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Effects of Light and Water Availability on the Performance of Hemlock Woolly Adelgid (Hemiptera: Adelgidae)

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Hickin, M. & Preisser, E. L. (2015). Effects of Light and Water Availability on the Performance Hemlock Woolly Adelgid (Hemiptera: Adelgidae). *Environmental Entomology*, 44(1): 128-135.
Available at: <http://dx.doi.org/10.1093/ee/nvu012>

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1 Subsection: plant-insect interactions

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3 **RUNNING TITLE:** Light and drought affect adelgid

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6 **TITLE:** Effects of light and water availability on the performance of hemlock woolly

7 adelgid (*Adelges tsugae*)

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17

18 **ABSTRACT**

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

38

39 **KEYWORDS** *Adelges tsugae*, *Tsuga canadensis*, plant-insect interactions

40

41 **INTRODUCTION**

42 The hemlock woolly adelgid (*Adelges tsugae* Annand) is an invasive species that poses a
43 major threat to eastern hemlock (*Tsuga canadensis* (L.) Carr.) on the east coast of the United
44 States. The adelgid was introduced to eastern Virginia in the early 1950s from Japan and spread
45 rapidly northward, reaching New England by 1985 (McClure 1989a). It completes two
46 generations per year and is obligately asexual in its invaded range (McClure 1989b), two factors
47 that have helped it spread rapidly through the Northeast. In its invaded range the adelgid feeds
48 exclusively on eastern and Carolina hemlock (*T. carolinensis*) and can kill mature trees in as little
49 as four years (McClure 1991), although some trees can survive for more than ten years (Orwig et
50 al. 2002). In its juvenile ‘crawler’ phase, *A. tsugae* crawlers can move within vegetation or be
51 passively dispersed among trees by wind, birds, or other vectors (McClure 1989b, Turner et al.
52 2011). Once it locates a suitable feeding site at the base of a hemlock needle, the crawler inserts
53 its stylet bundle and begins feeding on xylem ray parenchyma cells; it will stay in this feeding site
54 for the remainder of its life (Young et al. 1995). The adelgid is now found throughout New
55 England, ranging as far south as Georgia, and poses a significant threat to hemlocks in this region
56 (Orwig et al. 2012). Hemlocks are considered ‘foundation species’ in eastern forests, and their
57 loss will greatly impact both terrestrial and aquatic ecosystems as well as ecosystem processes
58 such as carbon sequestration and nutrient cycling (Ellison et al. 2005).

59 Researchers have recently begun addressing the mechanism(s) underlying the adelgid’s
60 rapid and lethal impact on hemlock trees. The adelgid has been shown to cause a systemic
61 hypersensitive response, a defensive response linked to plant stress, in hemlock trees (Radville et
62 al. 2011). The hypersensitive response, a common response to pathogens and sessile insect
63 herbivores, kills the tissue surrounding the feeding/infection site by starving it of water and

64 nutrients (Heath 2000). Perhaps as a result, *A. tsugae*-infested trees have a greater number of false
65 growth rings, bands of thick-walled latewood indicative of water stress, than uninfested trees
66 (Gonda-King et al. 2012). The adelgid is also known to affect other water-related parameters in
67 eastern hemlock, and to reduce overall tree water use by more than 40% (Domec et al. 2013).
68 Infestation by *A. tsugae* also increases amino acid concentrations at the site of the herbivore's
69 feeding: the largest increase is in proline, an amino acid that acts as an osmoprotectant (Gómez et
70 al. 2012). Furthermore, *A. tsugae* alters plant processes by decreasing stomatal conductance and
71 photosynthesis (Gonda-King et al. 2014).

72 Despite our improved understanding of the *A. tsugae*-hemlock interaction, the impact of
73 abiotic factors such as light and water availability on this relationship has not been assessed.
74 There is some evidence that water stress renders hemlocks more susceptible to *A. tsugae* damage
75 (Souto et al. 1996) and that trees decline more quickly on xeric versus mesic sites (Preisser et al.
76 2008). During a series of stand-level surveys, we have also noticed that understory hemlocks in
77 high-shade conditions appear to decline more quickly than do hemlocks growing in full sunlight
78 (E. Preisser, *personal observation*). One explanation for this result is that plants experiencing
79 stress may become more susceptible to herbivores (the plant stress hypothesis; White 1984).
80 Conversely, healthy and unstressed plants may provide high-quality resources necessary for
81 optimal herbivore growth (the plant vigor hypothesis; Price 1991).

82 We report the results of work testing how altered light and water availability affected
83 eastern hemlock, the progrediens generation of *A. tsugae*, and the *A. tsugae*-hemlock interaction.
84 Specifically, we assessed the response of greenhouse-grown hemlock saplings in a 2*2*2 factorial
85 experiment that crossed light (shade versus full-sun) and water (drought versus watered) with
86 progrediens-generation adelgids (presence versus absence). Since insects such as *A. tsugae* are

87 thought to do better on stressed trees, we hypothesized that *A. tsugae* would settle better and
88 survive longer on shaded, water-stressed trees. Since *A. tsugae* has also been shown to decrease
89 hemlock photosynthesis and stomatal conductance while increasing water potential, we further
90 hypothesized that the presence of *A. tsugae* would exacerbate the impact of abiotic stress on
91 eastern hemlock physiology.

92 MATERIALS AND METHODS

93 In February 2013, we purchased 165 two-year-old uninfested *Tsuga canadensis* saplings
94 (~0.5 m in height) from Van Pines Nursery (West Olive, Michigan). Upon arrival, each sapling
95 was individually planted into a 3.8L plastic pot with potting soil (Sun Gro Metro-Mix 830) and
96 watered. The potted trees were placed in a greenhouse at the University of Rhode Island
97 (Kingston RI) in a grid with 0.5m spacing; trees were rotated to a new randomly-chosen position
98 within the grid every two weeks. Each tree was fertilized two weeks post-transplantation with 175
99 ppm of 20-10-20 peat lite special. The fertilizer was applied using a five-second spray from a
100 Dosatron D14MZ2 direct injection proportioner. After three weeks, the trees were inspected and
101 five unhealthy trees were removed; each of the remaining 160 trees appeared healthy and had
102 begun to put on new growth. Forty of the 160 remaining trees were then randomly assigned to one
103 of four treatments: watered/full-sun, watered/shaded, water-stressed/full-sun, and water-
104 stressed/shaded. Within each 40-tree group, 20 randomly-selected trees were assigned to an
105 adelgid-infestation treatment and the other 20 trees were assigned to an adelgid-free control (see
106 below for details). This produced a total of eight 20-tree groups.

107 To create the watered and water-stressed treatments, the soil moisture in each pot was
108 measured every other day using an ML2x soil moisture probe and an HH1 readout (Dynamax
109 Inc., Austin, Texas) accurate to $\pm 1\%$. After soil moisture levels in the 160 pots were measured,

110 data from the 20 trees in each of the four watered treatments and 20 trees from each of the four
111 water-stressed treatments was averaged to generate a mean soil moisture in the four watered and
112 four water-stressed treatments. When average soil moisture in one of the watered treatments
113 dropped below 30%, all 20 trees in that treatment were watered to field capacity by slowly
114 watering each plant until water dripped quickly out of the bottom of the pot. When average soil
115 moisture in one of the water-stressed treatments dropped below 15%, all 20 trees in that treatment
116 were also watered to field capacity as described above. In the course of the experiment, trees in
117 the adelgid-present versus -absent treatments did not differ in their watering regime.

118 To create the light treatments, trees in the full-sun treatment were individually covered
119 with a 0.2 m³ bag of 10% shade cloth (ShadeClothStore, Libertyville, IL). Trees in the shaded
120 treatment were individually covered with a 0.2 m³ bag of 90% shade cloth. To minimize the
121 contact between the bags and the trees, we inserted three ~0.6 m bamboo stakes at the edge of
122 each pot (at 0, 120, and 240 radial degrees) before placing the bags on the trees; each tree's bag
123 rested on the bamboo stakes rather than the foliage.

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

128 Adelgids were applied to each of the trees in the infested treatments using adelgid-infested
129 foliage collected from Greenfield, Massachusetts. Foliage was attached to each tree using
130 standard protocols (Butin et al., 2007); briefly, we selected branches ~15 cm in length from
131 naturally growing hemlocks that were infested with adelgids. We only collected branches that
132 contained wool-bearing adelgids on >50% of the 15 cm segment. To control for the disturbance

133 associated with applying the foliage, uninfested foliage was applied to each tree in the uninfested
134 treatments using pest-free foliage collected from Barre, Vermont. When the inoculants were
135 checked four days later, few adelgid crawlers were visible; to ensure that the experimental trees
136 were fully infested, more adelgid-infested foliage was collected from the University of Rhode
137 Island campus (Kingston, RI). After checking the foliage to ensure that no non-adelgid pests were
138 present, a single branch was added to each of the trees in the infested treatment. Following this
139 round of inoculations, first-instar crawlers were clearly visible moving and settling on the trees.

140 **Plant measurements:** We measured growth, water potential, and gas exchange
141 parameters on each of the 160 experimental trees. To account for any initial differences in
142 hemlock seedling size, we measured the height of every tree from the soil surface to the tip of the
143 tallest branch. These initial measurements were used as covariates during analysis. We used a
144 Scholander pressure-bomb to make monthly water-potential measurements on each tree from
145 April to July. The April measurement was taken prior to adelgid inoculations, while the
146 May/June/July measurements were taken following the inoculations. Each measurement took two
147 to four consecutive days depending on the number of trees. Two hours before sunrise, two
148 clippings were taken from each tree. One clipping was ~6cm and included both old and new
149 growth; the other clipping was ~4cm and included only new growth. Approximately 0.05g of old-
150 growth needles were removed from the base of each 6cm clipping. After being weighed, the old-
151 growth needles were put into a coin envelope, placed into a 60° C drying oven for one week, and
152 reweighed. Relative water content was determined by subtracting dry weight from wet weight and
153 dividing by the wet weight. We took data in April, May, June and July; for the May sampling
154 experimental error precluded analysis of old growth samples. The same procedure was followed
155 using new-growth needles from the 4cm cutting to determine their relative water content. To take

156 water-potential measurements, the stem of each 6cm clipping was cut to reveal fresh vascular
157 cambium and individually placed into a pressure-bomb. Nitrogen gas was added to the chamber;
158 when fluid emerged from the xylem, the pressure in bars was recorded, and then converted to
159 MPa to get the water potential for the cutting.

160 At the same time we took monthly water-potential measurements, we also took gas
161 exchange measurements using a CIRAS-2 photosynthesis meter (PP Systems, Amesbury,
162 Massachusetts). We simultaneously measured photosynthesis, transpiration, and stomatal
163 conductance between 1 hour after sunrise and 11:30 am, with the CIRAS set as follows: ambient
164 light, CO₂ reference=390ppm, H₂O reference=100ppm. Three measurements were taken per
165 branch per tree and used to generate a mean value for each parameter. Because the needles in the
166 CIRAS cuvette did not fill the entire chamber, we took a picture of each branch while inside the
167 cuvette and calculated the needle area using ImageJ (Java Systems) in order to get the actual gas
168 exchange measurements. Because the old- and new-growth foliage grew so closely together on a
169 branch, we were unable to separately measure the photosynthetic rate, transpiration, and water
170 potential of new- versus old-growth foliage. High humidity in the greenhouse during July
171 prevented us from taking measurements at that time point.

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

179 adelgids per cm new- and old-growth foliage per tree. The experiment ended when all of the
180 progrediens-generation adelgids had either matured and reproduced or died.

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

191 **RESULTS**

192 **Adelgid performance:** Light affected *A. tsugae* settlement on old-growth but not new-
193 growth foliage (table 1). Settlement on old-growth foliage was 50% higher in the shade versus
194 light treatment, and 30% higher on water-stressed versus watered plants. The impact of light and
195 water on *A. tsugae* inhabiting old-growth foliage persisted over the course of the experiment: *A.*
196 *tsugae* density on old-growth foliage averaged 36% higher in the shaded treatment and 18%
197 higher in the water-stressed treatment (table 1; Figs. 1A,B). Adelgid density on new-growth
198 foliage was not affected by the treatments (Fig. 1B), and survival rates were similar in both old-
199 and new-growth foliage (table 1; Figs. 2B,D).

200 **Hemlock performance:** There was no main effect of adelgid infestation or water on any
201 of the plant performance variables (table 2; Figs. 3,4). Adelgid infestation did decrease water

202 potential in the watered treatment, but not in the water-stressed treatment (water*HWA
203 interaction in Table 2; Fig 4C). In contrast, there was a highly-significant main effect of light on
204 the RWC of both new- and old-growth foliage, photosynthetic rate, and water potential. The
205 RWC of foliage from shaded trees was 10-15% higher than for full-sun trees (Fig 3B). Full-sun
206 trees had higher rates of photosynthesis and transpiration in May, but not in June (time*light
207 interaction in Table 2; Figs. 4A,B). Finally, the water potential of full-sun trees was lower than
208 that of shaded trees throughout the experiment (Fig 4C).

209 DISCUSSION

210 Both light and water availability significantly affected adelgid settlement, but only on old-
211 growth foliage (where the majority of crawlers settled; Figs. 1A,B). Because *A. tsugae* survival
212 was consistent across treatments (Table 1), variation in *A. tsugae* settlement yielded differences in
213 *A. tsugae* density over the course of the experiment. Our results thus suggest that variation in
214 these abiotic factors can substantially alter adelgid population dynamics and may lead to
215 especially high-density infestations in shaded and xeric conditions. Since higher adelgid densities
216 should lead to more rapid hemlock decline, our results may help explain why trees growing in
217 low-light understory conditions or in drier areas appear particularly hard-hit by this pest.

218 While we expected that old- and new-growth foliage would differ in adelgid settlement,
219 the magnitude of the difference in adelgid settlement was striking. While the mobile crawlers
220 were found in high densities on both types of foliage, they were considered 'settled' only when
221 they began producing wool; a large fraction of crawlers on new-growth foliage never progressed
222 to this stage. As a result, there was often a clear line between settled, wool-producing insects on
223 old growth and black wool-free insects on new growth. Adelgids typically insert their stylet
224 bundle proximal to the plant and the needle abscission site (Young et al. 1995, Oten et al. 2014).

225 When settling on the current year's growth (e.g., the new growth in our study), however, adelgids
226 will insert their stylet bundle distal to the plant. This may result in needle abscission, or the insect
227 withdrawing its stylet bundle (Young et al. 1995); either outcome would likely prove fatal to
228 vulnerable crawlers. Although insects may be drawn to newly-produced foliage, the 'green' and
229 highly-flexible nature of this tissue may interfere with long-term stylet placement or favor needle
230 abscission. Our result is thus consistent with previous work showing that while the sistens
231 generation prefers the current year's growth (McClure 1991), the progrediens generation (which
232 we examined) settle preferentially on the previous year's growth.

233 Adelgid settlement on old-growth foliage was 50% higher on shaded versus full-sun trees,
234 a result that appears consistent with work on trees growing in forested habitats. Research into the
235 vertical stratification of adelgids found higher densities on lower branches than in the sunnier
236 upper canopy (Evans and Gregoire 2007). One reason for this may be that wool-free adelgids are
237 very fragile and prone to desiccation. Studies have shown that adelgid are very susceptible to cold
238 temperatures (Skinner et al. 2003), and ongoing research suggests that even brief periods of
239 intense summer heat can substantially decrease adelgid survival (J. Elkinton, *unpublished data*).
240 Furthermore, sun stress on a shade-adapted plant can cause the breakdown of photosystems,
241 proteins, and nucleic acids (Demmig-Adams and Adams III 1992). These light-stress-induced
242 problems cause the sap-feeding azalea lace bug *Stephanitis pyrioides* (Heteroptera: Tingidae) to
243 do better on shaded plants rather than ones grown in full sun (Trumbule and Denno 1995).
244 Although we did not test for the breakdown of photosystems, proteins, or nucleic acids, such
245 changes could have resulted in reduced adelgid settlement on full-sun trees. Further studies would
246 be useful to determine whether adelgid crawlers exhibit negative phototactic behavior that causes
247 them to move away from the sun, or if the sunlight itself is killing the insects once they settle.

248 The fact that adelgid settlement was 38% higher on water-stressed trees suggests that *A.*
249 *tsugae* may respond positively to some aspects of plant stress. Because plant morphology,
250 physiology, and water use can be negatively impacted by soil drought (Sperry et al. 2002), higher
251 settlement densities on water-stressed trees is consistent with the hypothesis that abiotic stress
252 renders some plants more susceptible to herbivores (White 1984). Our findings are also consistent
253 with work showing that piercing-sucking insects such as adelgids have higher relative growth
254 rates and reproductive potential on stressed plants (Koricheva et al. 1998). The adelgid may differ
255 from otherwise similar insects, however, in its ability to substantially alter water relations within
256 the tree. A recent field study found that adelgid presence lowered water potential by 45% relative
257 to uninfested trees (Gonda-King et al. 2014). This finding is consistent with another showing that
258 adelgid decreases water potential, hydraulic conductivity, and results in the production of wood
259 with no constitutive xylem ducts (Domec et al. 2013). This large impact on water relations within
260 the tree may result from the adelgid altering the tree to be an even more suitable host, and
261 suggests that the adelgid may do better at lower hydraulic conductivity and water potentials.

262 Despite high rates of *A. tsugae* settlement, the adelgid did not directly impact any of our
263 plant physiological measurements. This was surprising because herbivory is well-known to alter
264 plant morphology and physiology (Karban and Baldwin 2007), and adelgids have been shown to
265 affect hemlock water potential, photosynthesis, stomatal conductance, and tree water use (Domec
266 et al. 2013, Gonda-King et al. 2014). Adelgids did decrease water potential, but only in the well-
267 watered treatment: there was no similar effect in the water-stressed treatment (Table 2, Fig. 4C).
268 Since adelgids are known to cause water stress, we would have expected them to exacerbate the
269 decrease in water potential for water-stressed trees. Instead, adelgids significantly altered RWC
270 in the watered, but not the water-stressed, treatment. The fact that they did not alter the water

271 potential of the water-stressed trees may reflect the fact that while those trees are already
272 physiologically suitable for the insects, the watered trees are not (meaning that adelgids need to
273 induce water stress to improve their suitability). This may help explain why adelgids have a
274 greater impact on well-watered trees compared to those trees already experiencing water-stress.

275 Although some of our plant physiology results appear at odds with those of earlier studies
276 (Domec et al. 2013, Gonda-King et al. 2014), it is more likely that our short-term greenhouse
277 experiment was not long enough to detect adelgid-induced physiological changes within the plant.
278 While greenhouse studies provide the ability to precisely control soil moisture levels and other
279 abiotic factors, the environment was unavoidably different from what hemlocks would normally
280 encounter. While we carefully regulated greenhouse temperatures and rotated the trees bi-weekly
281 within the greenhouse, we cannot reject the hypothesis that the trees experienced some
282 greenhouse-related effects. While we are confident in our results, one future experiment might
283 involve repeating this work in a natural setting.

284 The short-term nature of our experiment is also likely responsible for the fact that there
285 was no direct impact of our water manipulation on any of our physiology measurements.
286 Furthermore, hemlocks are shade tolerant trees and the greenhouse conditions may have made the
287 physiological impacts more uniform across trees. In contrast, light availability had a substantial
288 effect on hemlock physiology, but there was no interaction between this factor and adelgid
289 presence (Table 2). The impact of light is unsurprising given its importance to plant growth
290 (Pacala et al. 1994), and since full-sun trees had 4.5x more light exposure than shaded trees, we
291 expected to see large physiological differences.

292 In conclusion, adelgid settlement was higher on the old growth of shaded and water-
293 stressed trees, but their survival was not altered by foliage age or either abiotic factor. Although

294 we expected the adelgid to exacerbate the impact of shading and drought stress, we found no
295 evidence that this was the case; this may, however, be the result of the relatively short duration of
296 our study. While we anticipated that adelgids would settle more readily on new-growth foliage,
297 their observed preference for old-growth foliage agrees with the work of McClure (1991), who
298 found the progrediens generation preferred settling on old-growth foliage; this may be due to the
299 fact that the newest-growth tissue available to settling progrediens is so ‘green’ that it interferes
300 with stylet bundle insertion and feeding. From a management perspective, the fact that adelgid
301 settlement was so much greater on shaded trees suggests that silvicultural interventions to
302 increase light levels for particularly valuable trees might prove worthwhile. The fact that
303 settlement was higher on water-stressed versus well-watered trees also suggests a possible
304 mechanism for the findings of large-scale surveys that reveal higher rates of adelgid-related
305 mortality for trees growing in xeric conditions (Preisser et al. 2008). From a broader perspective,
306 our results also suggest that management strategies targeting adelgid settlement may prove
307 fruitful in slowing or reducing pest-related hemlock mortality.

308 **ACKNOWLEDGEMENTS**

309 L. Gonda-King provided guidance with experimental design. W. Botta, D. Martins, and J. Rafter
310 assisted with soil moisture measurements and density counts. The comments of three anonymous
311 reviewers substantially improved the quality of this manuscript. The material is based upon work
312 supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture,
313 under Agreement No 2011-67013-30142, by the National Science Foundation under grant no.
314 DEB-1256769, and by RI-AES Hatch Grant RI00HI-4004.

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TABLES

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Table 1: Results of statistical analysis of *A. tsugae*-related variables. Values in bold are

385

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

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388 Table 2: Results of statistical analysis of hemlock-related variables. Values in bold are
 389 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

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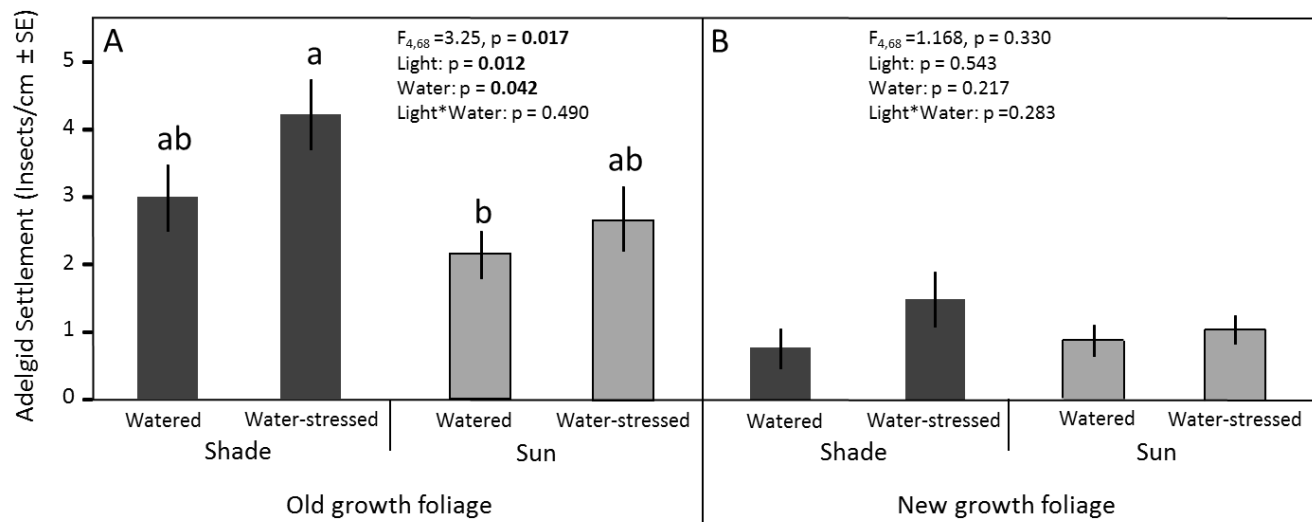
392 **FIGURE LEGENDS**

393 Figure 1. Settlement densities of adelgids. Darker bars represent shaded treatments, lighter
394 bars are full-sun treatments. (A) Settlement densities (\pm SE) on old growth. (B) Settlement
395 densities (\pm SE) on new growth. These data are represented in the 6 June time point in Fig. 2A, C.
396 Figure 2. Density and survival of adelgids throughout the summer. Solid markers represent
397 shaded treatments, open markers represent full-sun treatments, circles represent watered trees, and
398 triangles represent water-stressed trees. (A) Density of adelgid (\pm SE) on old growth. (B) Percent
399 survival of adelgid (\pm SE) on old growth. (C) Density of adelgid (\pm SE) on new growth. (D)
400 Percent survival of adelgid (\pm SE) on new growth. Data from the first time point (6 June) in (A)
401 and (C) are represented in Fig 1 A, B, to help enhance the interpretation of the settlement results.

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

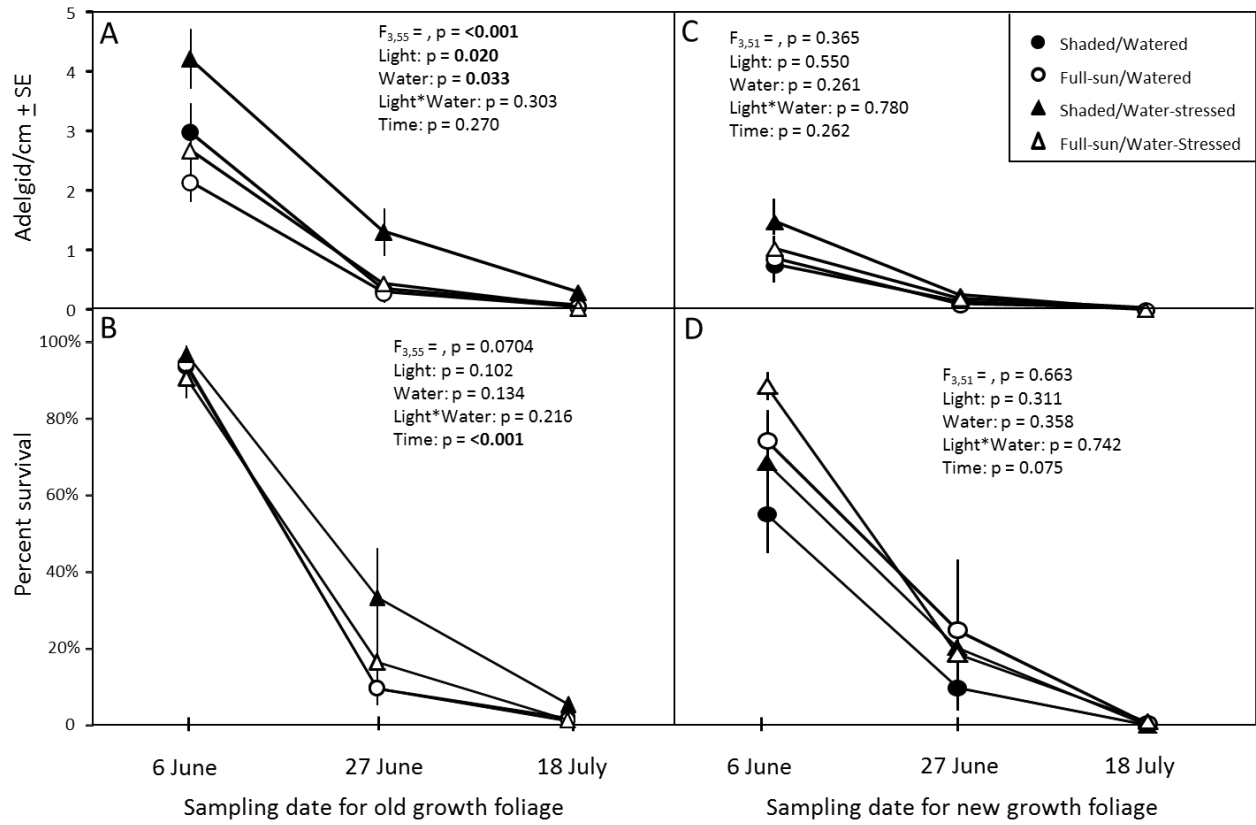
408 Figure 4. (A) Photosynthesis (\pm SE), (B) transpiration (\pm SE), and (C) water potential (\pm
409 SE) of trees after adelgid were added. Legend is the same as in fig 3. Photosynthetic rate, and
410 water potential were significantly affected by light, There was also a time*light interaction
411 because photosynthetic rates and transpiration were higher for full-sun trees in May but not in
412 June. Water potential was lower for full-sun trees throughout the experiment. Adelgids had no
413 effect on photosynthesis, transpiration, or water potential.

414 Figure 1.



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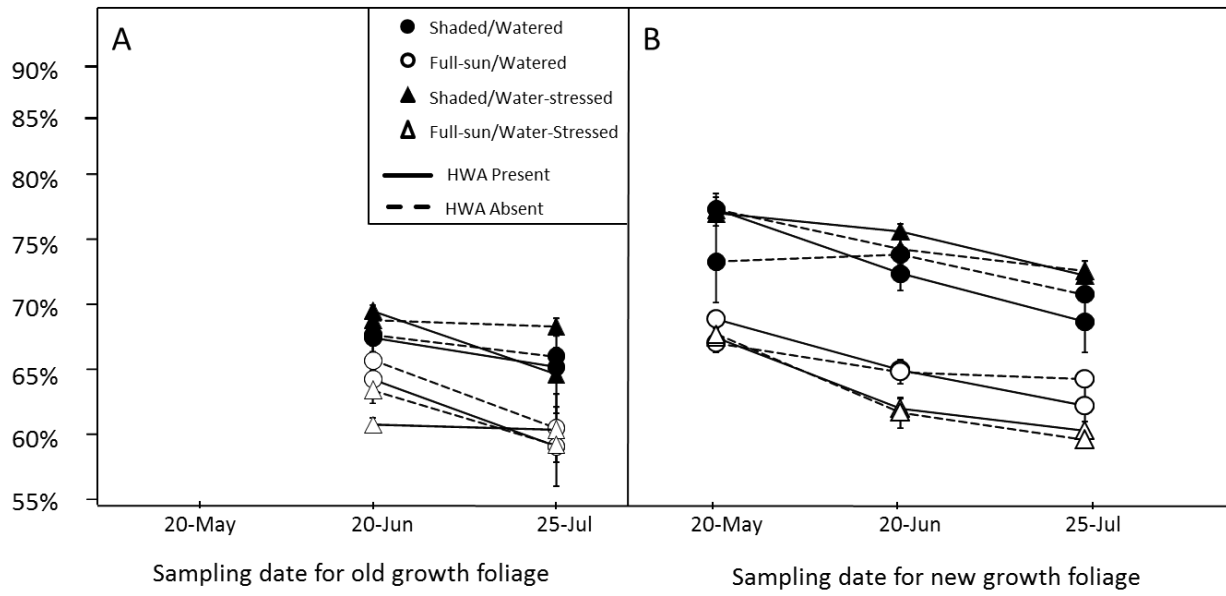
416 Figure 2.



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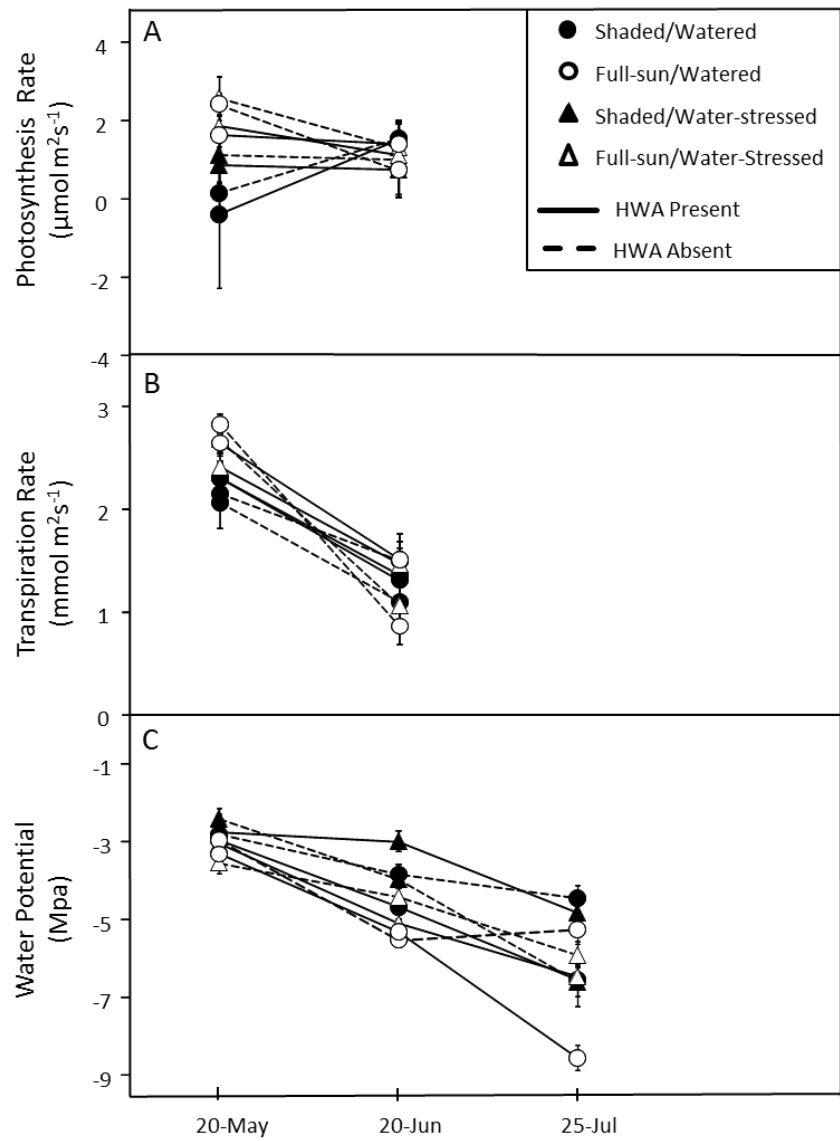
419 Figure 3.



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422 Figure 4.



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