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

3

4 Steven T. Brantley^{1, 2,*}, Albert E. Mayfield III³, Robert M. Jetton⁴, Chelcy F. Miniati¹,
5 David R. Zietlow¹, Cindi L. Brown¹, and James R. Rhea⁵

6 ¹USDA Forest Service, Southern Research Station, Coweeta Hydrologic Lab, 3160
7 Coweeta Lab Road, Otto, NC, 28763, USA

8 ²Present address: Joseph W. Jones Ecological Research Center, 3988 Jones Center Drive,
9 Newton, GA, 39870, USA

10 ³USDA Forest Service, Southern Research Station, 200 W. T. Weaver Boulevard,
11 Asheville, NC, 28804, USA

12 ⁴Camcore, Department of Forestry and Environmental Resources, 2720 Faucette Drive,
13 3229 Jordan Hall Addition, North Carolina State University, Raleigh, NC, 27695, USA

14 ⁵USDA Forest Service, Forest Health Protection Region 8 Field Office, 200 W. T.
15 Weaver Boulevard, Asheville, NC, 28804, USA

16 *Corresponding author, e-mail: sbrantle@jonesctr.org

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 0–90% 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

40 forests, our results suggest that silvicultural treatments such as forest thinning that
41 increase light exposure may reduce HWA abundance and lead to better tree C balance,
42 and may be an effective component of large-scale conservation and restoration strategies.

43 **Keywords:** *Adelges tsugae*, invasive species, nonstructural carbohydrates, shade, *Tsuga*
44 *canadensis*

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; Lovett *et al.* 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.*, 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 & 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 & 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 & Reardon, 2011), host gene conservation (Jetton *et*

68 *al.*, 2013), and enhancement of host resistance (Montgomery *et al.*, 2009). Although
69 chemical control has been used with great success to temporarily protect individual trees,
70 financial costs, logistical constraints of manually treating large areas, and environmental
71 constraints greatly limit its use as a sustainable strategy for protecting natural stands
72 (Vose *et al.*, 2013). Biological control shows some promise as several released predatory
73 species are successfully establishing in eastern forests (Mausel *et al.*, 2010; Hakeem *et*
74 *al.*, 2013; Vose *et al.*, 2013; Mayfield *et al.* 2015; D.R. Zietlow, *unpublished data*); but
75 the time required for widespread predator proliferation and dispersal, and timing predator
76 release effectively for outbreak species such as HWA, may limit the effectiveness of
77 biological control as a stand-alone strategy. Coupling these control strategies with forest
78 management practices that improve tree host resistance and increase stand resilience
79 (*sensu* Holling, 1973) may provide a long-term management solution, but direct evidence
80 supporting the efficacy of such treatments is scant.

81 One possible strategy to reduce the effects of HWA in infested hemlock stands is
82 to increase light availability for surviving eastern hemlock trees through silvicultural
83 treatments such as thinning or selective harvests (Fajvan, 2008). Eastern hemlock is one
84 of the most shade-tolerant tree species in eastern North America and can survive in
85 environments where as much as 99% of incident sunlight is intercepted by the canopy
86 (Bourdeau & Laverick, 1958; Hadley, 2000). However, growth in such environments is
87 slow and individual trees may not reach full height unless openings in the canopy allow
88 enough light into the understory for trees to grow more rapidly (Bourdeau & Laverick,
89 1958; Spurr & Barnes, 1980; Brissette & Kenefic, 2000). For example, in a long-term
90 study in Maine, eastern hemlock recruitment, regeneration, and growth all responded

positively to selective harvesting that created large canopy openings (Brissette & 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 & 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 & Elkinton, 2015; Mayfield & Jetton, 2013; Hickin & 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 & Jetton, 2013), and HWA settlement was higher on second year growth under high- compared to low- shade treatments (Hickin & 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 & Elkinton, 2005; 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 17–100% (McAvoy *et al.*, 2014); however, the progrediens 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 x 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 x 1 x 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,

183 PA, USA) and potted (Fafard® 3B growing mix, Sun Gro Horticulture, Agawam, MA,
184 USA) in 6 L plastic containers in May 2013. Seedlings were treated with 15 g of
185 Multicoat 15-16-17 fertilizer (Haifa Group, Altamont Springs, FL, USA) on the soil
186 surface, and watered with tap water. Five seedlings were added to each shade tent on 12
187 Jul 2013. Extra cypress mulch was packed around each pot to a depth of 10–15 cm to
188 insulate the roots and soil during the winter. Seedlings were watered regularly (twice
189 weekly for 2–4 hours, adjusted as needed to local weather and rainfall conditions) during
190 the spring, summer and fall with a button dripper irrigation system (Drip Depot, Inc.,
191 White City, OR) supplying 1.9 L water per hour to the soil surface of each pot. With the
192 exception of momentarily uncovering the tents or removing seedlings to make
193 measurements or infest seedling with adelgids, seedlings remained in the shade tents for
194 the remainder of the study which ended in May 2015.

195

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

198

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 minutes 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 & 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 $\frac{3}{4}$ inch (~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 (amyloglucosidase) 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 & 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 ~90km 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 manufactured 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 ($\text{Log}_{10} [\text{density} + 1]$) 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 ± 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

337 explained 52% of the variability in live HWA sistens density in Jan 2015 ($F_{1,19} = 19.6$, P
 338 < 0.001). In Jan 2015, no adelgids were observed on seedlings exposed to 30% shade or
 339 less, and densities greater than 1 adelgid dm^{-1} were observed only under 70% and 90%
 340 shade.

341
 342 Figure 2. Measured PPFD for shade treatments compared to manufacturers rating for
 343 shade cloth (top panel, $P = 0.006$, $n = 25$, $R^2 = 0.99$); and minimum (▼), mean (●), and
 344 maximum (▲) monthly relative humidity (middle panel) and air temperature (bottom
 345 panel) for all treatments.

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

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 $P > 0.05$).

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).

372

373 Figure 4. Average (± 1 standard error) leaf chlorophyll fluorescence (F_v/F_m) of eastern374 hemlock saplings growing under varying levels of shade ($n = 4$ replicates per shade

375 treatment) from Mar 2014 through Apr 2015. Color gradient of symbols and values in

376 legend correspond to the percent shade. All saplings were experimentally infested with

377 hemlock woolly adelgid in Mar 2014.

Figure 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.

383

384 Figure 6. Variation in foliar nitrogen concentration (% dry mass $\pm$ SE) of planted

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

408

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

Figure 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.

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 & 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 & 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 counterintuitively, 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 & Wiseniewski, 2012). Even moderate shade can prevent
 such damage (Gusta & 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.*
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; Brantley *et al.*, 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 & 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.

Acknowledgements: We thank Carol Harper, Marika Lapham, Bryan Mudder, Corinne Muldoon, Barbara Reynolds, Ben Smith, Andy Tait, Andy Whittier and all other USDA Forest Service, UNC-Asheville, NCSU, and UGA technicians and others who have contributed to this study. We also thank Jeffrey Warren, John Riggins, and Haley Ritger for providing thoughtful reviews on previous versions of this manuscript. Funding for this research was provided by the USDA Forest Service Southern Research Station and USDA Forest Service Forest Health Protection. Any opinions, findings, conclusions or recommendations expressed in the material are those of the authors and do not necessarily reflect the views of the USDA.

572 **Literature Cited**

- 573 Atkins MD, Hall AA. 1969. Effect of light and temperature on the activity of balsam
 574 woolly aphid crawlers. *The Canadian Entomologist* **101**: 481–488.
- 575 Bourdeau PF, Laverick ML. 1958. Tolerance and photosynthetic ability to light intensity
 576 in white pine, red pine, hemlock, and *Alianthus* seedlings. *Forest Science* **4**: 196–
 577 207.
- 578 Brantley ST, Ford CR, Vose JM. 2013. Future species composition will affect forest
 579 water use after loss of eastern hemlock from southern Appalachian forests.
 580 *Ecological Applications* **23**: 777–790.
- 581 Brantley ST, Miniatt CF, Elliott KJ, Laseter SH, Vose JM. 2015. Changes to southern
 582 Appalachian water yield and stormflow after the loss of a foundation species.
 583 *Ecohydrology* **8**: 518–528.
- 584 Brissette JC, Kenefic LS. 2000. Eastern hemlock response to even- and uneven-age
 585 management in the Acadian forest: results from the Penobscot Experimental
 586 Forest long-term silviculture study. In: McManus KA, Shields KS, Souto DR, eds.
 587 *Proceedings: Symposium on sustainable management of hemlock ecosystems in*
 588 *eastern North America*. USDA Forest Service Northeastern Forest Experiment
 589 Station General Technical Report NE-267, Newtown Square, PA, USA, 23–28.
- 590 Broeckling CD, Salom SM. 2003. Volatile emissions of eastern hemlock, *Tsuga*
 591 *canadensis*, and the influence of hemlock woolly adelgid. *Phytochemistry* **62**:
 592 175–180.

- 593 Cowles RS, Montgomery ME, Cheah CA. 2006. Activity and residues of imidacloprid
 594 applied to soil and tree trunks to control hemlock woolly adelgid (Hemiptera:
 595 Adelgidae) in forests. *Journal of Economic Entomology* **99**: 1258–1267.
- 596 Domec JC, Rivera LN, King JS, Peszlen I, Hain F, Frampton J. 2013. Hemlock woolly
 597 adelgid (*Adelges tsugae*) infestation affects water and carbon relations of eastern
 598 hemlock (*Tsuga canadensis*) and Carolina hemlock (*Tsuga caroliniana*). *New*
 599 *Phytologist* **199**: 452–463.
- 600 Ellison AM, Bank MS, Clinton BD, Colburn EA, Elliott KJ, Ford CR, Foster DR,
 601 Kloeppel BD, Knoepp JD, Lovett GM, *et al.* 2005. Loss of foundation species:
 602 consequences for the structure and dynamics of forested ecosystems. *Frontiers in*
 603 *Ecology and the Environment* **9**: 479–486.
- 604 Fajvan MA. 2008. The role of silvicultural thinning in eastern forests threatened by
 605 hemlock woolly adelgid (*Adelges tsugae*). In: Deal RL, ed. *Integrated restoration*
 606 *of forested ecosystems to achieve multiresource benefits: proceedings of the 2007*
 607 *national silviculture workshop*. USDA Forest Service Pacific Northwest Research
 608 Station General Technical Report PNW-733, 247–256.
- 609 Ford CR, Vose JM. 2007. *Tsuga canadensis* (L.) Carr. mortality will impact hydrologic
 610 processes in southern Appalachian forest ecosystems. *Ecological Applications* **17**:
 611 1156–1167.
- 612 Ford CR, Elliott KJ, Clinton BD, Kloeppel BD, Vose JM. 2012. Forest dynamics
 613 following hemlock mortality in the southern Appalachians. *Oikos* **121**: 523–536.
- 614 Flower CE, Knight KS, Gonzalez-Meler MA. 2013. Impacts of the emerald ash-borer
 615 (*Agilus planipennis* Fairmaire) induced ash (*Fraxinus* spp.) mortality on forest

- 616 carbon cycling and successional dynamics in the eastern United States. *Biological*
 617 *Invasions* **15**: 931–944.
- 618 Gomez S, Orians CM, Preisser EL. 2012. Exotic herbivores on a shared native host:
 619 tissue quality after individual, simultaneous, and sequential attack. *Oecologia*
 620 **169**: 1015–1024.
- 621 Gonda-King L, Gomez S, Martin JL, Orians CM, Preisser EL. 2014. Tree responses to an
 622 invasive sap-feeding insect. *Plant Ecology* **215**: 297–304.
- 623 Gusta LV, Wiseniewski M. 2012. Frost tolerance in plants. In: Shabala S, ed. *Plant Stress*
 624 *Physiology*. London, UK: CAB International.
- 625 Hadley JL. 2000. Understory microclimate and photosynthetic response of saplings in an
 626 old-growth eastern hemlock (*Tsuga canadensis* L.) forest. *Ecoscience* **7**: 66–72.
- 627 Hakeem A, Grant JF, Wiggins GJ, Lambdin PL, Hale FA, Buckley DS, Rhea JR,
 628 Parkman JP, Taylor G. 2013. Factors affecting establishment and recovery of
 629 *Sasajiscymnus tsugae* (Coleoptera: Coccinellidae), an introduced predator of
 630 Hemlock Woolly Adelgid (Hemiptera: Adelgidae) on Eastern Hemlock (Pinales:
 631 Pinaceae) *Environmental Entomology* **42**: 1272–1280.
- 632 Hamilton JG, Zangerl AR, DeLucia EH, Berenbaum MR. 2001. The carbon-nutrient
 633 balance hypothesis: its rise and fall. *Ecology Letters* **4**: 86–95.
- 634 Hickin M, Preisser EL. 2015. Effects of light and water availability on the performance
 635 of hemlock woolly adelgid (Hemiptera: Adelgidae). *Environmental Entomology*
 636 **44**: 128–135.
- 637 Holling CS. 1973. Resilience and stability of ecological systems. *Annual Review of*
 638 *Ecology and Systematics* **4**: 1–23.

- 639 Ingwell L, Brady J, Fitzpatrick M, Maynard B, Casagrande R, Preisser E. 2009.
 640 Intraspecific variation in *Tsuga canadensis* foliar chemistry. *Northeastern*
 641 *Naturalist* **16**: 585–594.
- 642 Jetton RM, Whittier WA, Dvorak WS, Rhea JR. 2013. Conserved *ex situ* genetic
 643 resources of eastern and Carolina hemlock: Eastern North American conifers
 644 threatened by the hemlock woolly adelgid. *Tree Planters Notes* **56**: 59–71.
- 645 Lagalante A, Montgomery M. 2003. Analysis of terpenoids from hemlock (*Tsuga*)
 646 species by solid-phase microextraction/gas chromatography/ion-trap mass
 647 spectrometry. *Journal of Agricultural and Food Chemistry* **51**: 2115–2120.
- 648 Lagalante AF, Lewis N, Montgomery ME, Shields KS. 2006. Temporal and spatial
 649 variation of terpenoids in Eastern Hemlock (*Tsuga canadensis*) in relation to
 650 feeding by *Adelges tsugae*. *Journal of Chemical Ecology* **32**: 2389–2403.
- 651 Lagalante AF, Montgomery ME, Calvosa FC, Mirzabeigi MN. 2007. Characterization of
 652 terpenoid volatiles from cultivars of Eastern Hemlock (*Tsuga canadensis*).
 653 *Journal of Agriculture and Food Chemistry* **55**: 10850–10856.
- 654 Larcher W. 2003. Physiological Plant Ecology, 4th Edition. New York, USA: Springer.
- 655 Lovett GM, Canham CD, Arthur MA, Weathers KC, Fitzhugh RD. 2006. Forest
 656 ecosystem responses to exotic pests and pathogens in eastern North America.
 657 *Bioscience* **56**: 395–405.
- 658 Lovett GM, Arthur MA, Weathers KC, Griffin JM. 2010. Long-term changes in forest
 659 carbon and nitrogen cycling caused by an introduced pest/pathogen complex.
 660 *Ecosystems* **13**: 1188–1200.

- 661 Lovett GM, Weiss M, Liebhold AM, Holmes TP, Leung B, Lambert KF, Orwig DA,
 662 Campbell FT, Rosenthal J, McCullough DG, et al. Nonnative forest insects and
 663 pathogens in the United States: Impacts and policy options. *Ecological*
 664 *Applications*: In press. DOI 10.1890/15-1176.1.
- 665 Mausel DL, Salom SM, Kok LT, Davis GA. 2010. Establishment of the Hemlock Woolly
 666 Adelgid predator, *Laricobius nigrinus* (Coleoptera: Derodontidae), in the Eastern
 667 United States. *Environmental Entomology* **39**: 440–448.
- 668 Mayfield AE, Jetton RM. 2013. A shady situation: evaluating the effect of shade on
 669 hemlock woolly adelgid densities on potted hemlock seedlings. P. 43 In: W.P.
 670 Shepherd (ed.), *Proceedings of the 55th Southern Forest Insect Work Conference*,
 671 23-26 July 2013, New Orleans, LA, USA.
- 672 Mayfield AE III, Reynolds BC, Coots CI, Havill NP, Brownie C, Tait AR, Hanula JL,
 673 Joseph SV, Galloway AB. 2015. Establishment, hybridization and impact of
 674 *Laricobius* predators on insecticide-treated hemlocks: exploring integrated
 675 management of the hemlock woolly adelgid. *Forest Ecology and Management*
 676 **335**: 1-10.
- 677 McAvoy TJ, Roberts EA, Johnson NG, McCoy T, Schneeberger NF, Salom SM. 2014.
 678 Hemlock woolly adelgid sistens mortality during the winter of 2013-2014 and
 679 progrediens recovery. Department of Entomology, Virginia Tech, Blacksburg,
 680 VA. 13 p.
- 681 McClure MS. 1989. Evidence of a polymorphic life cycle in the hemlock woolly adelgid,
 682 *Adelges tsugae* (Homoptera: Adelgidae). *Annals of the Entomological Society of*
 683 *America* **82**: 50–54.

- 684 McClure MS. 1991. Density-dependent feedback and population cycles in *Adelges tsugae*
 685 (Homoptera: Adelgidae) on *Tsuga canadensis*. *Environmental Entomology* **20**:
 686 258–264.
- 687 McKenzie EA, Elkinton JS, Casagrande RA, Pressier EI, Mayer M. 2014. Terpene
 688 chemistry of eastern hemlocks resistant to hemlock woolly adelgid. *Journal of*
 689 *Chemical Ecology* **40**: 1003–1012.
- 690 McManamay RH, Resler LM, Campbell JB, McManamay RA. 2011. Assessing the
 691 Impacts of Balsam Woolly Adelgid (*Adelges piceae* Ratz.) and Anthropogenic
 692 Disturbance on the Stand Structure and Mortality of Fraser Fir [*Abies fraseri*
 693 (Pursh) Poir.] in the Black Mountains, North Carolina. *Castanea* **76**: 1–19.
- 694 McWilliams WH, Schmidt TL. 2000. Composition, structure, and sustainability of
 695 hemlock ecosystems in eastern North America. In: McManus KA, Shields KS,
 696 Souto D.R., eds. *Proceedings: Symposium on Sustainable Management of*
 697 *Hemlock Ecosystems in Eastern North America*. USDA Forest Service
 698 Northeastern Forest Experiment Station General Technical Report NE-267.
 699 Newtown Square, PA, USA, 5–10.
- 700 Mech AM. 2015. Abiotic and biotic factors influencing eastern hemlock (*Tsuga*
 701 *canadensis*) health and hemlock woolly adelgid (*Adelges tsugae*) success in the
 702 Southern Appalachian Mountains. PhD Dissertation, University of Georgia,
 703 Athens, GA, USA.
- 704 Miller-Pierce MR, Orwig DA, Pressier E. 2010. Effects of hemlock woolly adelgid and
 705 elongate hemlock scale on eastern hemlock growth and foliar chemistry. *Plant-*
 706 *Insect Interactions* **39**: 513–519.

- 707 Montgomery ME, Bentz SE, Olsen RT. 2009. Evaluation of hemlock (*Tsuga*) species and
708 hybrids for resistance to *Adelges tsugae* (Hemiptera: Adelgidae) using artificial
709 infestation. *Journal of Economic Entomology* **102**: 1247–1254.
- 710 Onken BP, Reardon RC. 2011. An overview and outlook for biological control of
711 hemlock woolly adelgid. In: Onken B, Reardon R, eds. *Implementation and Status*
712 *of Biological Control of the Hemlock Woolly Adelgid*. USDA Forest Service
713 Forest Health Technology Enterprise Team Publication FHTET- 2011-04,
714 Morgantown, WV, USA, 222–228.
- 715 Orwig DA, Foster DR, Mausel DL. 2002. Landscape patterns of hemlock decline in New
716 England due to the introduced hemlock woolly adelgid. *Journal of Biogeography*
717 **29**: 1475–1487.
- 718 Orwig D, Thompson J, Povak N, Manner M, Niebyl D. 2012. A foundation tree at the
719 precipice: *Tsuga canadensis* health after the arrival of *Adelges tsugae* in central
720 New England. *Ecosphere* **3**: Article 10.
- 721 Parker BL, Skinner M, Gouli S, Ashikaga T, Teillon HB. 1998. Survival of hemlock
722 woolly adelgid (Homoptera: Adelgidae) at low temperatures. *Forest Science* **44**:
723 414-420.
- 724 Parker BL, Skinner M, Gouli S, Ashikaga T, Teillon HB. 1999. Low lethal temperature
725 for hemlock woolly adelgid (Homoptera: Adelgidae). *Environmental Entomology*
726 **28**: 1085-1091.
- 727 Pearce RS. 2001. Plant freezing and damage. *Annals of Botany* **87**: 417–424.

- 728 Pontius JA, Hallett RA, Jenkins JC. 2006. Foliar chemistry linked to infestation and
 729 susceptibility to hemlock woolly adelgid (Homoptera: Adelgidae). *Environmental*
 730 *Entomology* **35**: 112–120.
- 731 Pontius J, Hallett R, Martin M. 2002. Examining the role of foliar chemistry in Hemlock
 732 Woolly Adelgid infestation and hemlock decline. In: Reardon R, Onken B,
 733 Lashomb J, ed. *Proceedings: Symposium on the Hemlock Woolly Adelgid in*
 734 *Eastern North America*. US Forest Service, New Brunswick, NJ, USA, 86–99.
- 735 Radville L, Chaves A, Pressier EL. 2011. Variation in plant defense against invasive
 736 herbivores: evidence for a hypersensitive response in eastern hemlock (*Tsuga*
 737 *canadensis*). *Journal of Chemical Ecology* 37: 592–597.
- 738 Rubino L, Sherley C, Sirulink AG, Tuininga AR, Lewis JD. 2015. Invasive insect effects
 739 on nitrogen cycling and host physiology are not tightly linked. *Tree Physiology*
 740 **35**: 124–133.
- 741 Skinner M, Parker BL, Gouli S, Ashikaga T. 2003. Regional responses of hemlock
 742 woolly adelgid (Homoptera: Adelgidae) to low temperatures. *Environmental*
 743 *Entomology* **32**: 523–528.
- 744 Spurr SH, Barnes BV. 1980. *Forest Ecology, third edition*. New York, USA: John Wiley
 745 & Sons.
- 746 Stadler B, Müller T, Orwig D, Cobb R. 2005. Hemlock Woolly Adelgid in New England
 747 forests: canopy impacts transforming ecosystem processes and landscapes.
 748 *Ecosystems* **8**: 233–247.

- 749 Sussky EM, Elkinton JS. 2015. Survival and near extinction of hemlock woolly adelgid
 750 (Hemiptera: Adelgidae) during summer aestivation in a hemlock plantation.
 751 *Environmental Entomology* **44**: 153–159.
- 752 Vose JM, Wear DN, Mayfield III AE, Nelson CD. 2013. Hemlock woolly adelgid in the
 753 southern Appalachians: control strategies, ecological impacts, and potential
 754 management responses. *Forest Ecology and Management* **291**: 209–219.
- 755 Ward E, Deans JD. 1993. A simple method for the routine extraction and quantification
 756 of non-structural sugars in tree tissues. *Forestry* **66**: 171–180.
- 757 Webster JR, Morkeski K, Wojculewski CA, Niederlehner BR, Benfield EF, Elliott KJ.
 758 2012. Effects of hemlock mortality on streams in the southern Appalachian
 759 Mountains. *The American Midland Naturalist* **168**: 112–131.
- 760 Williams JP, Hanavan RP, Rock BN, Minocha SC, Linder E. 2016. Influence of hemlock
 761 woolly adelgid infestation on the physiological and reflectance characteristics of
 762 eastern hemlock. *Canadian Journal of Forest Research* **46**: 410–426.
- 763 Young RF, Shields KS, Berlyn GP. 1995. Hemlock Woolly Adelgid (Homoptera:
 764 Adelgidae): stylet bundle insertion and feeding sites. *Annals of the Entomological*
 765 *Society of America* **88**: 827–835.
- 766 Zvereva E, Lanta V, Kozlov M. 2010. Effects of sap-feeding insect herbivores on growth
 767 and reproduction of woody plants: a meta-analysis of experimental studies.
 768 *Oecologia* **163**: 949–960.