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Tree Responses to an Invasive Sap-Feeding Insect

Liahna Gonda-King

Sara Gomez

Jessica L. Martin

Colin M. Orians

Evan L. Preisser

University of Rhode Island, preisser@uri.edu

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Tree responses to an invasive sap-feeding insect

Liahna Gonda-King^{*a}, Sara Gómez^{a,b}, Jessica L. Martin^b, Colin M. Orians^b,
and Evan L. Preisser^a

^aDepartment of Biological Sciences, University of Rhode Island, Kingston, USA

^bDepartment of Biology, Tufts University, Medford, USA

^{*}Corresponding author: liahna.gonda.king@gmail.com

Abstract

Although sap-feeding insects are known to negatively affect plant growth and physiology, less is known about sap-feeding insects on woody plants. *Adelges tsugae* (Annand Hemiptera: Adelgidae), the hemlock woolly adelgid, is an invasive sap-feeding insect in eastern North America that feeds on and kills *Tsuga canadensis* (L. Carrière), eastern hemlock. In the summer, newly hatched nymphs crawl to young unattacked tissue, settle and immediately enter diapause (aestivation) while attached to hemlock. We assessed the effect of *A. tsugae* infestation on *T. canadensis* growth and physiology by analyzing hemlock growth, water potential, photosynthesis, stomatal conductance, and foliar nitrogen (%N). *A. tsugae* infestation decreased eastern hemlock growth, and photosynthesis. Additionally, adelgid-infested hemlocks exhibited signs of water stress that included reductions in water potential and stomatal conductance. These responses shed light on possible mechanisms of adelgid-induced mortality.

Key-words: *Tsuga canadensis*, water potential, gas exchange, % nitrogen, plant-insect interactions

Introduction

Sap-feeders often have long-lasting physiological impacts on their host plant. These physiological changes are driven by both changes in plant nutrients (Masters & Brown 1992; McClure 1980) and the production of secondary chemicals (Haukioja et al. 1990; Karban & Myers 1989). By removing nutrients from the plants' xylem or phloem, sap-feeding insect herbivores decrease plant growth, photosynthesis rates, and reproduction (Candolfi *et al.* 1993; Meyer 1993). In fact, in some systems sap-feeding insects cause greater damage to woody plants than do defoliators (Zvereva et al. 2010).

Relatively few studies have investigated the effects of sap-feeding herbivores on woody species (reviewed in Zvereva et al. 2010). Conifers may be especially susceptible to sap-feeders because they allocate more storage compounds to foliage as opposed to deciduous trees that store resources in their roots, stems, and other tissues inaccessible to sap feeders (Chapin et al. 1990; Hester et al. 2004; Krause & Raffa 1996b). The lack of such stored resources may make conifers more likely to succumb to intense sap-feeding events (Fernandes 1990; Furuta & Aloo 1994; Paine 2000). The invasive hemlock woolly adelgid (*Adelges tsugae* Annand Hemiptera: Adelgidae) is a specialist sap-feeding insect currently decimating eastern hemlock (*Tsuga canadensis* (L.) Carrière), a foundation species in eastern North American forests. *Adelges tsugae* feed by inserting their stylet bundle at the base of a needle into the ray parenchyma tissue (Young et al. 1995) and can kill mature hemlocks within four years of infestation (McClure 1991). Once *A. tsugae* selects a feeding place, it remains sessile throughout its entire life cycle.

Adelges tsugae has two generations in its invaded range; each generation passes through four larval instars before becoming adults. The April – June *progre dien* generation lays eggs that hatch in July; the *sistens* generation that emerges remains on hemlock until the following April

when the cycle starts again (McClure 1989). While the progrediens generation feeds continuously throughout their shorter life cycle, the sistens generation enters diapause, a period of arrested growth and metabolic depression, immediately after hatching and settling on young plant tissue with no previous direct exposure to *A. tsugae*. Summer diapause, also known as aestivation, in *A. tsugae* is primarily induced by temperature (Salom 2001); Lagalante et al. (2006) suggest that *A. tsugae* enters diapause to avoid seasonal increases in plant terpenoids.

The aim of this study was to determine how *A. tsugae* impacts hemlock anatomy and physiology. Specifically, we assessed hemlock growth during its growing season (April-July) and quantified physiological plant responses as a proxy for host performance (water potential, photosynthesis, stomatal conductance, and foliar nitrogen concentration) once the foliage had fully matured. We took these measurements in September when the sistens generation is in diapause and in October, immediately after *A. tsugae* resumed feeding (Fig. 1).

Materials and methods

Study Site: In April 2011, one-year-old *T. canadensis* seedlings (~0.3m in height) were purchased from Van Pines (West Olive, MI, USA) and planted in a grid in a randomized complete block design at the Kingston Wildlife Research Station (Kingston, RI). Treatments were randomly assigned within each row of the grid and each row contained every treatment. Trees (n=15 per treatment) were either experimentally inoculated with *A. tsugae* or remained uninfested. Insect inoculations were applied in April 2011 and 2012 following a standard protocol (see Butin et al. 2007). Briefly, adelgid-infested hemlock branches collected from naturally-infested trees were attached to the trees in the insect treatment while control trees received uninfested branches. Each sapling was enclosed in a cylindrical wire cage (0.3-m diameter, 0.9-m height) covered by a mesh bag (Agribon-15, Johnny's Selected Seeds,

Waterville, ME, USA; 90% light transmission) to prevent cross-contamination. Prior to each physiological sampling (see details below), we counted the number of *A. tsugae* present/cm of sampled foliage in branches from *A. tsugae*-infested trees.

Hemlock Growth: In April, at the beginning of the plant growing season, two terminal branches and two side branches per tree (n=15) were marked. For each marked branch, we measured length of new growth starting at bud break (April 28, 2012) and counted the number of buds (starting on June 15) on the newly produced growth (hereafter referred to as 'secondary buds'). We measured growth and counted buds every two weeks thereafter until July 30, 2012 (when the production of new growth ceased).

Water Potential: On September 8 and October 27, 2012 we measured pre-dawn shoot water potential on 12 randomly-chosen trees per treatment in the *A. tsugae*-infested and control treatments. Between 4:00-5:30 am on each date, we collected one ~5 cm cutting from a terminal branch from each tree, wrapped it in a wet paper towel, placed it in a bag and immediately brought it back to the laboratory in a cooler. Each branch was placed in the pressure chamber of a Scholander pressure bomb (3005 Plant Water Status Console, Soil Moisture Equipment, Santa Barbara, CA, USA) and we recorded the pressure at which xylem appeared visible at the tip of the branch under a magnifying glass.

Gas Exchange: We measured gas exchange of new growth (2012) foliage on a terminal branch on each tree used to quantify water potential. Measurements were conducted between 9:00-11:00 am on September 9 and October 26, 2012. After each measurement, foliage inside the cuvette was excised and photographed; we quantified total needle area using ImageJ 1.44 software (Abràmoff et al. 2004) to use in gas exchange calculations. To determine gas exchange rates we used a CIRAS-2 portable photosynthesis system (PP systems, Haverhill, MA, USA)

with a 2.5 cm² cuvette and a CIRAS-2 LED light source of 1500 μmolm⁻²s⁻¹, a CO² concentration of 390 ppm, air flow rate at 350 cm³s⁻¹ and leaf temperature of 25° C.

Total % Nitrogen: We collected new growth needles and stems from 2012 growth for *A. tsugae*-infested (n=15) and control trees (n=15) in September and October. These needles and stems were oven dried to constant weight at 70°C and then finely ground. We determined total %N through analysing ~2-3 mg of each sample by dry combustion with a CHNOS analyzer (vario Micro cube, Elementar Americas, Mt. Laurel, NJ).

Statistical Analyses: All statistical analyses were performed using JMP 10.0 with each data point being the mean response variable per tree per sampling date. We used repeated-measures ANOVA to analyze the effect of time (repeated factor), treatment, branch type (terminal or side) and all interactions on growth and bud number. We used repeated-measures ANOVA to analyze the main effects of treatment and time, and the treatment*time interaction, on the following variables: water potential, net photosynthesis, stomatal conductance, and %N. We used linear regression to assess the within-treatment correlation between *A. tsugae* density and water potential, photosynthesis, and stomatal conductance for both time points. We checked all data for normality, homogeneity of variance and sphericity. Water potential data were log transformed in order to meet ANOVA assumptions. For analyses that did not meet the assumptions of sphericity, Greenhouse-Geisser corrected p-values are reported.

Results

Hemlock Growth: *Adelges tsugae* infestation had a significant effect on hemlock growth ($F_{1,52}=7.16$, $P=0.010$; Figs 2A and 2B) and bud production ($F_{1,52}=12.34$, $P=0.0009$; Figs 2C, 2D). By the end of the growing season, terminal and side branches on control trees were 41% and 57% longer, respectively, than on *A. tsugae*-infested trees. There was a significant

time*treatment interaction ($F_{6,312}=136.77$, $P=0.0078$) and time*tissue type interaction ($F_{6,312}=12.67$, $P<0.001$). However, there was no time*treatment*tissue interaction. Branch type had a significant effect on growth and bud production. Terminal branches grew more ($F_{1,52}=16.49$, $P<0.001$) and produced more secondary buds ($F_{1,52}=23.78$, $P<0.001$) than side branches. The number of buds increased over time ($F_{3,156}=2.52$, $P<0.001$) and there was a time*tissue interaction ($F_{3,156}=5.22$, $P=0.003$). There were no time*treatment or time*treatment*tissue interactions.

Water Potential: Water potential was 45% higher in control branches than *A. tsugae*-infested branches ($F_{1,20}=11.36$, $P=0.003$; Fig. 3). Water potential changed over time ($F_{1,20}=7.93$, $P=0.011$), but there was no time*treatment interaction ($F_{1,20}=0.02$, $P=0.900$). On both dates, there was a significant negative correlation between *A. tsugae* density and water potential (September: $F_{1,35}=9.55$, $P=0.006$, $R_2=0.313$; October: $F_{1,21}=7.38$, $P=0.010$, $R_2=0.123$).

Gas Exchange: Net photosynthetic rates and stomatal conductance were lower in *A. tsugae*-infested trees ($F_{1,22}=7.70$ and 14.75 , $P=0.011$ and <0.001 respectively; Figs 4A,B) compared to control trees. Photosynthesis and stomatal conductance were greater in October ($F_{1,22}=37.89$ and 111.04 , respectively, both $P<0.001$). There was a significant time*treatment interaction for both gas exchange variables ($F_{1,22}=9.43$ and 11.35 , $P=0.006$ and 0.003 , respectively). There was a negative correlation between *A. tsugae* density and stomatal conductance for both September ($F_{1,21}=6.38$, $P=0.020$, $R_2=0.233$) and October ($F_{1,22}=7.18$, $P=0.013$, $R_2=0.238$). There was no relationship between *A. tsugae* density and photosynthesis for September ($F_{1,21}=1.06$, $P=0.314$) but was marginally significant for October ($F_{1,22}=4.02$, $P=0.057$).

% Nitrogen: Regardless of insect treatment, %N was higher in needles compared to stems ($F_{1,51}=200.87$, $P<0.001$; Figs 5A,B). Adelgid-infested foliage had higher %N concentration than control trees ($F_{1,51}=8.47$, $P=0.0053$). Interestingly, %N was greater in infested trees in September but not October (time*treatment interaction; $F_{1,51}=12.59$, $P<0.001$). When *A. tsugae* were in aestivation (September), %N content was 13% higher in *A. tsugae* -infested needles and 27% higher in *A. tsugae*-infested stems. However, when *A. tsugae* were actively feeding in October the %N content differed by less than 1% for both needles and stems compared to control trees.

Discussion

Our study investigated the effects of an insect's presence on various plant performance traits. We found that *A. tsugae* had significant impacts on hemlock growth. The progrediens generation settles and begins to feed immediately before the start of the hemlock growing season. By the end of the growing season, terminal branches on control trees were 41% longer and had 56% more new buds than *A. tsugae*-infested trees. The effect of *A. tsugae* was even more pronounced on side branches. Side branches on uninfested trees grew 56% more and had 120% more new buds than *A. tsugae*-infested trees. The significant treatment*time interactions for both terminal and side branches suggests that *A. tsugae* has a greater negative impact later in the growing season. While other studies have shown that *A. tsugae* has a detrimental impact on hemlock growth (McClure 1991; Miller-Pierce et al. 2010), we showed that *A. tsugae*-infested trees have significantly less lateral growth and buds than uninfested trees. If these trends continue, such difference in branch architecture could have profound effects on small insects with limited active dispersal such as *A. tsugae*. Lateral and increased branching can promote

herbivore tolerance by increasing sectorized subunits within a plant and augmenting resource capture (reviewed in Stowe *et al.* 2000).

The negative effect of *A. tsugae* continued even after hemlock growth ceased. Adelgid-infested trees had lower photosynthetic and stomatal conductance rates; this difference was evident in September but was magnified in October. In September, *A. tsugae* reduced photosynthesis and stomatal conductance by 10% and 29%, respectively, compared to control trees. In October, however, photosynthesis and stomatal conductance were 36% and 41% lower in infested trees. The hemlocks' magnified response to *A. tsugae* presence in October is likely due to the fact that *A. tsugae* have broken aestivation and are actively feeding by the October time point (Fig. 1). This is congruent with the idea that sap-feeding insects generally tend to decrease photosynthesis (reviewed in Zvereva *et al.* 2010) and suggests that this occurs even when a sap-feeding insect is present but inactive.

The impact of *A. tsugae* may be caused by physical injury or chemical cues associated with the initial stylet insertion. Stylet insertion by other sucking insects has been shown to cause long-lasting anatomical and physiological changes (Ecale & Backus 1995; Ladd & Rawlins 1965; Shackel *et al.* 2005). *Adelges tsugae* secretes a salivary sheath when feeding at the base of hemlock needles that Young *et al.* (1995) suggests may be 'toxic' and responsible for the disproportional negative impact on hemlock growth caused by *A. tsugae* feeding. In support of the 'toxic' saliva hypothesis, Radville *et al.* (2011) found that *A. tsugae* elicits a local and systemic hypersensitive response in hemlock. Alternatively, the adelgid's impact on photosynthesis and stomatal conductance might be explained by long-lasting changes in nutrient allocation originated when *A. tsugae* feeding.

Sap-feeding insects often act as resource sinks (Inbar et al. 1995; Kaplan et al. 2011) that can compete with natural plant sinks (i.e. actively growing tissues). This appears to be true when *A. tsugae* is actively feeding but not when in diapause, as indicated by a 13% decrease in %N after *A. tsugae* resumes feeding in October. During diapause, *A. tsugae*-infested needles and stems have higher %N than control trees, but once *A. tsugae* begin feeding %N does not differ between *A. tsugae*-infested and controls. *Adelges tsugae* feeding is known to alter local and systemic foliar nitrogen content (Gómez et al. 2012; Miller-Pierce et al. 2010; Stadler et al. 2005). Nitrogen sink competition between feeding herbivores and new foliage alters leaf nutrient status, resulting in decreased photosynthesis (Larson 1998). Since *A. tsugae* feed on photosynthate from xylem ray parenchyma cells (Young et al. 1995) that transfer and store nutrients it is likely *A. tsugae* induce greater sinks when feeding than when in diapause. Although the increase in %N of *A. tsugae*-infested trees might increase photosynthesis since most leaf N is incorporated into photosynthetic enzymes and pigments (Evans 1989), we instead documented consistent decreases in photosynthesis. *Adelges tsugae* feeding increases free amino acids (Gómez et al. 2012), a finding that would explain the increase in total %N found in our study.

High %N (White 1984), low photosynthetic activity and low stomatal conductance (Dang et al. 1997; Epron & Dreyer 1993; Farquhar & Sharkey 1982) are often linked with water stress. Hemlocks exhibited greater water stress (i.e., more negative water potential) both when *A. tsugae* were in diapause and feeding. For both time points, this effect was density dependent; water potential was negatively correlated with *A. tsugae* density. Adelgid-induced water stress may result from the increased formation of false rings in *A. tsugae*-infested branches (Gonda-King et al. 2012, Domec et al. 2013). False rings are bands of abnormal wood within an annual ring that

consist of thick-walled xylem cells and which may hinder water transport efficiency (Mitchell 1967). If reductions in water potential are driven by false ring formation, this permanent change in wood anatomy would have a long-lasting impact on water relations and may explain why *A. tsugae* feeding impacts tree physiology.

The *A. tsugae*-hemlock interaction provides an ideal system to study how the presence of sap-feeding insects impact host-plant physiology and performance. Overall, our results suggest that *A. tsugae*-infested trees are water stressed as shown by decreased hemlock growth, water potential, photosynthesis and stomatal conductance congruent with other hypotheses). These physiological changes in *A. tsugae*-infested trees may shed light on possible mechanisms behind *A. tsugae*-induced death. We suggest taking long-term measurements on *A. tsugae*-infested trees, from infestation to mortality, to better clarify the mechanism of *A. tsugae*-induced death.

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Figures

Figure 1. Timing of growth and physiological measurements in relation to the life-histories of *A. tsugae* and *T. canadensis*.

Figure 2. Average ($\pm$ SE) new growth length and number of new buds in hemlocks infested with *A. tsugae* (dark gray) or uninfested (light gray) in terminal (A, C) and side branches (B, D) from bud break (April) to the end of the growing season (July).

Figure 3. Average ($\pm$ SE) water potential measurements in *A. tsugae*-infested (dark gray) and control trees (light gray). Figure 4 . Average ($\pm$ SE) photosynthesis (A) and stomatal conductance (B) in control (light gray) and *A. tsugae*-infested plants (dark gray).

Figure 5. % nitrogen in *A. tsugae*-infested (dark gray) and uninfested needles (light gray) (A) and stems (B).

Figure 1

Figure 2

Figure 3

448
449
450

Figure 4

451
452
453

Figure 5