and a subsample of 10 shoots from each group were used to estimate number of ovisacs per 40 cm shoot and the number of eggs per ovisac (based on dissection of 10 ovisacs per shoot under a dissecting microscope). Heavily and lightly infested 40-cm shoots had a mean (SE) density of 257 (47) and 108 (17) ovisacs per shoot, respectively, whereas both groups averaged 15 eggs per ovisac. Four shoots (two heavily and two lightly infested) were attached to each seedling on 19 Mar 2014. An estimated 11,000 progrediens eggs were applied to each seedling in this manner. The HWA progrediens generation settled on the 2013 seedling shoot growth and subsequently gave rise to a sistens generation—i.e. the overwintering generation—that settled on the 2014 shoot growth. Woolly ovisacs, each 1–2 mm diameter, produced by the progrediens and sistens generations were counted on 9 Jun 2014 and 21 Jan 2015, respectively. Ovisac counts were made on five 10-cm shoot tips (one terminal and four laterals) and were averaged to produce a seedling mean in adelgids/decimeter.

2.4. Leaf-level physiology

Leaf-level, instantaneous, steady-state (i.e., dark-adapted) chlorophyll fluorescence was measured five times between March 2014 and April 2015 (LI-6400-40, Li-Cor Biosciences, Lincoln, NE, USA). Fluorescence measurements were taken on both one and two-year old leaf cohorts for each replicate ($n = 4$) per shade treatment ($n = 5$) per time period ($n = 5$) and were subsequently averaged across cohorts. Leaves were dark-adapted for ≥ 30 min before fluorescence measurements were taken. Maximum quantum efficiency of photosystem II (PSII, F_v/F_m , unitless) was calculated as $(F_m - F_o)/F_m$, where F_o is the fluorescence signal (relative units) when all reaction centers are open and F_m is the fluorescence signal (relative units) under a saturating light source that activates all reaction centers.

Ambient net photosynthesis (A_{net} , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ leaf area s}^{-1}$) and stomatal conductance (g_s , $\text{mol H}_2\text{O m}^{-2} \text{ leaf area s}^{-1}$) were measured on first and second cohort leaves in Sep 2014 and on first cohort leaves in Apr 2015 using a portable infrared gas analyzer (LI-6400-40, Li-Cor Biosciences, Lincoln, NE, USA). Standard conditions for all treatments were as follows: leaf temperature was set to 20 °C, $[\text{CO}_2]$ was 400 ppm, and relative humidity was 50–70%. Photosynthetically active photon flux density (PPFD, $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) in the chamber simulated typical mid-day light levels indicated for each individual treatment. Specific PPFD settings were 200, 600, 1000, 1400 or 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ to represent the 90, 70, 50, 30 or 0% shade treatments, respectively. All but the

90% shade treatment were well above the light saturation point (350 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) for eastern hemlock reported in Hadley (2000). Gas exchange data were filtered to exclude negative values of g_s and transpiration, or if internal $[\text{CO}_2]$ fell outside 100–500 ppm.

2.5. Tissue carbohydrates

Stem (bark only), root, and cohort-specific leaves were analyzed for total soluble sugar and starch content (i.e., total nonstructural carbohydrates, TNC) using methods described by Ward and Deans (1993). Samples were collected from plants during leaf-level measurements from March 2014 to April 2015, with leaves sampled most often, followed by bark (including outer bark and cambium), and roots. Roots were sampled with a $\frac{3}{4}$ in. (~ 19 mm) diameter soil sampler, and washed and sorted from soil and organic matter. Soluble sugars were extracted from a subsample of dried, ground tissues using water. Additional subsamples underwent an enzymatic (amylglucosidase) degradation to convert starch to soluble sugars. The extracted samples were subsequently quantified colorimetrically (Astoria 2 Flow Analyzer, Astoria Pacific International) using *p*-hydroxybenzoic acid hydrazide (*p*-HBAH) with in line acid hydrolysis and expressed as glucose concentrations. Changes to the manifold as described by Ward and Deans (1993) were made; most notably, we increased the HCl concentration and decreased the flow of *p*-HBAH. Starch was quantified by the difference in the total non-structural carbohydrates and the soluble sugars. For quality control, certified and in-house reference standards were extracted with the samples, blanks were included, samples were spiked with known concentrations, and samples were compared with results from other labs. The absolute error for the extraction of the reference standards compared to actual was 8.3% TNC, 12.5% starch and 12.2% sugar. Mean recovery of the reference was 98% TNC, 91% starch and 102% sugar. The average (SD) blank value, as glucose, was 1.5 mg L^{-1} (2.5). The method detection limit, at 99% confidence level, was 1.4 mg L^{-1} glucose. The average TNC and sugar sample values were 379 and 290 mg L^{-1} (as glucose) respectively. Spiked and un-spiked samples were highly correlated ($R = 0.87, 0.85$ and 0.88 for TNC, starch and sugar, respectively), as were samples from an inter-lab comparison ($R = 0.83$).

2.6. Foliar nitrogen

Foliage was collected from terminal branches from each tent in Sep 2014 and Apr 2015. Samples were stored on ice, transported to the lab, and then stored in a freezer until processed. Because they were also analyzed for total nonstructural carbohydrate content, all samples were thawed, dried at 100 °C for one hour prior to drying at 60 °C for three days and then ground (Spex 8000D Mixer/Mill). Dried and ground samples were then analyzed for nitrogen (Thermo Electron Corp., Flash EA 1112, NC method) following the Dumas method. All leaf chemical analyses were conducted at the USDA Forest Service Coweeta Hydrologic Laboratory in Otto, NC, located ~ 90 km from nursery plot and followed standard QA/QC.

2.7. Seedling height, stem and shoot growth measurements

Seedling height and basal diameter were measured in Jun 2013 coincident with placement of seedlings in the shade tents as well as 3 and 14 months after initial HWA infestation (Jun 2014 and May 2015, respectively). Basal diameter (± 0.1 cm) was measured twice, 90° apart. Diameter and height increment (cm) were calculated for the Jun 2013–May 2015 period. To estimate new shoot growth in the 2014 growing season, we measured and averaged

the length of five shoots per seedling (the terminal shoot and four lateral shoots separated by $\sim 90^\circ$) in Jan 2015. New growth was defined as growth from bud scale to shoot tip (excluding any side shoots).

2.8. Data analysis

Spot measurements of PPFD were averaged by shade treatment and compared to manufacturer shade cloth rating using linear regression. To test for variation in T and RH among shade treatments, T and RH were modeled as a function of shade treatment using a repeated-measures, mixed effects ANOVA with unstructured variance and repeated observations on monthly values (PROC Mixed, SAS v9.2, SAS Institute, Inc., Cary, NC, USA).

Non-linear regression was used to test for effects of shade treatment on HWA density and hemlock growth parameters. HWA density was modeled using the exponential function $y = a^{bx}$, where y is HWA density, x is percent shade, and a and b are estimated regression coefficients. HWA density in adelgids per dm was log-transformed ($\log_{10}[\text{density} + 1]$) to reduce heteroscedasticity. To test for variations in hemlock growth among shade treatments, height and diameter increments and new shoot length were modeled using the quadratic function $y = y_0 + ax + bx^2$, where y is hemlock growth, x is percent shade, a and b are estimated regression coefficients, and y_0 is the intercept (SigmaPlot 12.5, Systat Software, Inc.).

To test for variations in leaf fluorescence (F_v/F_m), leaf gas exchange (A_{net} and g_s), tissue carbohydrates, and foliar [N] among seasons and shade treatments, each parameter was modeled as a function of shade treatment and season using repeated-measures, mixed effects ANOVA with unstructured variance and repeated observations on each tent through time (PROC Mixed). Significant differences among shade treatments for each season were tested using a post-hoc test on least squares mean estimates. Relationships between shade and leaf physiology (F_v/F_m , A_{net} , and g_s) for each season and relationships between F_v/F_m and A_{net} , or g_s were also tested using Pearson correlation. We also determined how net photosynthesis varied as a function of foliar [N] (Proc CORR, SAS 9.4), and finally, we tested whether net photosynthesis varied as a function of season, shade and their interaction with foliar [N] as a co-variate (Proc GLM, SAS 9.4).

3. Results

3.1. Shade treatments

Mean light attenuation (% full sun) for the shade treatments were 31 ± 3 , 57 ± 3 , 74 ± 3 and $91 \pm 3\%$ for the assigned 30, 50, 70, and 90% treatments, respectively (Fig. 2). Treatments only affected incident light, and did not affect other aspects of microclimate. Minimum, mean, and maximum temperature and minimum, mean, and maximum relative humidity did not vary among shade treatments (Fig. 2, all $P > 0.05$).

3.2. Effect of shade on HWA density

The density of live, settled HWA progrediens adults in Jun 2014 increased markedly with increasing shade, consistent with our hypothesis (Fig. 3). Shade explained 58% of the variability in HWA progrediens density ($F_{1, 19} = 25.1$, $P = 0.001$). Mean densities of more than 8 adelgids dm^{-1} were observed only under 70% and 90% shade. This positive relationship persisted into the subsequent adelgid generation, when shade explained 52% of the variability in live HWA sistens density in Jan 2015 ($F_{1, 19} = 19.6$, $P < 0.001$). In Jan

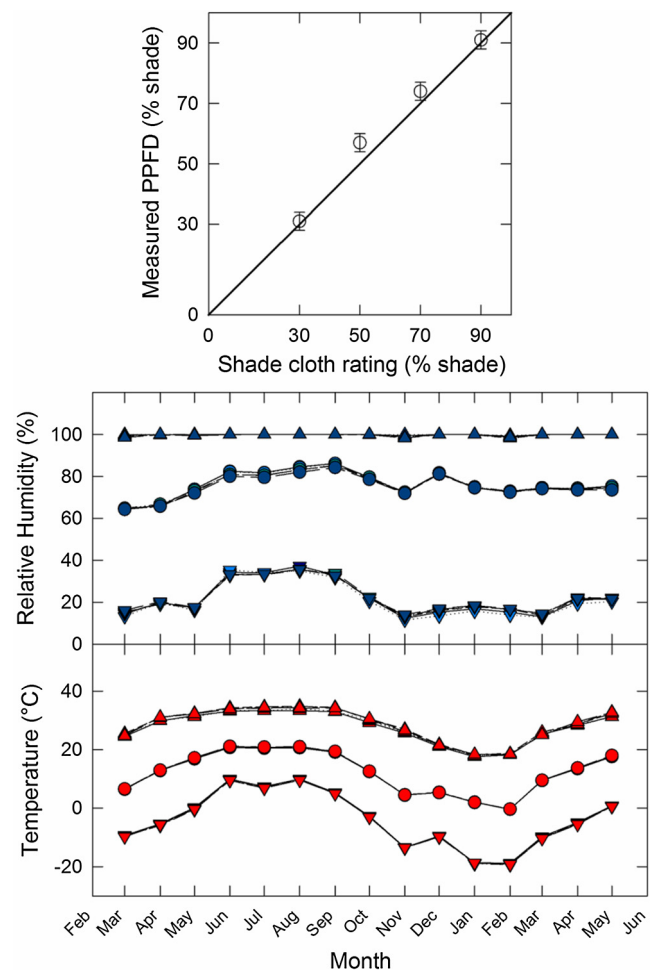


Fig. 2. Measured PPFD for shade treatments compared to manufacturers rating for shade cloth (top panel, $P = 0.006$, $n = 25$, $R^2 = 0.99$); and minimum (▼), mean (●), and maximum (▲) monthly relative humidity (middle panel) and air temperature (bottom panel) for all treatments.

2015, no adelgids were observed on seedlings exposed to 30% shade or less, and densities greater than 1 adelgid dm^{-1} were observed only under 70% and 90% shade.

3.3. Leaf-level physiology

Shade treatment had marked effects on leaf physiology and this effect changed through time. Seedlings' maximum efficiency of PSII (F_v/F_m) varied both seasonally and among shade treatments (season effect $F_{4, 75} = 83.65$, $P < 0.001$; shade effect: $F_{4, 75} = 8.85$, $P < 0.001$; Fig. 4). Across all shade treatments, F_v/F_m was lowest in Mar 2014 and highest during Sep 2014. F_v/F_m was positively correlated with shade in Mar 2014 (i.e., higher shade equated to lower stress, $R = 0.88$, $n = 25$, $P < 0.001$). F_v/F_m was not significantly correlated with shade for subsequent sampling periods (all $P > 0.05$). Early in the study (Sep 2014), A_{net} and g_s were positively correlated with shade ($R = 0.90$, $n = 25$, $P < 0.001$, Fig. 5). However, later in the study this trend had changed. A_{net} and g_s were not significantly correlated with shade in Apr 2015 ($R = 0.43$, $n = 25$, $P = 0.473$, Fig. 5). Rather, A_{net} and g_s were highest in intermediate shade treatments, lower in higher shade treatments, and lowest in full sunlight (Fig. 5). Neither A_{net} nor g_s were correlated with F_v/F_m during either period where gas exchange was measured (all $P > 0.05$).

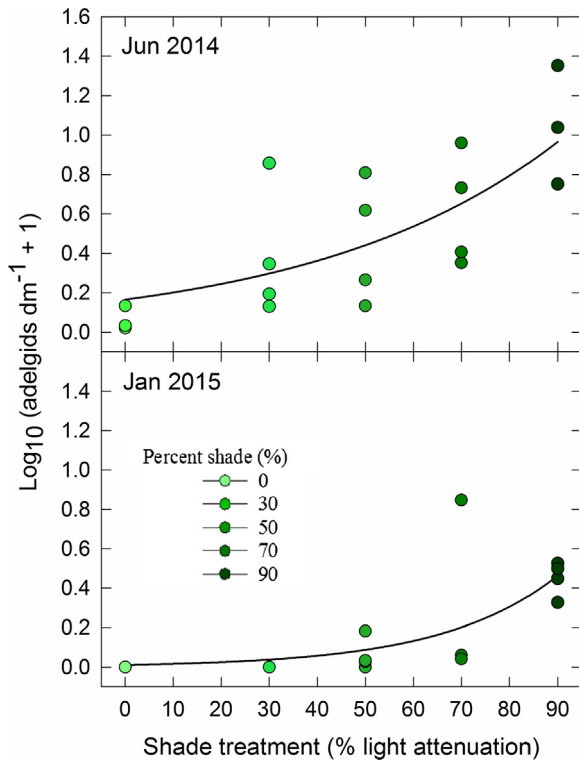


Fig. 3. Relationship between percent shade and HWA density on eastern hemlock seedlings in June 2014 (top, progrediens generation) and Jan 2015 (bottom, sistens generation).

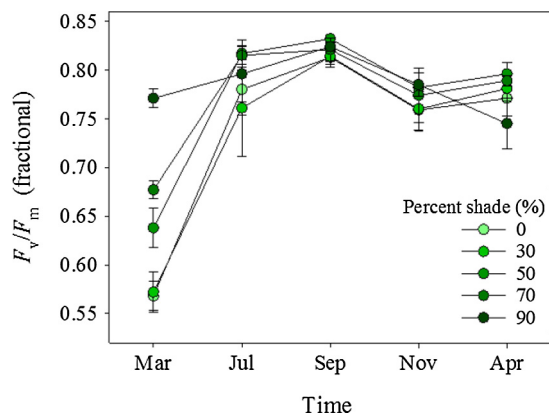


Fig. 4. Average (± 1 standard error) leaf chlorophyll fluorescence (F_v/F_m) of eastern hemlock saplings growing under varying levels of shade ($n = 4$ replicates per shade treatment) from Mar 2014 through Apr 2015. Color gradient of symbols and values in legend correspond to the percent shade. All saplings were experimentally infested with hemlock woolly adelgid in Mar 2014.

Both A_{net} and g_s were strongly related to foliar [N]. Seedlings under greater shade had higher foliar [N] (shade effect $F_{4, 37} = 22.66$, $P < 0.001$; season effect $F_{1, 37} = 27.14$, $P < 0.001$; NS interaction) (Fig. 6). The greater the foliar [N], the higher net photosynthesis was ($R = 0.533$, $n = 38$, $P < 0.001$). Although we found that net photosynthesis tended to increase with increasing shade, utilizing foliar [N] as a covariate, we found that net photosynthesis didn't vary among shade treatments or seasons ([N] effect $F_{1, 37} = 16.60$, $P < 0.004$; shade effect $F_{4, 37} = 1.61$, $P = 0.20$; season effect $F_{1, 37} = 2.67$, $P = 0.114$; NS interaction).

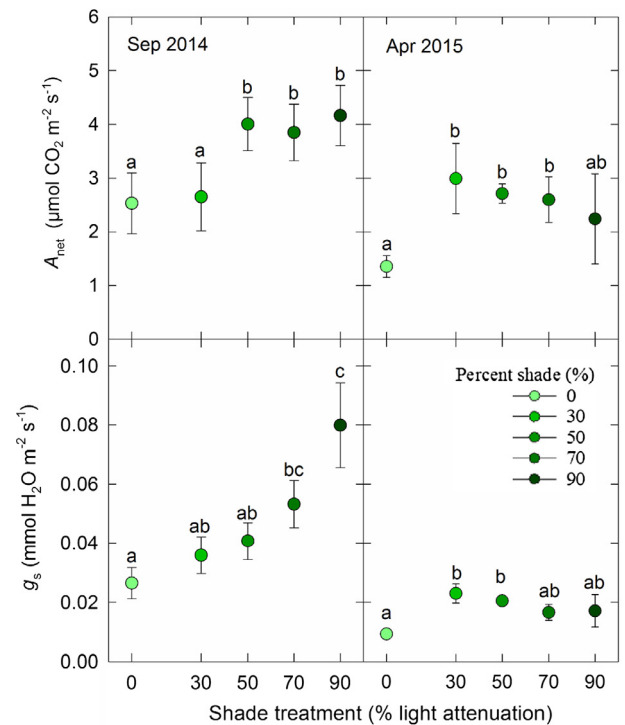


Fig. 5. Leaf level net assimilation (A_{net} , top panels) and stomatal conductance (g_s , bottom panels) for nursery-grown hemlock seedlings under varying shade treatments after artificial infestation with hemlock woolly adelgid. Significant differences among treatments are represented with different lowercase letters.

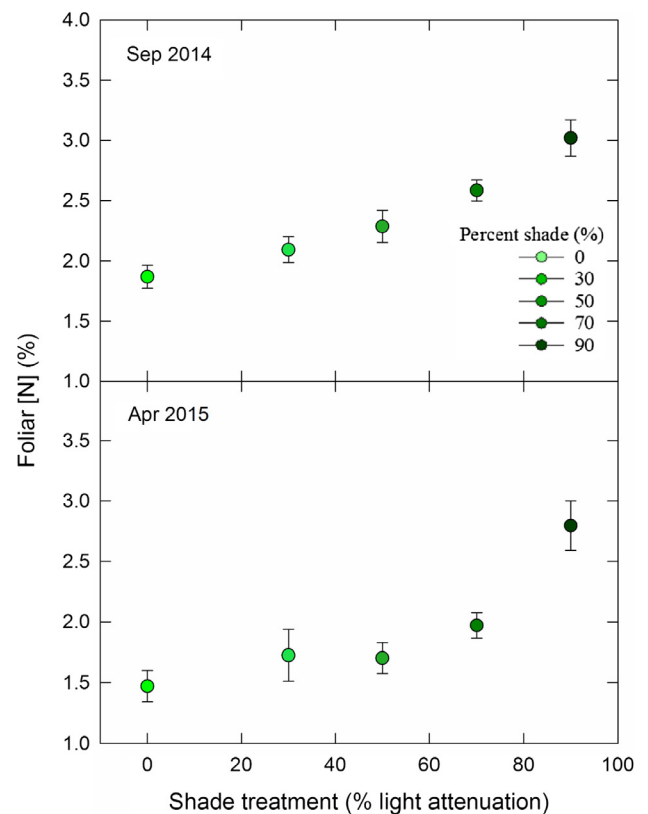


Fig. 6. Variation in foliar nitrogen concentration (% dry mass \pm SE) of planted hemlock seedlings among shade treatments.

3.4. Tissue nonstructural carbohydrates

Leaf sugar and starch concentrations did not vary significantly among shade treatments before infestation (Mar 2014, $F_{4, 15} = 1.33$, $P = 0.30$ and $F_{4, 15} = 2.11$, $P = 0.13$, respectively), but declined with shade after infestation (Jul 2014, $F_{4, 15} = 3.72$, $P = 0.03$ and $F_{4, 15} = 3.16$, $P < 0.001$) (Fig. 7). Leaf starch concentrations showed significant negative effects of shade in Sep 2014 ($F_{4, 15} = 4.64$, $P = 0.01$). Bark starch concentrations varied in Apr 2015 when 0, 30, 50 and 70% shade treatments were ~3-fold higher than the 90% shade treatment ($F_{4, 15} = 3.64$, $P = 0.03$, Fig. 7). Root starch concentrations varied significantly among shade treatments for Sep 2014 and Apr 2015 ($F_{4, 15} = 9.56$, $P < 0.001$ and $F_{4, 15} = 4.51$, $P = 0.02$, respectively, Fig. 7) and the 90% shade treatment had the lowest root starch concentration during both periods. In the

final sampling period, root starch concentrations were highest in the 50% shade treatment. Starch concentrations, in general, were positively correlated with sugar concentrations in the leaves and roots (all $P < 0.05$) except during Apr 2015. Sugar concentrations were not correlated with starch concentrations in the bark tissue for any period (all $P > 0.05$).

3.5. Effect of shade on growth

All three measures of growth were relatively unaffected by shade levels of 50% or less, but decreased as shade increased to 70% and 90% (Fig. 8). Diameter increment ($F_{2, 19} = 4.9$; $P = 0.02$, $R^2 = 0.37$), height increment ($F_{2, 19} = 8.8$; $P < 0.01$; $R^2 = 0.51$), and new shoot length ($F_{2, 19} = 5.2$; $P = 0.02$; $R^2 = 0.38$) all declined with increasing shade. Negative height growth was observed in some of

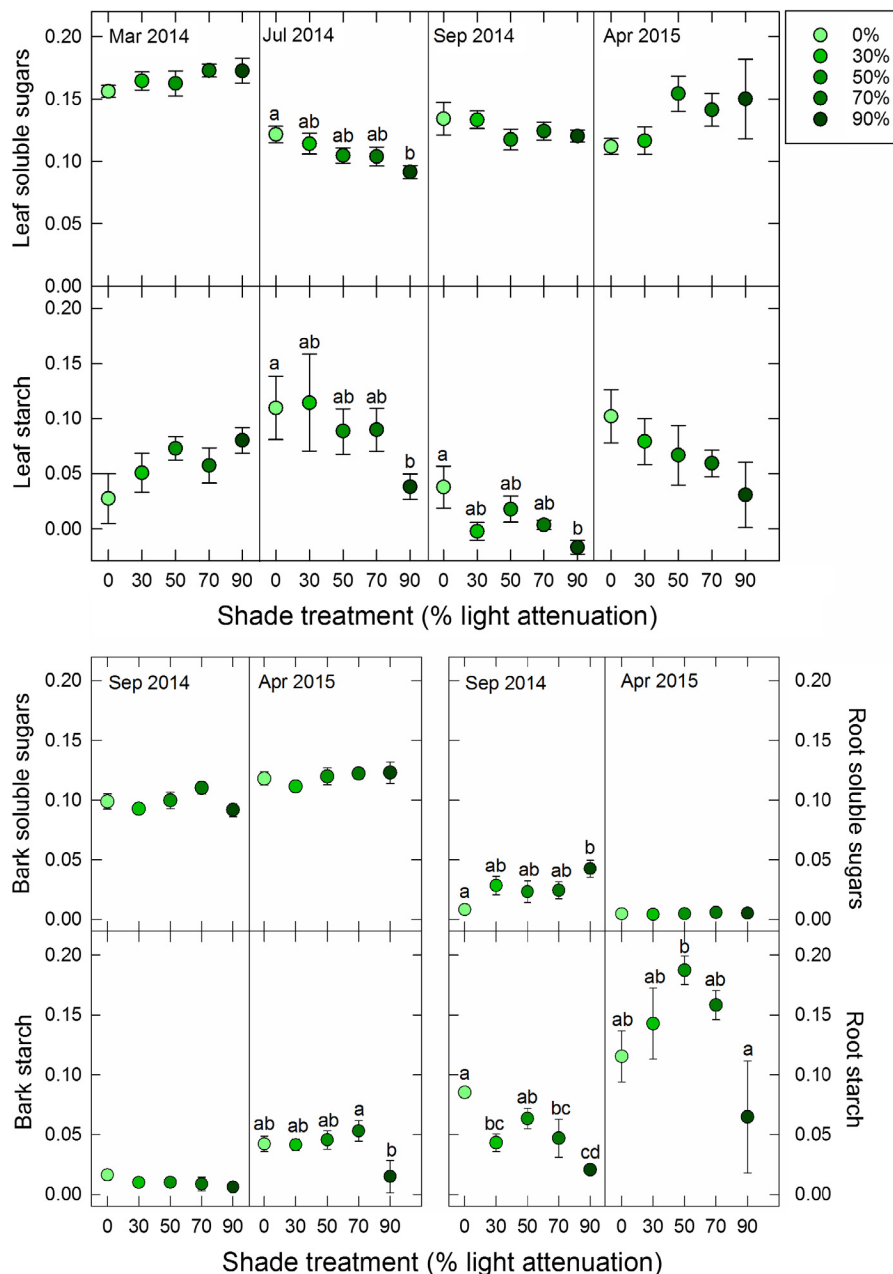


Fig. 7. Average (\pm SE) sugar and starch concentrations (mg mg^{-1} dry tissue mass) of leaf (top), bark (bottom left), and roots (bottom right) over time for eastern hemlock saplings growing under varying levels of shade between Mar 2014 and Apr 2015. All saplings were experimentally infested with hemlock woolly adelgid after the Mar 2014 sampling. Significant differences among light treatments are noted with lowercase letters.

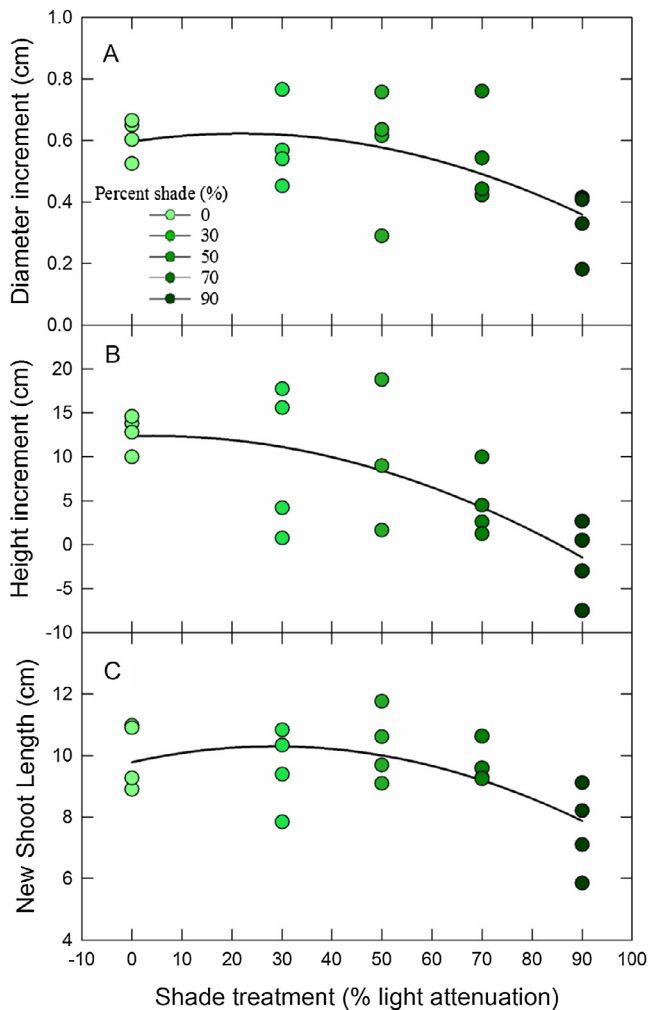


Fig. 8. Relationships between percent shade and eastern hemlock seedling growth parameters: (A) diameter increment June 2013–May 2015, (B) height increment June 2013–May 2015, and (C) length of new shoot produced in 2014.

the seedlings in the 90% shade treatments due to dieback of terminal shoots (Fig. 8).

4. Discussion

4.1. Overall benefits of moderate increases in light

Light treatments of 30% of full sunlight or above were associated with both reduced HWA infestation and enhanced carbon balance and growth in eastern hemlock seedlings in the presence of HWA. Light treatments $\geq 30\%$ of full sunlight had a negative effect on HWA density; i.e., more light resulted in fewer live adelgids. However, full or near-full (i.e. 100 or 70%) sunlight was associated with increased leaf stress early in the study in the absence of HWA. High light levels in the presence of HWA also resulted in lower instantaneous leaf-level C assimilation, even with lower HWA densities. While high light treatments reduced instantaneous leaf gas exchange, the net effect of the high light treatment was a higher TNC content and increased plant growth, especially under moderate light levels where adelgid density was lower. Thus, the tradeoff of reduced C assimilation from highlight was not enough to eliminate the positive effects of increased light on adelgid density. These effects did not require full sunlight which by itself might have negative consequences for plant carbon

balance. These results suggest that the deeply shaded environments characteristic of many natural hemlock stands are not optimal for *T. canadensis* survival and growth in the presence of HWA. However, light levels did not need to be 100% of full sunlight to see reduced HWA infestation levels and substantial benefits for plant C balance, as these were seen at all treatments other than 90% shade.

4.2. Light effects on adelgids

The inverse relationship between ambient light and adelgid density suggests that HWA infestations on understory hemlock trees may be more severe in more heavily shaded environments. These results are consistent with the findings of [Hickin and Preisser \(2015\)](#) who observed higher densities of both live adelgids (those that produced wool) and all settled adelgids (including nymphs that died before producing wool) under low- compared to high-light. [Hickin and Preisser \(2015\)](#) found no effect of light on percent survival of settled adelgids, suggesting a negative effect of sunlight on crawlers before they settle to feed. Negative phototactic behavior has been observed in crawlers of the balsam woolly adelgid, which dropped from host material in greater numbers under elevated light level conditions than under shade ([Atkins and Hall, 1969](#)). Experiments examining the direct effects of light on HWA crawler behavior and survival may be useful in elucidating the mechanisms behind the relationship between light and adelgid density on the host. Although we anticipated that reducing shade would affect both minimum and maximum temperatures, air temperature did not differ significantly among light treatments in this experiment and the effect of light on HWA density appeared to be independent of temperature effects.

Our experiment did not distinguish whether the observed light-related differences in live adelgid density were due to direct effects on the insects, or indirect effects mediated through the host plant. Lower HWA densities on seedlings in high light could have been the result of improved host resistance by seedlings as a consequence of improved seedling C balance. Evidence for improved plant chemical defense against herbivores due to resource-driven enhancement of carbon-nutrient balance has not been consistent across a wide range of studies ([Hamilton et al., 2001](#)). However, a number of studies have identified potential relationships between adelgid performance and inter- or intraspecific variability in hemlock tissue chemistry, including terpenoid content in leaves and twigs ([Lagalante and Montgomery, 2003](#); [Lagalante et al., 2006, 2007](#); [McKenzie et al., 2014](#)) and cation concentrations in leaves ([Pontius et al., 2002, 2006](#); [Ingwell et al., 2009](#)). Because HWA is technically a twig feeder, additional studies linking sunlight to host chemistry in twig tissues may be more consistent toward understanding the patterns of adelgid density observed in this and similar studies.

4.3. Light effects on leaf physiology and plant carbon balance

Leaf-level stress and gas exchange were also strongly related to light treatment, albeit counter intuitively, as assimilation rates generally decreased with increasing light and lower levels of infestation. This finding contrasts with other studies that have shown negative gas-exchange responses to HWA infestation and other sap-feeding insects ([Zvereva et al., 2010](#); [Gonda-King et al., 2014](#); [Rubino et al., 2015](#)) but agrees with studies that have shown possible compensatory increases in photosystem function during the early stages of infestation ([Williams et al., 2016](#)). In the current study, the negative relationship between light treatment and assimilation may have resulted from a variety of factors including damage to photosystem II from excessive visible light following freezing, lower N concentration in light-exposed leaves, or some

combination of factors over the course of the study (Pearce, 2001; Larcher, 2003). For example, leaves in spring 2014 in all but the 90% shade treatment had the lowest F_v/F_m observed during the study suggesting severe photosystem damage. This pre-infestation difference in stress may have been the result of freeze damage from the previous, unusually-cold winter when a polar-vortex resulted in a number of minimum nighttime temperatures as low as -18°C at the study site. Nighttime temperatures well below freezing for several hours followed by high irradiance in the less shaded treatments may have caused significant leaf damage due to photobleaching (Gusta and Wiseniewski, 2012). Even moderate shade can prevent such damage (Gusta and Wiseniewski, 2012) and this would explain the wide variation F_v/F_m in Mar 2014. Later in the study after leaves had recovered from the severe freeze event in early 2014, the inverse relationship between assimilation/conductance and light treatment was most likely due to differences in foliar [N]. Foliar [N] often correlates with photosynthetic capacity and has been shown to increase with HWA infestation (Stadler et al., 2005; Gomez et al., 2012; Domec et al., 2013; Rubino et al., 2015). This generally suggests increased photosynthetic capacity in infested trees, although increased assimilation has not been a universal response to higher foliar N (Gonda-King et al., 2014; Rubino et al., 2015). The negative relationship between light and gas exchange observed later in the study suggests a dilution of leaf N concentration from increased growth in seedlings exposed to higher light (Miller-Pierce et al., 2010).

Although there was a negative relationship between instantaneous assimilation rates and increasing light in the presence of HWA, C balance increased in higher light treatments. This suggests that lower levels of HWA infestation due to the light treatment may have reduced carbohydrate extraction by hemlock woolly adelgid. Ultimately, there was generally a strong positive relationship between light treatment and both TNC storage and seedling growth over the course of the study. Although infested trees in other studies have demonstrated a negative C balance, those responses were not necessarily due to reduced photosynthetic capacity (Stadler et al., 2005; Domec et al., 2013; Gonda-King et al., 2014). Moderate light levels (30 and 50% full sunlight) appeared optimum in terms of both reducing HWA populations, providing more light for photosynthesis, and protecting seedlings from either freeze damage or other photosystem stress.

4.4. Potential management implications

Although these results still need to be replicated under field conditions before final management recommendations can be made, they have important implications for future eastern hemlock conservation efforts. Many questions remain unresolved as to how other confounding environmental factors may affect hemlock survival and growth in natural stands where management practices such as thinning and selective harvests increase available light for remaining hemlock. For example, removing a large proportion of canopy trees would likely reduce stand-level transpiration resulting in increased soil moisture, thus also providing a water subsidy for remaining hemlock (Brantley et al., 2013, 2015). HWA infestation has been shown to have deleterious effects on tree hydraulic function and result in a decrease in leaf water potential (Domec et al., 2013; Hickin and Preisser, 2015). Thus, higher soil moisture would likely translate to lower hydraulic resistance in the stem, increased stomatal conductance, and improved C fixation. HWA has also been shown to reduce lateral shoot growth and possibly reduce branch-to-branch contact in infested stands (McClure, 1991; Gonda-King et al., 2014). Thinning dense hemlock

stands to increase light might further hinder HWA movement within the canopy. Considering the combined effects of fewer new buds from HWA infestation (which HWA prefer), HWA-induced changes in hemlock growth and architecture, and the negative response of adelgids to light, thinning or selection harvests might slow the spread and severity of infestation and increase survival in infested stands. While these findings should be interpreted cautiously, they do suggest a path forward for future manipulative studies in infested hemlock stands.

However, large canopy openings (e.g., a shelterwood harvest or group selection that leaves understory hemlock in full sunlight) may also induce additional stress on surviving hemlock adapted to deeply shaded environments and increase competition from shade-intolerant species. Although our results showed no effect of shade treatment on air temperature, substantial changes in ambient light in forested understories would likely increase the extreme range of both air and soil temperatures, and thus might induce either heat or cold stress on HWA populations, depending on location and season. Heat stress would be more likely in the southern end of the hemlock range where this study occurred. In northern parts of the species range, in higher elevation stands, and during extreme cold events, increased exposure of hemlock to extreme cold might increase tree stress through damage to PSII. Canopy removal may increase the susceptibility of young trees to freeze damage in winter. Still, seedlings in the less shaded treatments in this study recovered from freeze damage by late spring and showed levels of leaf stress more similar to other treatments over the remainder of the study. Although stress from increased temperature variation will likely have negative effects on tree physiology over the short-term, hemlock trees may be able to tolerate these stresses better than adelgids and the net effect might actually improve tree survival and growth.

This work improves our understanding of how the ambient light environment influences both HWA densities and C balance of infested trees, provides additional experimental support for the carbon starvation mechanism of mortality, and suggests strategies to improve survival and growth of remaining hemlock. Although there are tradeoffs involved with increasing light and these results should be tested in the field, these findings suggest that silvicultural practices might promote recovery of surviving infested hemlock and enhance resistance of advanced hemlock regeneration facing new HWA pressure. Silvicultural practices such as thinning, shelterwood harvest, or selection harvests that increase light availability to regenerating hemlock might be particularly beneficial in sites where biological control agents are established and/or cold winter temperatures help control adelgid populations. In the end, this type of integrated pest management approach may be the best long-term strategy for conserving and restoring eastern hemlock in eastern North America.

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