

Effect of an exotic herbivore, *Adelges tsugae*, on photosynthesis of a highly susceptible *Tsuga* host, with notes on conspecifics

Lori A. Nelson · Dylan N. Dillaway ·
Lynne K. Rieske

Received: 23 April 2013 / Accepted: 14 November 2013
© Springer Science+Business Media Dordrecht 2013

Abstract Hemlocks are significant components of temperate forests of Asia and North America, and in eastern North America, they are threatened by an exotic herbivore, the hemlock woolly adelgid, *Adelges tsugae*. The adelgid is native to Asia and northwestern North America, but is highly invasive in eastern North America where natural enemies are unable to regulate populations and eastern hemlock, *Tsuga canadensis*, is highly susceptible. In order to gain a better understanding of the metabolic effects of *A. tsugae* on eastern hemlock, we evaluated its effects on photosynthesis and also evaluated photosynthesis on *Tsuga* species from various geographic origins. We measured light-saturated photosynthesis (A_{sat}) and dark respiration of *T. canadensis* that were infested with adelgid and found a significant decrease in A_{sat} and a small but significant increase in dark respiration, suggesting that *A. tsugae* triggers a physiological response in eastern hemlock by decreasing metabolic activity. In a separate experiment, we also measured A_{sat} of five different hemlock species, including eastern hemlock, the Pacific Northwestern

T. heterophylla and *T. mertensiana*, and the Asian *T. diversifolia* and *T. chinensis*. Only weakly significant differences in A_{sat} were found, with the highest rate in the eastern North American *T. canadensis* and the lowest in the Pacific Northwestern *T. mertensiana*. The relatively high photosynthetic rate of *T. canadensis* could possibly play a role in its susceptibility to *A. tsugae*. A better understanding of this metabolic response could help develop effective management strategies for combating the highly invasive *A. tsugae*.

Keywords Eastern hemlock · Hemlock woolly adelgid · *Adelges tsugae* · Invasive

Introduction

Hemlocks (Fam: Pinaceae, genus: *Tsuga*) are coniferous evergreens widely distributed throughout northern temperate regions. *Tsuga* are well adapted or limited to mesic, cool, temperate areas with high rainfall, relatively cool summers, and minimal water stress (Godman and Lancaster 1990; Means 1990; Packee 1990; Farjon 1990). They are slow growing, shade tolerant, and regenerate poorly. Hemlock is useful for sawtimber, small diameter timber, and pulp, the bark for tanning leather and the needles for teas and perfumes (Godman and Lancaster 1990). Hemlocks are also widely utilized in the horticultural industry; they are excellent landscape trees, and numerous named cultivars have been developed from several *Tsuga* species (Swartley 1984). There are four *Tsuga* species in North America and five in Asia (Farjon 1990).

While hemlocks are significant economically, their principal importance is ecological (see Wang 1961; Godman and Lancaster 1990; Means 1990). Eastern hemlock,

Communicated by Handling Editors: Walter Carson and Heikki Hokkanen.

L. A. Nelson · L. K. Rieske (✉)
Department of Entomology, University of Kentucky,
S-225 Ag North, Lexington, KY 40546-0091, USA
e-mail: lrieske@uky.edu

Present Address:

L. A. Nelson
Department of Radiology, University of Virginia,
480 Ray C. Hunt Dr., Charlottesville, VA 22908, USA

D. N. Dillaway
Center for Natural Resource Management and Protection,
Unity College, 90 Quaker Hill Road, Unity, ME 04988, USA

T. canadensis L. Carrière, and to a lesser extent the more geographically limited con-generic, Carolina hemlock, *T. caroliniana* Engl., play a key role as foundation species in forests of eastern North America (Snyder et al. 2002; Ross et al. 2003). Eastern hemlock is shade tolerant, slow growing, and long-lived (Godman and Lancaster 1990). The canopy is dense, significantly reducing light penetration, and modulating air, soil, and water temperatures beneath the canopy (Godman and Lancaster 1990; Yorks et al. 2003; Ford and Vose 2007; Nuckolls et al. 2009). Understory associates are sparse (Rankin and Tramer 2002). Eastern hemlock needles decompose slowly, resulting in low rates of nitrogen mineralization and nitrification and affecting soil chemistry (Finzi et al. 1998; Jenkins et al. 1999; Yorks et al. 2000). The coarse woody debris generated from eastern hemlock remains in streams longer than that of deciduous species, creating microhabitats and altering sedimentation rates, flow dynamics, and nutrient cycling (Ellison et al. 2005). The resulting habitats support distinct riparian macroarthropods (Adkins and Rieske 2013), diverse fish assemblages (Ross et al. 2003), and benthic macroinvertebrate communities (Snyder et al. 2002; Adkins and Rieske 2013). Eastern hemlock provides key wildlife habitat and serves as an esthetic backdrop for much of the forested lands of the eastern USA (Godman and Lancaster 1990).

Hemlocks in eastern North America are threatened by an exotic and highly invasive insect herbivore, the hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae). The adelgid is native to Asia and northwestern North America (Havill et al. 2006), and hemlocks in these geographic regions have co-evolved with the adelgid to develop some level of resistance (McClure 1992; Montgomery et al. 2009). In addition, these co-evolved hemlock hosts support a complex of natural enemies that help regulate *A. tsugae* populations (Mausel 2005; Kohler et al. 2008). *Adelges tsugae* is a relatively new arrival in eastern North America, and *T. canadensis* has no co-evolutionary history with the herbivore and therefore very little resistance (Havill et al. 2006). Consequently, *A. tsugae* is highly invasive in the region.

In eastern North America, *A. tsugae* has two generations per year, each consisting of a brief highly mobile stage and a longer sessile stage. Newly hatched nymphs (“crawlers”) are mobile and disperse to suitable feeding sites. They settle at the base of a hemlock needle immediately proximal to the abscission layer (Young et al. 1995) and insert their stylet deep into the plant tissue, becoming sessile for the duration of their life cycle. *Adelges tsugae* feed on starches in xylem ray parenchyma cells, which deplete starch reserves (Young et al. 1995).

Plant starches are produced via photosynthesis (Mauseth 2003), and sap-feeding herbivores can reduce woody plant photosynthesis up to 27 % (Zvereva et al. 2010). This suggests that *A. tsugae* not only depletes starch resources in hemlock

trees, but also prevents starch replacement by inhibiting photosynthesis. This depletion of starches within hemlock storage cells leads to needle loss, branch dieback, and eventual tree mortality (McClure et al. 2001). Hemlock mortality occurs 4–10 years after infestation in the northeastern United States, but can occur much quicker in the south (McClure 1991; Orwig and Foster 1998; Trotter and Shields 2009).

Various hemlock species, particularly those of Asian origin, demonstrate adelgid resistance (Bentz et al. 2002; Del Tredici and Kitajima 2004; Montgomery et al. 2009; Weston and Harper 2009), but the exact mechanisms of this resistance are not fully understood. Terpenoid profiles (Lagalante and Montgomery 2003; Lagalante et al. 2006), foliar chemistry (Pontius et al. 2006), or phenological asynchrony (Nelson 2012) may play roles in resistance. In addition, the exact physiological effects of the hemlock woolly adelgid on its host plant are not well known. *Adelges tsugae* may affect hemlock host plants by inducing the production of monoterpenes (Broeckling and Salom 2003), inducing a systemic hypersensitive response (Radville et al. 2011), causing the formation of false rings that restrict water flow (Gonda-King et al. 2012), affecting stomatal conductance, or otherwise altering plant water relations (Domec et al. 2013). A better understanding of hemlock physiology, the physiological impacts of *A. tsugae* feeding, and hemlock response to the adelgid will improve our knowledge of resistance mechanism(s) and contribute to the knowledge base required to develop effective management strategies for combating this highly invasive herbivore.

We evaluated light-saturated photosynthesis (A_{sat}) on several *Tsuga* species with two main objectives. We first evaluated A_{sat} of eastern hemlock, on adelgid-infested and non-infested trees, to determine how *A. tsugae* affects hemlock photosynthesis. We hypothesized that *A. tsugae* feeding on eastern hemlock would reduce photosynthetic rates. We also hypothesized that *A. tsugae* would have negligible effects on dark respiration rates of eastern hemlock, and so we evaluated dark respiration on adelgid-infested and non-infested trees. Our second objective was to measure A_{sat} on five hemlock species that exist in similar habitats in geographically distinct regions. We further hypothesized that any differences may play a role in susceptibility to *A. tsugae*. Our overall goal was to evaluate hemlock physiology and the effects of *A. tsugae* by focusing on light-saturated photosynthetic rates.

Methods

Adelgid-infested *T. canadensis*

Photosynthesis was evaluated on eastern hemlock trees growing at the University of Kentucky’s Robinson

Experimental Forest (Breathitt Co.). Ten *T. canadensis* were planted in a forest gap in 2007–2008 as 3–4-year-old trees, 4.5 m apart. These trees are lightly shaded by surrounding canopy trees, they are not watered, and competing vegetation is manually removed intermittently. Selected branches from a subset of trees ($N = 3$) were artificially infested with *A. tsugae* on April 1, 2011. Infested branch tips (~ 10 cm) were collected from areas of natural infestation and arranged in bundles of 20–25 ovisacs. One bundle was placed on top of the terminal end of each selected hemlock branch and held in place loosely with a clip (Butin et al. 2007). Sleeve cages were constructed from nylon mesh (110 count, Dynamesh, West Chicago, IL) sewn on three sides and wired closed at the basal end. Large (60×60 cm) or small (60×30 cm) cages were used depending on the size of particular branches (Nelson and Rieske 2014). Random sampling of 15 field-collected ovisacs revealed an average of $80(\pm 9)$ eggs per ovisac, making the artificial infestation rates 1600–2,000 adelgid eggs per caged branch, typical of densities found in surrounding infestations.

In October 2011, following the emergence of adelgid sistens nymphs from summer estivation, three adelgid-infested and three adelgid-free *T. canadensis* were randomly chosen to evaluate light-saturated photosynthesis. Three branches were selected from each adelgid-free tree and six branches, three adelgid-infested (~ 60 ovisacs per branch) and three adelgid-free were selected from the adelgid-infested trees. Thus, there were three treatments, (1) adelgid-free branch on an adelgid-free tree (o/o), (2) adelgid-free branch on an infested tree (o/+), and (3) infested branch on an infested tree (+/+) ($N = 9$ branches per treatment).

Sleeve cages were removed prior to photosynthesis measurements, and light-saturated photosynthesis (A_{sat} , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) was measured using a Li-Cor LI-6400 portable photosynthesis system (LI-COR Biosciences, Inc. Lincoln, NE) fitted with a 6400-02B LED light source and a 2×3 cm leaf chamber. A two-liter plastic bottle of ambient air was used as a buffer volume and attached to the inlet air stream with plastic tubing. Several liters of buffer volume is sufficient to dampen fluctuations of incoming CO_2 and keep sample chamber CO_2 relatively stable (LI-COR Biosciences 2004). The conifer configuration was selected; the light source was set to a quantum flux of $1,500 \mu\text{mol m}^{-2} \text{ s}^{-1}$; and the flow rate was set to $200 \mu\text{mol s}^{-1}$. Sample and reference gas analyzers were matched prior to initialization of measurements and when necessary during measurements. The cuvette was clamped onto the hemlock branch with care in order to maximize the amount of leaf area in the cuvette without overlapping needles. Needles were left in the chamber for 2 min to allow for stabilization, after which time A_{sat} was recorded.

The leaf chamber was wiped with alcohol between readings to avoid transferring *A. tsugae* to uninfested branches. All readings were taken on October 3, 2011 between 1200 and 1,400 h. The sky was slightly overcast with a thin layer of clouds, and the temperature was ~ 19 °C.

In order to adjust A_{sat} measurements for differences in leaf area inside the cuvette, a reference grid was constructed with a 2×3 cm window representative of the LI-6400 leaf chamber. The grid was placed over each branch so that only a 6 cm^2 section of foliage was visible; this was digitally photographed from above. Photographs were cropped to the 6 cm^2 section, and Adobe Photoshop 5.0 software (Adobe Systems, San Jose, CA) was used to select only the photosynthetic leaf area (needles) while deleting non-photosynthetic stem. The number of pixels in the leaves was divided by the total number of pixels, and this proportion was multiplied by 6 cm^2 to determine the photosynthetic surface area. All photosynthesis measurements were divided by this photosynthetic surface area to derive an area-based photosynthetic rate (Hadley 2000; Ishii et al. 2007).

To evaluate the extent to which adelgid respiration affects A_{sat} , respiration of adelgid-infested and adelgid-free hemlocks were measured. Hemlock woolly adelgid are sessile feeders with an elongate feeding stylet deeply embedded in host plant tissue; they are intimately associated with their host plant. As such, evaluating adelgid CO_2 emission independent of the host plant is problematic. Three adelgid-infested eastern hemlocks were located with wild populations of *A. tsugae*. Trees were of a size comparable with those in the common garden (< 3 m). From the adelgid-infested trees, two branches were selected, again using the criteria of ~ 60 ovisacs per branch to define “infested” (above). Dark respiration measurements were taken at (1) an adelgid-free point on an adelgid-infested branch (no adelgid ovisacs within the chamber), (2) an adjacent adelgid-infested point on the same selected branch (~ 13 adelgid ovisacs within the leaf chamber), and (3) an adelgid-free branch on the same infested tree (again, no adelgid ovisacs). Readings were taken on 20 and 29 November between 1100 and 1400 hours, when sistens nymphs are actively feeding; skies were slightly overcast with a thin cloud layer and temperatures were ~ 11 – 18 °C. There were three dark respiration measurements of each treatment on each tree using the Li-Cor 6400 system. Thus, we had three treatments on three trees yielding nine respiration measurements per tree, for a total of 27 measurements. All settings on the 6400 were similar for A_{sat} and dark respiration measurements with the exception of the light source. The light source was kept off for dark respiration measurements. Upon clamping onto the branch, readings were allowed to equilibrate (~ 5 min) prior to taking the measurement. Readings were then adjusted for

cuvetted leaf area identically to those used for photosynthetic measurements.

We estimated the portion of CO_2 attributed solely to adelgid respiration, by subtracting the respiration value obtained from the adelgid-free point on the infested branch (treatment 1) from the respiration value obtained from the adelgid-infested point on the same branch (treatment 2) and used that as our estimated adelgid respiration rate ($0.2495 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). We then added the respiration value as a constant to the area-based A_{sat} to estimate adjusted gross photosynthesis. Analysis of variance was used to compare log transformed area-based A_{sat} and area-based respiration across adelgid infestation treatments. Tukey's HSD was used for means separation (SAS 9.2 Cary NC).

Tsuga of varying geographic origin

Photosynthesis was evaluated on hemlock trees growing in a common garden at the University of Kentucky's Spindletop Research Farm (Fayette Co.). The common garden design provides a mechanism to reduce inter- and intra-specific variability due to abiotic factors, to focus solely on interspecific variation. Five hemlock species, including eastern, the Pacific Northwestern *T. heterophylla* (Raf.) Sargent and *T. mertensiana* (Bong.) Carrière, and the Asian *T. diversifolia* (Maxim.) Masters and *T. chinensis* (Franchet) E. Pritzel, were planted in 2007–2008 as 3–4-year-old trees. Ten trees of each species were planted 3 m apart in randomized blocks. Trees are protected on three sides by 70 % shade fabric (Dewitt, Sikeston, MO) and irrigated with ~19 l of water per tree two times a week through the summer. Trees were fertilized with 15-15-15, N-P-K slow release fertilizer at planting (Osmocote, Sierra Chemical, Milipitas, CA), and competing vegetation is suppressed with landscape fabric (DuPont, Wilmington, DE). All trees at the Spindletop common garden are free of adelgid.

Three healthy trees of each species were chosen, and three branches at equal heights on the tree were selected ($N = 9$ branches per species). Light-saturated photosynthesis (A_{sat}) was measured over the course of three days (22, 27, and 29 September 2011) using methods described above; one tree of each species was measured per day. The weather was consistent between days with clear, sunny skies and temperatures between 20 and 23 °C. All measurements were taken between 1100 and 1300 hours.

A_{sat} , adjusted for leaf area, was compared across hemlock species using analysis of variance and Tukey's HSD.

Results

Adelgid feeding caused measureable differences in A_{sat} of *T. canadensis*. Rates differed among trees ($F_{5,21} = 2.66$,

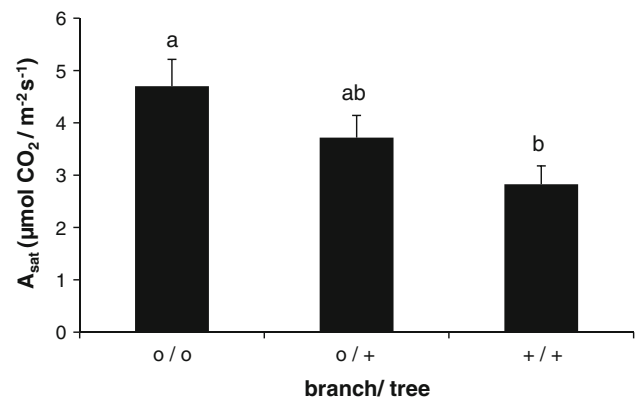


Fig. 1 Effect of *A. tsugae* feeding on eastern hemlock light-saturated photosynthesis (A_{sat} , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) ($F_{2,20} = 5.27$, $P = 0.02$). o/o indicates uninfested tree; o/+ indicates uninfested branch on an infested tree; +/+ indicates infested branch on an infested tree

$P = 0.05$), so tree was included as a block effect in our analysis. Adelgid-infested branches on infested trees had a lower A_{sat} than did adelgid-free trees ($F_{2,20} = 5.27$, $P = 0.02$). Adelgid-free branches on infested trees had intermediate rates of photosynthesis and did not differ from the other treatments (Fig. 1).

Adelgid colonization and feeding also resulted in a small but measureable increase in dark respiration ($F_{3,23} = 3.78$; $P = 0.02$). Respiration was not influenced by tree ($P = 0.47$), so tree was excluded from our analysis. Respiration measured at the point of adelgid feeding (~13 ovisacs) was greater ($F_{4,22} = 3.12$; $P = 0.04$) than respiration measured from an adjacent adelgid-free point on the same branch, which did not differ from adelgid-free branches of the same tree (Fig. 2).

Using our estimate of adelgid respiration, gross photosynthesis (area-based A_{sat} + area-based respiration) on adelgid-free trees was greater than on adelgid-infested trees (Fig. 3).

We found only weak differences ($F_{4,40} = 2.10$, $P = 0.09$) in A_{sat} between *Tsuga* species (Fig. 4), with the highest rate in the eastern North American *T. canadensis* and the lowest in the Pacific Northwestern *T. mertensiana*. The remaining hemlocks evaluated were intermediate with respect to photosynthetic rate and did not differ between either extreme. Rates did not differ between trees of the same species ($F_{2,42} = 0.67$, $P = 0.52$), so tree effect was not included in our analysis. When *T. mertensiana* was removed from the analysis, A_{sat} in the remaining four species did not differ ($F_{3,32} = 1.98$, $P = 0.14$).

Discussion

The extreme susceptibility of eastern hemlock to the hemlock woolly adelgid contributes to the highly invasive

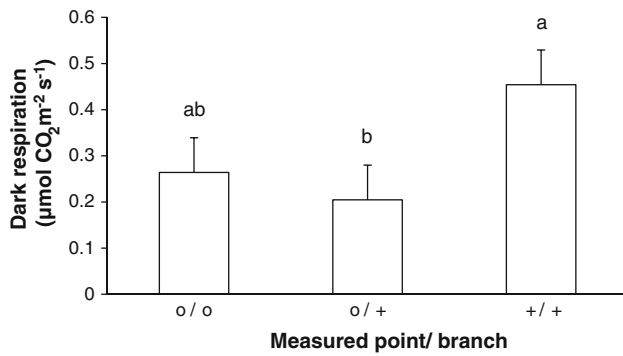


Fig. 2 Effect of *A. tsugae* feeding on eastern hemlock dark respiration ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) ($F_{2,22} = 5.45$, $P = 0.01$). o/o indicates uninfested point on an uninfested branch; o/+ indicates uninfested point on an infested branch; +/+ indicates infested point (~13 ovisacs) on an infested branch

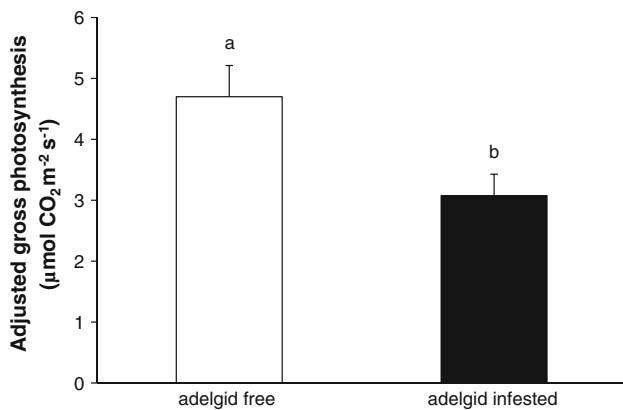


Fig. 3 Adjusted gross photosynthesis (area-based light-saturated photosynthesis—area-based dark respiration, $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) rate of adelgid-infested adelgid-free *T. canadensis* ($F_{1,15} = 7.83$, $P = 0.01$)

nature of this exotic herbivore in eastern North America. Numerous studies have investigated hemlock resistance to the adelgid (McClure 1992; Bentz et al. 2002; Jetton et al. 2008; Montgomery et al. 2009; Weston and Harper 2009; Joseph et al. 2011; Nelson et al. in review), but the mechanisms and extent of that resistance are not fully understood. In an effort to gain a deeper understanding of the invasive nature of the adelgid in the context of host plant susceptibility, we sought to understand the physiological effects of *A. tsugae* on eastern hemlock photosynthesis, and also to investigate potential differences in photosynthetic characteristics among *Tsuga* species from various geographic origins.

Light-saturated photosynthesis was reduced on adelgid-infested eastern hemlock relative to adelgid-free hemlock. Coupled with minimal CO₂ emission associated with adelgid respiration, our results demonstrate a clear and significant reduction in gross photosynthesis associated

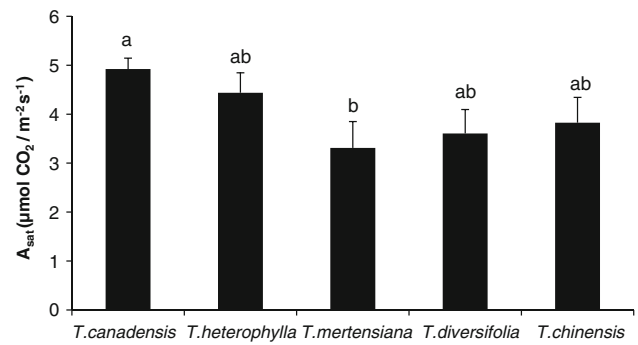


Fig. 4 A_{sat} (area-based light-saturated photosynthetic rates) of five adelgid-free *Tsuga* spp. of various geographic origins growing in a common garden ($F_{4,40} = 2.10$, $P = 0.09$)

with adelgid feeding, suggesting that *A. tsugae* feeding alters the ability of *T. canadensis* to photosynthesize. This physiological response associated with photosynthetic ability is likely coupled with an induced hypersensitive response (Radville et al. 2011), and induced effects on water relations (Gonda-King et al. 2012; Domec et al. 2013) and chemical defenses (Broeckling and Salom 2003), in determining eastern hemlock susceptibility to the adelgid. The decrease in photosynthesis we observed on adelgid-infested branches of infested trees could be due to an inability of *T. canadensis* to compensate for insect attack (Zvereva et al. (2010).

While the above-ground storage capacity of evergreen species is still not well understood, studies have shown that foliar carbohydrates are accumulated and in turn used for subsequent growth and metabolism (Gordon and Larson 1970; Glerum and Balatinecz 1980; Glerum 1980). If foliar carbohydrate storage is indeed the main reserve for new foliage growth in eastern hemlock, adelgid feeding could seriously reduce tree vigor, leading to an inability to provide adequate carbohydrate stores for new growth flushes and general maintenance of tissues. In addition, since adelgids are sessile, feeding and subsequent carbohydrate depletion is continuous, unlike discrete feeding and defoliation events by more mobile sap-feeding insects such as leafhoppers. This continual depletion of carbohydrate stores ultimately results in widespread cell death and a reduction in both active leaf area and photosynthetic capacity (Radville et al. 2011); this appears to be insurmountable for *T. canadensis*.

The majority of studies analyzing tree metabolic response to insect feeding (folivory) report an increase in photosynthetic rates associated with attack (Ericsson et al. 1980; Hunter and Visser 1988). In these cases it appears that the increase in metabolism is a mechanism used to rejuvenate lost carbohydrate stores in leaves, thereby compensating for some reduction in leaf area, at least in the short-term. However, the response of *T. canadensis* to

adelgid feeding that we observed is not consistent with a metabolic compensatory response. Instead it appears to be consistent with the long-term response reported by Radville et al. (2011) and the findings of a meta-analysis of sap-feeding insects (Zvereva et al. 2010). It is possible that because there is no actual loss in leaf area, the typical compensatory response to insect attack is never triggered in the tree. However, the mechanisms triggering an increase in photosynthetic rates to leaf area loss is unknown.

The presence of sleeve cages in our study, used to contain adelgids on adelgid-infested branches, could act as a confounding factor. Sleeve cages may alter branch microhabitat (Nelson and Rieske 2014), potentially affecting long-term photosynthetic rates. However, sleeve cages in our study were removed prior to photosynthesis readings.

Unfortunately, our study did not include southern Japanese hemlock, *T. sieboldii* Carrière, the ancestral host of eastern North America's *A. tsugae* (Havill et al. 2006). It would be interesting to evaluate adelgid-induced physiological responses in the host species with which the adelgid evolved, and also evaluate any physiological similarities of *T. sieboldii* in the context of its congeners.

The relatively low A_{sat} of the Pacific Northwestern mountain hemlock, *T. mertensiana*, adds to other attributes that make it somewhat anomalous among the *Tsuga*. In the past, mountain hemlock was classified in a separate genus as *Hesperopeuce mertensiana* (Bong.) Rydb. (Lemmon 1890), and it is currently placed in its own section within the *Tsuga* (Farjon 1990). Mountain hemlock grows in cold maritime climates on upper elevation subalpine slopes and mountain crests from northern California to Alaska (Means 1990), most often on north-facing slopes. Its habitat and geographic range overlap considerably with the congeneric *T. heterophylla* (Godman and Lancaster 1990; Packee 1990; Farjon 1990), so habitat and geography alone cannot explain the differences in photosynthetic rates we observed. In contrast to other *Tsuga* species, *T. mertensiana* leaves are less flattened and arise around the shoot, and stomata are present on both the upper and lower leaf surfaces (Farjon 1990). These morphological differences may contribute to measurable differences in A_{sat} .

Additional studies are needed to more fully understand the physiological effects of the hemlock woolly adelgid on its hemlock hosts. A more thorough knowledge of the physiological responses associated with adelgid-induced hemlock mortality will enhance our understanding of resistance mechanisms, ultimately enabling us to improve eastern hemlock conservation efforts in eastern North America.

Acknowledgments The authors thank Joshua Adkins, Luke Dodd, Ignazio Graziosi, Abe Levin-Nielsen and Melanie Sprinkle for

assistance in the field, and Millie Hamilton who provided assistance with the LICOR 6400 portable photosynthesis system. Dan Potter and John Obrycki reviewed earlier versions of this manuscript. This work was supported by USDA Forest Service FHP EM and MacIntire Stennis funds from the Kentucky Agricultural Experiment Station and is published as Experiment Station #12-08-114.

References

- Adkins JK, Rieske LK (2013) Loss of a foundation forest species due to an exotic invader impacts terrestrial arthropod communities. *For Ecol Manag* 295:126–135. doi:10.1016/j.foreco.2013.01.012
- Bentz SE, Riedel LGH, Pooler MR, Townsend AM (2002) Hybridization and self-compatibility in controlled pollinations of eastern North American and Asian hemlock (*Tsuga*) species. *J Arboric* 28:200–205
- Broeckling CD, Salom SM (2003) Volatile emissions of eastern hemlock, *Tsuga canadensis*, and the influence of hemlock woolly adelgid. *Phytochemistry* 62:175–180
- Butin E, Preisser E, Elkinton J (2007) Factors affecting settlement rate of the hemlock woolly adelgid, *Adelges tsugae*, on eastern hemlock, *Tsuga canadensis*. *Agric. Forest Entomol.* 9:215–219
- Del Tredici P, Kitajima A (2004) Introduction and cultivation of Chinese hemlock (*Tsuga chinensis*) and its resistance to hemlock woolly adelgid (*Adelges tsugae*). *J Arboric* 30:282–287
- Domec JC, Rivera LN, King JS, Peszlen I, Hain F, Smith B, Frampton J (2013) Hemlock woolly adelgid (*Adelges tsugae*) infestation affects water and carbon relations of eastern hemlock (*Tsuga canadensis*) and Carolina hemlock (*Tsuga caroliniana*). *New Phytol* 199(2):452–463. doi:10.1111/nph.12263
- Ellison AM, Bank MS, Clinton BD, Colburn EA, Elliott K, Ford CR, Foster DR, Kloeppel BD, Knoepp JD, Lovett GM, Mohan J, Orwig DA, Rodenhouse NL, Sobczak WV, Stinson KA, Stone JK, Swan CM, Thompson J, Von Holle B, Webster JR (2005) Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. *Front Ecol Environ* 3:479–486
- Ericsson A, Hellkvist J, Hiller-Hadalgstromer K, Larsson S, Matton-Djos, Tenow O (1980) Consumption and pine growth—hypotheses on effects on growth processes by needle-eating insects. In: Persson T (ed) Structure and function of northern coniferous forests: an ecosystem study. *Ecological Bulletins* 32:537–545
- Farjon A (1990) Pinaceae: Drawings and descriptions of the genera *Abies*, *Cedrus*, *Pseudolarix*, *Keteleeria*, *Nothotsuga*, *Tsuga*, *Cathaya*, *Pseudotsuga*, *Larix*, and *Picea*, 121st edn. Koeltz Scientific Books, Königstein
- Finzi A, Van Breemen N, Canham CD (1998) Canopy tree-soil interactions within temperate forests: species effects on soil carbon and nitrogen. *Ecol Appl* 8:440–446
- Ford CR, Vose JM (2007) *Tsuga canadensis* (L.) Carr. mortality will impact hydrologic processes in southern Appalachian forest ecosystems. *Ecol Appl* 17:1156–1167
- Glerum C (1980) Food sinks and food reserves of trees in temperate climates. *N Z J For Sci* 10:176–185
- Glerum C, Balatinecz JJ (1980) Formation and distribution of food reserves during autumn and their subsequent utilization in jack pine. *Can J Botany* 58:40–54
- Godman RM, Lancaster K (1990) *Tsuga canadensis*. In: Burns RM, Honkala BH (eds) *Silvics of North America vol 1. Conifers*. Agricultural Handbook 654. USDA Forest Service, Washington, DC
- Gonda-King L, Radville L, Preisser EL (2012) False ring formation in eastern hemlock branches: impacts of hemlock woolly adelgid and elongate hemlock scale. *Environ Entomol* 41:523–531. doi:10.1603/EN11227

- Gordon JC, Larson PR (1970) Redistribution of ^{14}C -labeled reserve food in young red pines during shoot elongation. For Sci 16:14–20
- Hadley JL (2000) Understory microclimate and photosynthetic response of saplings in an old-growth eastern hemlock (*Tsuga canadensis* L.) forest. Ecoscience 7:66–72
- Havill NP, Montgomery ME, Yu GY, Shiyake S, Caccone A (2006) Mitochondrial DNA from hemlock woolly adelgid (Hemiptera: Adelgidae) suggests cryptic speciation and pinpoints the source of the introduction to eastern North America. Ann Entomol Soc Am 99:195–203
- Hunter JJ, Visser JH (1988) The effect of partial defoliation, leaf position and developmental stage of the vine on the photosynthetic activity of *Vitis vinifera* L. cv Cabernet Sauvignon. S Afr J Enol Vitic 9:9–15
- Ishii H, Kitaoka S, Fujisaki T, Maruyama Y, Koike T (2007) Plasticity of shoot and needle morphology and photosynthesis of two *Picea* species with different site preferences in northern Japan. Tree Physiol 27:1595–1605
- Jenkins JC, Aber JD, Canham CD (1999) Hemlock woolly adelgid impacts on community structure and N cycling rates in eastern hemlock forests. Can J For Res 29:630–645
- Jetton RM, Dvorak WS, Whittier WA (2008) Ecological and genetic factors that define the natural distribution of Carolina hemlock in the southeastern United States and their role in ex situ conservation. For Ecol Manag 255:3212–3221
- Joseph SV, Braman SK, Hanula JL (2011) Effects of fertilization of four hemlock species on *Adelges tsugae* (Hemiptera: Adelgidae) growth and feeding preference of predators. J Econ Entomol 104:288–298
- Kohler GR, Stiefel VL, Wallin KF, Ross DW (2008) Predators associated with the hemlock woolly adelgid (Hemiptera: Adelgidae) in the Pacific Northwest. Environ Entomol 37:494–504
- Lagalante AF, Montgomery ME (2003) Analysis of terpenoids from hemlock (*Tsuga*) species by solid-phase microextraction/gas chromatography/ion-trap mass spectrometry. J Agric Food Chem 51:2115–2120
- Lagalante AF, Lewis N, Montgomery ME, Shields KS (2006) Temporal and spatial variation of terpenoids in eastern hemlock (*Tsuga canadensis*) in relation to feeding by *Adelges tsugae*. J Chem Ecol 32:2389–2403
- Lemmon JG (1890) Cone-bearers of California. Third biennial report of the California state Board of Forestry for the years 1887–99, to Governor R. W. Waterman. California State Board of Forestry, Sacramento
- LI-COR Biosciences Inc (2004) Using the LI-6400/LI-6400XT Portable Photosynthesis System. Lincoln, Nebraska
- Mausel DL (2005) Observations on fecundity and natural enemies of hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae) in Seattle, Washington. Pan-Pac Entomol 81:97–98
- Mauseth JD (2003) Botany: an introduction to plant biology, 3rd edn. Jones and Barlett Publishers Inc., Sudbury
- McClure MS (1991) Density-dependent feedback and population-cycles in *Adelges tsugae* (Homoptera, Adelgidae) on *Tsuga canadensis*. Environ Entomol 20:258–264
- McClure MS (1992) Hemlock woolly adelgid. Am Nurseryman 175:82–89
- McClure MS, Salom SM, Shields KS (2001) Hemlock woolly adelgid. USDA Forest Service FHTET-2001-03. Morgantown, WV
- Means JE (1990) Mountain hemlock, *Tsuga mertensiana* (Bong.) Carr. In: Burns RM, Honkala BH (eds), Silvics of North America vol 1. Conifers. Agricultural Handbook 654. USDA Forest Service, Washington, DC
- Montgomery ME, Bentz SE, Olsen RT (2009) Evaluation of hemlock (*Tsuga*) species and hybrids for resistance to *Adelges tsugae* (Hemiptera: Adelgidae) using artificial infestation. J Econ Entomol 102:1247–1254
- Nelson LA (2012) Geographically distinct *Tsuga*: Variation in tree physiology, host resistance, and predator response. MS Thesis, Department of Entomology, University of Kentucky, Lexington. 88 p
- Nelson LA, Rieske LK (2014) Microclimatic variation within sleeve cages in ecological studies. J Insect Sci (in press)
- Nuckolls AE, Wurzbarger N, Ford CR, Hendrick RL, Vose JM, Kloepfel BD (2009) Hemlock declines rapidly with hemlock woolly adelgid infestation: impacts on the carbon cycle of southern Appalachian forests. Ecosystems 12:179–190
- Orwig DA, Foster DR (1998) Forest response to the introduced hemlock woolly adelgid in southern New England, USA. J Torrey Bot Soc 125:60–73
- Packee EC (1990) *Tsuga heterophylla*. In: Burns RM, Honkala BH (eds), Silvics of North America volume 1. Conifers. Agricultural Handbook 654. USDA Forest Service, Washington, DC
- Pontius JA, Hallett RA, Jenkins JC (2006) Foliar chemistry linked to infestation and susceptibility to hemlock woolly adelgid (Homoptera: Adelgidae). Environ Entomol 35:112–120
- Radville L, Chaves A, Preisser EL (2011) Variation in plant defense against invasive herbivores: evidence for a hypersensitive response in eastern hemlocks (*Tsuga canadensis*). J Chem Ecol 37:592–597
- Rankin W, Tramer EJ (2002) The gap dynamics of canopy trees of a *Tsuga canadensis* forest community. Northeast Nat 9:391–406
- Ross R, Bennett R, Snyder C, Smith D, Young J, Lemarie D (2003) Influence of eastern hemlock (*Tsuga canadensis* L.) on fish community structure and function in headwater streams of the Delaware River basin. Ecol Freshw Fish 12:60–65
- Snyder CD, Young JA, Lemarie DP, Smith DR (2002) Influence of eastern hemlock (*Tsuga canadensis*) forests on aquatic invertebrate assemblages in headwater streams. Can J Fish Aquat Sci 59:262–275
- Swartley JC (1984) The cultivated hemlocks. Timber Press, Portland, OR
- Trotter RT III, Shields KS (2009) Variation in winter survival of the invasive hemlock woolly adelgid (Hemiptera: Adelgidae) across the eastern United States. Environ Entomol 38:577–587
- Wang CW (1961) The forests of China with a survey of grassland and desert vegetation. Maria Moors Cabot Foundation Publication 5. Harvard University, Cambridge
- Weston PA, Harper RW (2009) Potential of *Tsuga* spp. from Western North America and Asia as replacements for eastern hemlock (*Tsuga canadensis*). Arboric Urban Forest 35:5–9
- Yorks TE, Jenkins JC, Leopold DJ, Raynal DJ, Orwig DA (2000) Influences of eastern hemlock mortality on nutrient cycling. In: Proceedings: symposium on sustainable management of hemlock ecosystems in eastern North America. Northeastern Research Station, pp 126–133
- Yorks TE, Leopold DJ, Raynal DJ (2003) Effects of *Tsuga canadensis* mortality on soil water chemistry and understory vegetation: possible consequences of an invasive insect herbivore. Can J For Res 33:1525–1537
- Young RF, Shields KS, Berlyn GP (1995) Hemlock woolly adelgid (Homoptera, Adelgidae)—stylet bundle insertion and feeding sites. Ann Entomol Soc Am 88:827–835
- Zvereva EL, Lanta V, Kozlov MV (2010) Effects of sap-feeding insect herbivores on growth and reproduction of woody plants: a meta-analysis of experimental studies. Oecologia 163:949–960