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Effects of Light and Water Availability on the Performance of Hemlock Woolly Adelgid (*Adelges Tsugae*)

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EFFECTS OF LIGHT AND WATER AVAILABILITY ON
THE PERFORMANCE OF HEMLOCK WOOLLY

ADELGID (*ADELGES TSUGAE*)

BY

MAURI HICKIN

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF

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MASTER OF SCIENCE IN BIOLOGICAL SCIENCES THESIS
OF
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2015

ABSTRACT

Eastern hemlock (*Tsuga canadensis* [L.] Carriere) is a dominant shade-tolerant tree in northeastern United States that has been declining since the arrival of the hemlock woolly adelgid (*Adelges tsugae* Annand). Determining where *A. tsugae* settles under different abiotic conditions is important in understanding the insect's expansion. Resource availability such as light and water can affect herbivore selectivity and damage. We examined how *A. tsugae* settlement and survival were affected by differences in light intensity and water availability, and how adelgid affected tree performance growing in these different abiotic treatments. In a greenhouse at the University of Rhode Island, we conducted an experiment in which the factors light (full-sun, shaded), water (water-stressed, watered), and adelgid (infested, insect-free) were fully crossed for a total of eight treatments (20 two-year-old hemlock saplings per treatment). We measured photosynthesis, transpiration, water potential, relative water content, adelgid density and survival throughout the experiment. Adelgid settlement was higher on the old-growth foliage of shaded and water-stressed trees, but their survival was not altered by foliage age or either abiotic factor. The trees responded more to the light treatments than the water treatments. Light treatments caused a difference in relative water content, photosynthetic rate, transpiration and water potential, however, water availability did not alter these variables. Adelgid did not enhance the impact of these abiotic treatments. Further studies are needed to get a better understanding of how these abiotic factors impact adelgid densities and tree health, and to determine why adelgid settlement was higher in the shaded treatments.

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PREFACE

This thesis “Effects of light and water availability on the performance of hemlock woolly adelgid (*Adelges tsugae*)” is being submitted in manuscript form. This has been accepted for publication in Environmental Entomology.

TABLE OF CONTENTS

ABSTRACT	ii
ACKNOWLEDGMENTS	iii
PREFACE.....	iv
TABLE OF CONTENTS.....	v
LIST OF TABLES	vi
LIST OF FIGURES	vii
CHAPTER 1	1
ABSTRACT.....	2
INTRODUCTION	3
METHODS	5
RESULTS	10
DISCUSSION	11
ACKNOWLEDGEMENTS	17
TABLES.....	18
FIGURES	20
LITERATURE CITED	24

LIST OF TABLES

TABLE	PAGE
Table 1. Results of statistical analysis of <i>A.tsugae</i> -related variables. Values in bold are significant at $P < 0.05$	1
Table 2. Results of statistical analysis of hemlock-related variables. Values in bold are significant at $P < 0.05$	11

LIST OF FIGURES

FIGURE	PAGE
Figure 1. Settlement densities of adelgids. Darker bars represent shaded treatments, lighter bars are full-sun treatments. Watered and water-stressed are labeled below. . .	1
Figure 2. Density and survival of adelgids throughout the summer.	13
Figure 3. Percent relative water content (\pm SE) in needles on old growth and new growth.	19
Figure 4. Photosynthesis (\pm SE), transpiration (\pm SE), and water potential (\pm SE) of trees after adelgids were added.....	21

CHAPTER 1

“Effects of light and water availability on the performance of hemlock woolly adelgid (*Adelges tsugae*)”

By

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Published in Environmental Entomology

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ABSTRACT

Eastern hemlock (*Tsuga canadensis* [L.] Carriere) is a dominant shade-tolerant tree in northeastern United States that has been declining since the arrival of the hemlock woolly adelgid (*Adelges tsugae* Annand). Determining where *A. tsugae* settles under different abiotic conditions is important in understanding the insect's expansion. Resource availability such as light and water can affect herbivore selectivity and damage. We examined how *A. tsugae* settlement and survival were affected by differences in light intensity and water availability, and how adelgid affected tree performance growing in these different abiotic treatments. In a greenhouse at the University of Rhode Island, we conducted an experiment in which the factors light (full-sun, shaded), water (water-stressed, watered), and adelgid (infested, insect-free) were fully crossed for a total of eight treatments (20 two-year-old hemlock saplings per treatment). We measured photosynthesis, transpiration, water potential, relative water content, adelgid density and survival throughout the experiment. Adelgid settlement was higher on the old-growth foliage of shaded and water-stressed trees, but their survival was not altered by foliage age or either abiotic factor. The trees responded more to the light treatments than the water treatments. Light treatments caused a difference in relative water content, photosynthetic rate, transpiration and water potential, however, water availability did not alter these variables. Adelgid did not enhance the impact of these abiotic treatments. Further studies are needed to get a better understanding of how these abiotic factors impact

adelgid densities and tree health, and to determine why adelgid settlement was higher in the shaded treatments.

INTRODUCTION

The hemlock woolly adelgid (*Adelges tsugae* Annand) is an invasive species that poses a major threat to eastern hemlock (*Tsuga canadensis* (L.) Carr.) on the east coast of the United States. The adelgid was introduced to eastern Virginia in the early 1950s from Japan and spread rapidly northward, reaching New England by 1985 (McClure 1989a). It completes two generations per year and is obligately asexual in its invaded range (McClure 1989b), two factors that have helped it spread rapidly through the Northeast. In its invaded range the adelgid feeds exclusively on eastern and Carolina hemlock (*T. carolinensis*) and can kill mature trees in as little as four years (McClure 1991), although some trees can survive for more than ten years (Orwig et al. 2002). In its juvenile ‘crawler’ phase, *A. tsugae* crawlers can move within vegetation or be passively dispersed among trees by wind, birds, or other vectors (McClure 1989b, Turner et al. 2011). Once it locates a suitable feeding site at the base of a hemlock needle, the crawler inserts its stylet bundle and begins feeding on xylem ray parenchyma cells; it will stay in this feeding site for the remainder of its life (Young et al. 1995). The adelgid is now found throughout New England, ranging as far south as Georgia, and poses a significant threat to hemlocks in this region (Orwig et al. 2012). Hemlocks are considered ‘foundation species’ in eastern forests, and their loss will greatly impact both terrestrial and aquatic ecosystems as well as ecosystem processes such as carbon sequestration and nutrient cycling (Ellison et al. 2005).

Researchers have recently begun addressing the mechanism(s) underlying the adelgid's rapid and lethal impact on hemlock trees. The adelgid has been shown to cause a systemic hypersensitive response, a defensive response linked to plant stress, in hemlock trees (Radville et al. 2011). The hypersensitive response, a common response to pathogens and sessile insect herbivores, kills the tissue surrounding the feeding/infection site by starving it of water and nutrients (Heath 2000). Perhaps as a result, *A. tsugae*-infested trees have a greater number of false growth rings, bands of thick-walled latewood indicative of water stress, than uninfested trees (Gonda-King et al. 2012). The adelgid is also known to affect other water-related parameters in eastern hemlock, and to reduce overall tree water use by more than 40% (Domec et al. 2013). Infestation by *A. tsugae* also increases amino acid concentrations at the site of the herbivore's feeding: the largest increase is in proline, an amino acid that acts as an osmoprotectant (Gómez et al. 2012). Furthermore, *A. tsugae* alters plant processes by decreasing stomatal conductance and photosynthesis (Gonda-King et al. 2014).

Despite our improved understanding of the *A. tsugae*-hemlock interaction, the impact of abiotic factors such as light and water availability on this relationship has not been assessed. There is some evidence that water stress renders hemlocks more susceptible to *A. tsugae* damage (Souto et al. 1996) and that trees decline more quickly on xeric versus mesic sites (Sivaramakrishnan and Berlyn 2000, Preisser et al. 2008). During a series of stand-level surveys, we have also noticed that understory hemlocks in high-shade conditions appear to decline more quickly than do hemlocks growing in full sunlight (E. Preisser, *personal observation*). One explanation for this result is that plants experiencing stress may become more susceptible to herbivores (the plant stress

hypothesis; White 1984) . Conversely, healthy and unstressed plants may provide high-quality resources necessary for optimal herbivore growth (the plant vigor hypothesis; Price 1991).

We report the results of work testing how altered light and water availability affected eastern hemlock, *A. tsugae*, and the *A. tsugae*-hemlock interaction. Specifically, we assessed the response of greenhouse-grown hemlock saplings in a 2*2*2 factorial experiment that crossed light (shade versus full-sun) and water (drought versus watered) with adelgid presence (versus absences). Since insects such as *A. tsugae* are generally thought to do better on stressed trees, we hypothesized that *A. tsugae* would settle better and survive longer on shaded, water-stressed trees. Since *A. tsugae* has also been shown to decrease hemlock photosynthesis and stomatal conductance while increasing water potential, we further hypothesized that the presence of *A. tsugae* would exacerbate the impact of abiotic stress on eastern hemlock physiology.

METHODS

In February 2013, we purchased 165 two-year-old uninfested *Tsuga canadensis* saplings (~0.5 m in height) from Van Pines Nursery (West Olive, Michigan). Upon arrival, each sapling was individually planted into a 3.8L plastic pot with potting soil (Sun Gro Metro-Mix 830) and watered. The potted trees were placed in a greenhouse at the University of Rhode Island (Kingston RI) in a grid with 0.5m spacing; trees were rotated to a new randomly-chosen position within the grid every two weeks. Each tree was fertilized two weeks post-transplantation with 175 ppm of

20-10-20 peat lite special. The fertilizer was applied using a five-second spray from a Dosatron D14MZ2 direct injection proportioner. After three weeks, the trees were inspected and five unhealthy trees were removed; each of the remaining 160 trees appeared healthy and had begun to put on new growth. Forty of the 160 remaining trees were then randomly assigned to one of four treatments: watered/full-sun, watered/shaded, water-stressed/full-sun, and water-stressed/shaded. Within each 40-tree group, 20 randomly-selected trees were assigned to an adelgid-infestation treatment and the other 20 trees were assigned to an adelgid-free control (see below for details). This produced a total of eight 20-tree groups.

To create the watered and water-stressed treatments, the soil moisture in each pot was measured every other day using an ML2x soil moisture probe and an HH1 readout (Dynamax Inc., Austin, Texas) accurate to $\pm 1\%$. After soil moisture levels in the 160 pots were measured, data from the 20 trees in each of the four watered treatments and 20 trees from each of the four water-stressed treatments was averaged to generate a mean soil moisture in the four watered and four water-stressed treatments. When average soil moisture in one of the watered treatments dropped below 30%, all 20 trees in that treatment were watered to field capacity by slowly watering each plant until water dripped quickly out of the bottom of the pot. When average soil moisture in one of the water-stressed treatments dropped below 15%, all 20 trees in that treatment were also watered to field capacity as described above.

To create the light treatments, trees in the full-sun treatment were individually covered with a 0.2 m³ bag of 10% shade cloth (ShadeClothStore, Libertyville, IL). Trees in the shaded treatment were individually covered with a 0.2 m³ bag of 90%

shade cloth. To minimize the contact between the bags and the trees, we inserted three ~0.6 m bamboo stakes at the edge of each pot (at 0, 120, and 240 radial degrees) before placing the bags on the trees; each tree's bag rested on the bamboo stakes rather than the foliage.

After six weeks of exposure to the watered/water-stressed and full-sun/shade treatments, the 40 trees in each of the four treatments were split equally into adelgid-infested and uninfested treatments (20 trees per treatment). Crossing the watered/water-stressed and full-sun/shade treatments with an adelgid infested/uninfested treatment created a total of eight 20-tree treatments.

Adelgids were applied to each of the trees in the infested treatments using adelgid-infested foliage collected from Greenfield, Massachusetts. Foliage was attached to each tree using standard protocols (Butin et al., 2007); Briefly, we selected branches from naturally growing hemlocks that were infested with adelgids. We preferably collected branches that contained wool-bearing adelgids on at least 50 percent of the 15cm segment chosen. To control for the disturbance associated with applying the foliage, uninfested foliage was applied to each tree in the uninfested treatments using pest-free foliage collected from Barre, Vermont. When the inoculants were checked four days later, few adelgid crawlers were visible; to ensure that the experimental trees were fully infested, more adelgid-infested foliage was collected from the University of Rhode Island campus (Kingston, RI). After checking the foliage to ensure that no non-adelgid pests were present, a single branch of it was added to each of the trees in the infested treatment. Following this round of inoculations, first-instar crawlers were clearly visible moving and settling on the trees.

Plant measurements: We measured growth, water potential, and gas exchange parameters on each of the 160 experimental trees. To account for any initial differences in hemlock seedlings, we measured stem diameter at soil surface, tree height from soil surface to the tip of the tallest branch, and the length of one randomly-selected terminal branch on every tree. These initial measurements were used as covariates during analysis. We used a Scholander pressure-bomb to make monthly water-potential measurements on each tree from April to July. The April measurement was taken prior to adelgid inoculations, while the May/June/July measurements were taken following the inoculations. Each measurement took two to four consecutive days depending on the number of trees. Two hours before sunrise, two clippings were taken from each tree. One clipping was ~6cm and included both old and new growth; the other clipping was ~4cm and included only new growth. Approximately 0.05g of old-growth needles were removed from the base of each 6cm clipping. After being weighed, the old-growth needles were put into a coin envelope, placed into a 60° C drying oven for one week, and reweighed. Relative water content was determined by subtracting dry weight from wet weight and dividing by the wet weight. We took data in April, May, June and July; for the May sampling experimental error precluded analysis of old growth samples. The same procedure was followed using new-growth needles from the 4cm cutting to determine their relative water content. To take water-potential measurements, the stem of each 6cm clipping was cut to reveal fresh vascular cambium and individually placed into a pressure-bomb. Nitrogen gas was added to the chamber; when fluid emerged from the xylem,

the pressure in bars was recorded, and then converted to MPa to get the water potential for the cutting.

At the same time we took monthly water-potential measurements, we also took gas exchange measurements using a CIRAS-2 photosynthesis meter (PP Systems, Amesbury, Massachusetts). We simultaneously measured photosynthesis, transpiration, and stomatal conductance between 1 hour after sunrise and 11:30 am, with the CIRAS set as follows: ambient light, CO₂ reference=390ppm, H₂O reference=100ppm. Three measurements were taken per branch per tree and used to generate a mean value for each parameter. Because the needles in the CIRAS cuvette did not fill the entire chamber, we took a picture of each branch while inside the cuvette and calculated the needle area using ImageJ (Java Systems) in order to get the actual gas exchange measurements. Due to the high humidity in the greenhouse, the July measurement could not be taken.

Insect Measurements: Starting in early June, we measured adelgid density on both new- and old-growth foliage on two randomly-selected branches per tree. On each branch, the length of new- and old-growth foliage was recorded and the density of both unsettled/dead (first-instar adelgids, distinguishable by their black coloration and lack of woolly covering) and mature (older adelgids, distinguishable by their larger size and white woolly covering) adelgid were counted. Density counts were taken every three weeks from early June through the end of the experiment; data from the two sampled branches was averaged to determine the number of settled and mature adelgids per cm new- and old-growth foliage per tree.

STATISTICAL ANALYSIS

Because new- and old-growth foliage responded very differently to our treatments, we analyzed them separately. We analyzed the effect of light and water on *A. tsugae* settlement using a two-way ANOVA, and assessed changes in *A. tsugae* density and survival over time using a two-way rm-ANOVA. Initial plant height was included in all analyses to account for pre-existing differences in size. We analyzed the effect of light, water, and *A. tsugae* infestation over time on relative water content ('RWC'), photosynthetic rate, transpiration, and water potential using a three-way rm-ANOVA. RWC was measured for both new- and old-growth foliage; because photosynthetic rate, transpiration, and water potential could not be measured separately on new- versus old-growth foliage, our analysis of this data does not differentiate between foliage types. All analyses were performed using JMP 10.0.2 (SAS Systems, Durham NC).

RESULTS

Adelgid performance: Light affected *A. tsugae* settlement on old-growth but not new-growth foliage (table 1). Settlement on old-growth foliage was 50% higher in the shade versus light treatment, and 30% higher on water-stressed versus watered plants. The impact of light and water on *A. tsugae* inhabiting old-growth foliage persisted over the course of the experiment: *A. tsugae* density on old-growth foliage averaged 36% higher in the shaded treatment and 18% higher in the water-stressed treatment (table 1; Figs. 1A,B). Adelgid density on new-growth foliage was not

affected by the treatments (Fig. 1B), and survival rates were similar in both old- and new-growth foliage (table 1; Figs. 2B,D).

Hemlock performance: There was no main effect of adelgid infestation or water on any of the plant performance variables (table 2; Figs. 3,4). Adelgid infestation did decrease water potential in the watered treatment, but not in the water-stressed treatment (water*HWA interaction in Table 2; Fig 4C). In contrast, there was a highly-significant main effect of light on the RWC of both new- and old-growth foliage, photosynthetic rate, and water potential. The RWC of foliage from shaded trees was 10-15% higher than for full-sun trees (Fig 3B). Full-sun trees had higher rates of photosynthesis and transpiration in May, but not in June (time*light interaction in Table 2; Figs. 4A,B). Finally, the water potential of full-sun trees was lower than that of shaded trees throughout the experiment (Fig 4C).

DISCUSSION

Both light and water availability significantly affected adelgid settlement, but only on old-growth foliage (where the majority of crawlers settled; Figs. 1A,B). Because *A. tsugae* survival was consistent across treatments (Table 1), the variation in *A. tsugae* settlement yielded differences in *A. tsugae* density over the course of the experiment. Our results thus suggest that variation in these abiotic factors can substantially alter adelgid population dynamics and may lead to especially high-density infestations in shaded and xeric conditions. Since higher adelgid densities should lead to more rapid hemlock decline, our results may help explain why trees

growing in low-light understory conditions or in drier areas appear particularly hard-hit by this pest.

While we expected that old- and new-growth foliage would differ in adelgid settlement, we were surprised that adelgids appeared to do better on old growth tissue. While the mobile crawlers were found in high densities on both types of foliage, they were considered ‘settled’ only when they began producing wool; a large fraction of crawlers on new-growth foliage never progressed to this stage. As a result, there was often a clear line between settled, wool-producing insects on old growth and black wool-free insects on new growth. Adelgids typically insert their stylet bundle proximal to the plant and the needle abscission site (Young et al. 1995, Oten et al. 2014). When settling on the current year’s growth (e.g., the new growth in our study), however, adelgids will insert their stylet bundle distal to the plant. This may result in needle abscission, or the insect withdrawing its stylet bundle (Young et al. 1995); either outcome would likely prove fatal to vulnerable crawlers. Although insects may be drawn to newly-produced foliage, the ‘green’ and highly-flexible nature of this tissue may interfere with long-term stylet placement or favor needle abscission. This is consistent with previous work showing that while the sistens generation prefers the current year’s growth (McClure 1991), the progrediens generation (which we examined) settle preferentially on the previous year’s growth.

Adelgid settlement on old-growth foliage was 50% higher on shaded versus full-sun trees. Although our study took place in a greenhouse, this result appears consistent with work on trees growing in forested habitats. Research into the vertical stratification of adelgids found higher densities on lower branches than in the sunnier

upper canopy (Evans and Gregoire 2007). One reason for this may be that wool-free adelgids are very fragile and prone to desiccation. Studies have shown that adelgid are very susceptible to cold temperatures (Skinner et al. 2003), and ongoing research suggests that even brief periods of intense summer heat can substantially decrease adelgid survival (J. Elkinton, *unpublished data*). Furthermore, sun stress on a shade-adapted plant can cause the breakdown of photosystems, proteins, and nucleic acids (Demmig-Adams and Adams III 1992). These light-stress-induced problems cause the sap-feeding azalea lace bug *Stephanitis pyrioides* (Heteroptera: Tingidae) to do better on shaded plants rather than ones grown in full sun (Trumbule and Denno 1995). Although we did not test for the breakdown of photosystems, proteins, or nucleic acids, such changes could have resulted in reduced adelgid settlement on full-sun trees. Further studies would be useful to determine whether adelgid crawlers exhibit negative phototaxis behavior that causes them to move away from the sun, or if the sunlight itself is killing the insects once they settle.

The fact that adelgid settlement was 38% higher on water-stressed trees suggests that *A. tsugae* may respond positively to some aspects of plant stress. Because plant morphology, physiology, and water use can be negatively impacted by soil drought (Sperry et al. 2002), high settlement densities on water-stressed trees supports the hypothesis that abiotic stress renders some plants more susceptible to herbivores (White 1984). Our findings are also consistent with work showing that piercing-sucking insects such as adelgids have higher relative growth rates and reproductive potential on stressed plants (Koricheva et al. 1998), but appear to disagree with work showing that other sap-feeders may not benefit from plant water

stress (Huberty and Denno 2004). The adelgid may be different from other similar insects, however, in its ability to substantially alter water relations within the tree. A recent field study found that adelgid presence lowered water potential by 45% relative to uninfested trees (Gonda-King et al. 2014). This finding is consistent with another showing that adelgid decreases water potential, hydraulic conductivity, and results in the production of wood with no constitutive xylem ducts (Domec et al. 2013). This large impact on water relations within the tree may be because the adelgid is altering the tree to be an even more suitable host, and that the adelgid actually does better when hydraulic conductivity and water potential are lowered.

Despite high rates of *A. tsugae* settlement, the adelgid did not directly impact any of our plant physiological measurements. This was surprising because herbivory is well-known to alter plant morphology and physiology (Karban and Baldwin 2007), and adelgids have been shown to affect hemlock water potential, photosynthesis, stomatal conductance, and tree water use (Domec et al. 2013, Gonda-King et al. 2014). Adelgids did decrease water potential, but only in the well-watered treatment: there was no similar effect in the water-stressed treatment (Table 2, Fig. 4C). Since adelgids are known to cause water-stress, we would've expected them to exacerbate the decrease in water potential for water-stressed trees. Since we saw that they significantly altered the watered treatment, we suspect that a water-stressed tree is a more suitable host for the insect. This may help explain why the adelgids have a greater impact on trees that are well-watered compared to the trees already experiencing water-stress.

Although most of our plant physiology results appear at odds with those of earlier studies (Domec et al. 2013, Gonda-King et al. 2014), it is probable that our short-term greenhouse experiment was not long enough to detect adelgid-induced physiological changes within the plant. A greenhouse study such as ours has its benefits, but also some disadvantages. A greenhouse is a perfect setting to control proper soil moisture levels and to control various abiotic conditions. However, growing a shade tolerant tree in a greenhouse causes potential issues. The trees may have experienced slightly hotter temperatures, despite our efforts to control for that. Also, having black shade cloth on the trees may have increased temperatures and impacted shoot tips. Since the trees were rotated bi-weekly, and were all kept in the same area, they experienced the same increases in temperature. The insects may have experienced some greenhouse-related effects. They crawled and settled very similarly to natural conditions, but by the end of the experiment most of the crawlers of the sistens generation did not settle. The lack of sisten settlement did not alter our study because we were interested in the progrediens generation and those insects survived for the duration of time we were interested in observing. While we are confident in our results, future experiments may look to repeat this in a natural setting to determine if the greenhouse had measureable ill-effects and I would recommend not using black shade cloth due to its ability to increase temperature. The short-term nature of our experiment is also likely responsible for the fact that there was no direct impact of our water manipulation on any of our physiology measurements. Furthermore, hemlocks are shade tolerant trees and the greenhouse conditions may have made the physiological impacts more uniform across trees. In contrast, light availability had a

substantial effect on hemlock physiology, but there was no interaction between this factor and adelgid presence (Table 2). The impact of light is unsurprising given its importance to plant growth (Pacala et al. 1994), and since full-sun trees had 4.5x more light exposure than shaded trees, we expected to see large physiological differences.

In conclusion, adelgid settlement was higher on the old growth of shaded and water-stressed trees, but their survival was not altered by foliage age or either abiotic factor. The trees responded more to the light treatments than the water treatments. Light treatments caused a difference in relative water content, photosynthetic rate, transpiration and water potential, but water availability did not alter this effect. Shaded trees had higher adelgid settlement, water potential, and relative water content in the foliage; they also had lower photosynthetic rates and lower transpiration. Although we expected the adelgid to exacerbate the impact of these abiotic factors, we found no evidence that this was the case which may be a product of the experiment being relatively short or greenhouse condition. Our study did show, however, that adelgid settle at higher rates on old growth, shaded trees, and trees experiencing water stress. The preferential settlement of the progrediens generation adelgids on old growth has been discussed in connection with other studies, but this is the first to document actual densities of these insects. There is a clear distinction between adelgid settlement on these two growth types. We speculate that it is due to foliage age and that the newest tissue is “greener” than the old growth and may be difficult for stylet bundle insertion and feeding. There may be other factors affecting adelgid settlement that could be studied further. Also preferential settlement of adelgid on shaded trees is clear, and may be a great opportunity for further studies. It is unclear whether this result is a

product of adelgid desiccation in the sunlight or negatively phototaxis behavior on the part of the crawlers. We did not detect a difference in plant gas exchange, or water potential for trees grown in shade versus sun, therefore there may be other light-induced changes that occur to deter adelgid settlement. These two main findings about adelgid settlement allow for more studies examining these insects in various light environments and settlement sites. We also showed increased settlement on water-stressed trees which may result from the adelgid creating an even more suitable host for itself. Previous research has shown that *A. tsugae* can have a large impact on hemlock water relations, a result consistent with our finding that adelgids affected the water potential of well-watered trees. These results may help to explain hemlock susceptibility to *A. tsugae* and why adelgid densities may vary within a tree.

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TABLES

Table 1: Results of statistical analysis of *A. tsugae*-related variables. Values in bold are significant at $P < 0.05$.

Model Term	<i>A. tsugae</i> settlement						<i>A. tsugae</i> density						<i>A. tsugae</i> survival					
	Old growth			New growth			Old growth			New growth			Old growth			New growth		
	DF	F	P	DF	F	P	DF	F	P	DF	F	P	DF	F	P	DF	F	P
Light	1,68	6.74	0.012	1,67	0.37	0.54	1,54	5.74	0.020	1,50	0.37	0.55	1,54	2.77	0.102	1,50	1.07	0.31
Water	1,68	4.31	0.042	1,67	1.56	0.22	1,54	4.80	0.033	1,50	1.30	0.26	1,54	2.32	0.134	1,50	0.86	0.36
Light*Water	1,68	0.48	0.490	1,67	1.17	0.28	1,54	1.08	0.303	1,50	0.08	0.78	1,54	1.57	0.216	1,50	0.11	0.74
Initial Height	1,68	1.24	0.269	1,67	3.49	0.07	1,54	1.14	0.289	1,50	3.03	0.09	1,54	0.30	0.587	1,50	0.88	0.35
Time	-	-	-	-	-	-	2,53	1.36	0.270	2,49	1.39	0.26	2,53	11.20	<0.001	2,49	2.76	0.07
Time*Light	-	-	-	-	-	-	2,53	2.06	0.139	2,49	0.15	0.86	2,53	0.48	0.622	2,49	2.12	0.13
Time*Water	-	-	-	-	-	-	2,53	0.38	0.680	2,49	1.38	0.26	2,53	1.50	0.232	2,49	0.51	0.61
Time*Light*Water	-	-	-	-	-	-	2,53	0.42	0.660	2,49	0.26	0.78	2,53	0.36	0.701	2,49	0.44	0.65
Time*Initial Height	-	-	-	-	-	-	2,53	3.25	0.047	2,49	0.89	0.42	2,53	0.28	0.756	2,49	0.74	0.48

Table 2: Results of statistical analysis of hemlock-related variables. Values in bold are significant at $P < 0.05$.

Model Term	<i>Relative Water Content</i>						Photosynthesis rate			Transpiration rate			Water potential		
	Old growth			New growth											
	DF	F	P	DF	F	P	DF	F	P	DF	F	P	DF	F	P
Light	1,114	35.63	<0.001	1,110	352.20	<0.001	1,129	5.03	0.03	1,129	1.73	0.19	1,114	5.97	0.02
Water	1,114	0.00	0.945	1,110	0.43	0.51	1,129	0.14	0.71	1,129	0.17	0.68	1,114	3.50	0.06
Adelgid	1,114	1.06	0.306	1,110	0.09	0.76	1,129	0.77	0.38	1,129	1.73	0.19	1,114	2.43	0.12
Light*Water	1,114	2.80	0.097	1,110	15.11	<0.001	1,129	0.03	0.85	1,129	0.52	0.47	1,114	0.03	0.85
Light*Adelgid	1,114	0.02	0.885	1,110	0.01	0.98	1,129	0.04	0.85	1,129	0.01	0.91	1,114	2.40	0.12
Water*Adelgid	1,114	0.01	0.951	1,110	0.51	0.48	1,129	0.04	0.84	1,129	1.11	0.29	1,114	5.96	0.02
Time	1,114	10.12	0.002	2,109	78.64	<0.001	1,129	0.17	0.68	1,129	124.53	<0.001	2,113	71.14	<0.001
Time*Light	1,114	1.03	0.313	2,109	1.89	0.16	1,129	4.61	0.03	1,129	4.99	0.03	2,113	1.37	0.26
Time*Water	1,114	0.37	0.545	2,109	0.30	0.74	1,129	1.22	0.27	1,129	1.39	0.24	2,113	1.05	0.35
Time*Adelgid	1,114	0.28	0.595	2,109	3.04	0.05	1,129	0.71	0.40	1,129	2.16	0.14	2,113	0.77	0.47
Time*Light*Water	1,114	1.65	0.202	2,109	4.07	0.02	1,129	1.17	0.28	1,129	0.01	0.92	2,113	0.50	0.61
Time*Light*Adelgid	1,114	2.67	0.105	2,109	0.29	0.75	1,129	0.03	0.86	1,129	5.42	0.02	2,113	1.06	0.35
Time*Water*Adelgid	1,114	0.04	0.846	2,109	2.59	0.08	1,129	0.15	0.70	1,129	0.23	0.63	2,113	1.43	0.24

FIGURES

Figure 1. Settlement densities of adelgids. Darker bars represent shaded treatments, lighter bars are full-sun treatments. Watered and water-stressed are labeled below. (A) Settlement densities (\pm SE) on old growth. (B) Settlement densities (\pm SE) new growth. These data are represented in the 6 June time point in Fig. 2A, C.

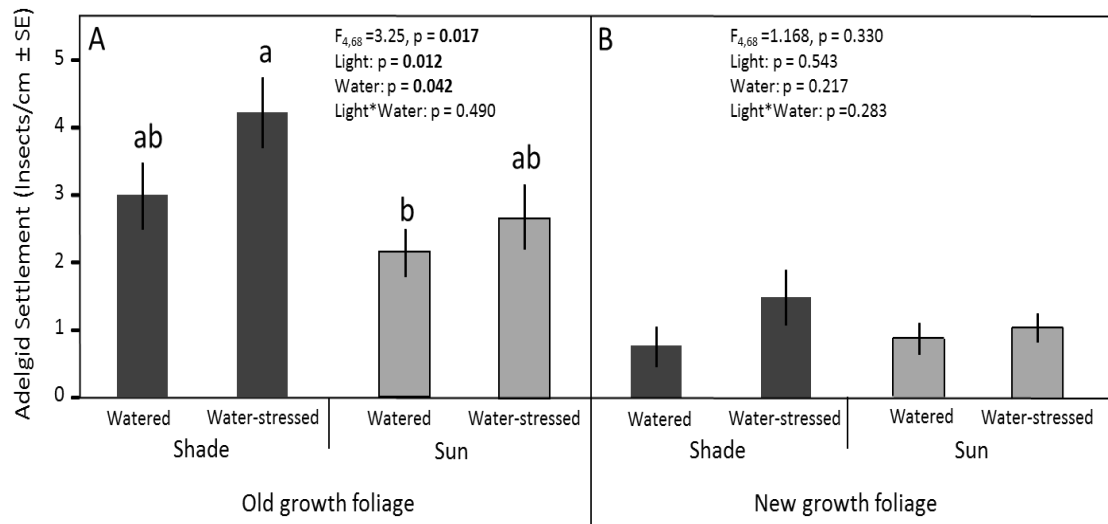


Figure 2. Density and survival of adelgids throughout the summer. Solid markers represent shaded treatments, open markers represent full-sun treatments, circles represent watered trees, and triangles represent water-stressed trees. (A) Density of adelgid (\pm SE) on old growth. (B) Percent survival of adelgid (\pm SE) on old growth. (C) Density of adelgid (\pm SE) on new growth. (D) Percent survival of adelgid (\pm SE) on new growth. Time point 6 June for (A) and (C) are represented in Fig 1 A, B, to help enhance the interpretation of the settlement results.

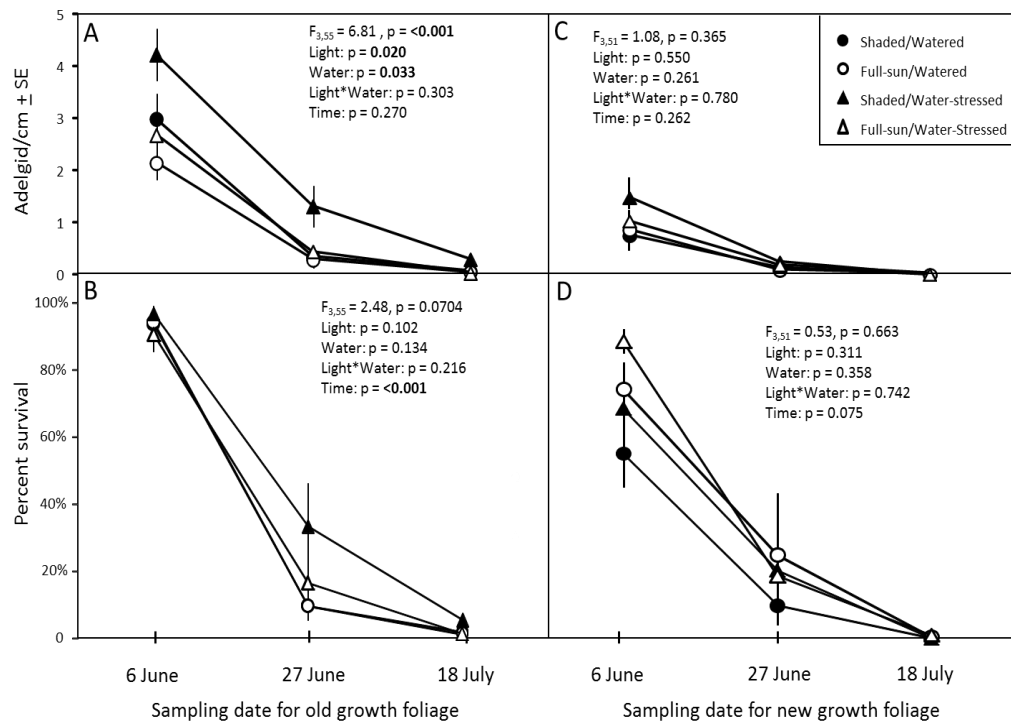


Figure 3. Percent relative water content (\pm SE) in needles on old growth (A) and new growth (B). The legend is the same as fig. 1 for abiotic treatments; solid lines connect adelgid-present treatments and dashed lines connect adelgid-absent treatments. Light availability significantly affected percent relative water content in new and old growth foliage (B). Old growth percent relative water content was 10%-15% higher in shaded trees than full-sun trees. However, adelgids had no effect on percent relative water content.

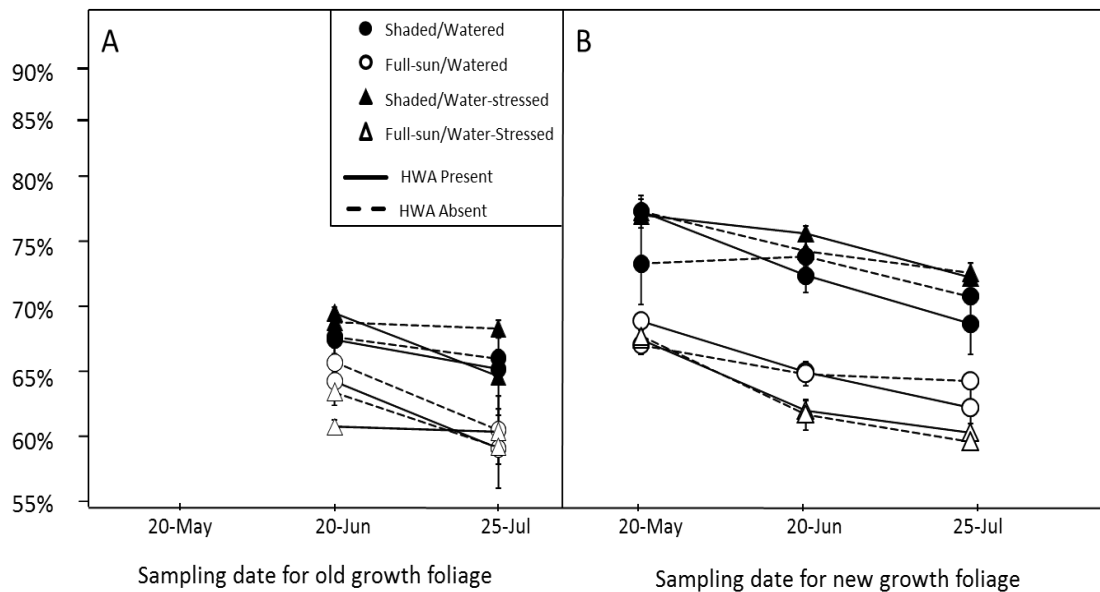
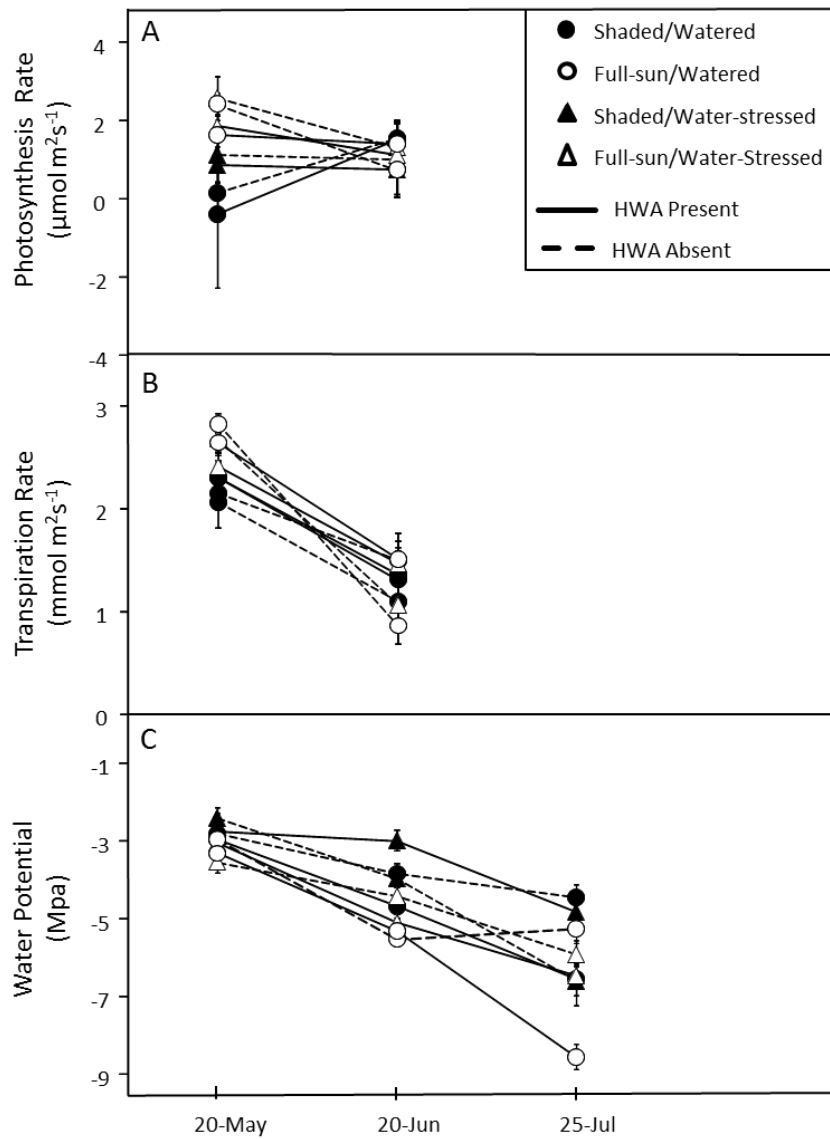


Figure 4. (A) Photosynthesis (\pm SE), (B) transpiration (\pm SE), and (C) water potential (\pm SE) of trees after adelgid were added. Legend is the same as in fig 2. Photosynthetic rate, and water potential were significantly affected by light, There was also a time*light interaction because photosynthetic rates and transpiration were higher for full-sun trees in May but not in June. Water potential was lower for full sun trees throughout the experiment. Adelgids had no effect on photosynthesis, transformation or water potential.



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