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Tree Responses to an Invasive Sap-Feeding Insect

Liahna Gonda-King

Sara Gomez

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Authors

Liahna Gonda-King, Sara Gomez, Jessica L. Martin, Colin M. Orians, and Evan L. Preisser

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Tree responses to an invasive sap-feeding insect

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

9

and Evan L. Preisser^a

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

11

^bDepartment of Biology, Tufts University, Medford, USA

12

*Corresponding author: liahna.gonda.king@gmail.com

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14 **Abstract**

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

26

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

29

30 **Introduction**

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

38 Relatively few studies have investigated the effects of sap-feeding herbivores on woody
39 species (reviewed in Zvereva et al. 2010). Conifers may be especially susceptible to sap-feeders
40 because they allocate more storage compounds to foliage as opposed to deciduous trees that store
41 resources in their roots, stems, and other tissues inaccessible to sap feeders (Chapin et al. 1990;
42 Hester et al. 2004; Krause & Raffa 1996b). The lack of such stored resources may make conifers
43 more likely to succumb to intense sap-feeding events (Fernandes 1990; Furuta & Aloo 1994;
44 Paine 2000). The invasive hemlock woolly adelgid (*Adelges tsugae* Annand Hemiptera:
45 Adelgidae) is a specialist sap-feeding insect currently decimating eastern hemlock (*Tsuga*
46 *canadensis* (L.) Carrière), a foundation species in eastern North American forests. *Adelges*
47 *tsugae* feed by inserting their stylet bundle at the base of a needle into the ray parenchyma tissue
48 (Young et al. 1995) and can kill mature hemlocks within four years of infestation (McClure
49 1991). Once *A. tsugae* selects a feeding place, it remains sessile throughout its entire life cycle.

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

53 when the cycle starts again (McClure 1989). While the progrediens generation feeds
54 continuously throughout their shorter life cycle, the sistens generation enters diapause, a period
55 of arrested growth and metabolic depression, immediately after hatching and settling on young
56 plant tissue with no previous direct exposure to *A. tsugae*. Summer diapause, also known as
57 aestivation, in *A. tsugae* is primarily induced by temperature (Salom 2001); Lagalante et al.
58 (2006) suggest that *A. tsugae* enters diapause to avoid seasonal increases in plant terpenoids.

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

65 **Materials and methods**

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

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

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

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

93 *Gas Exchange:* We measured gas exchange of new growth (2012) foliage on a terminal
94 branch on each tree used to quantify water potential. Measurements were conducted between
95 9:00-11:00 am on September 9 and October 26, 2012. After each measurement, foliage inside the
96 cuvette was excised and photographed; we quantified total needle area using ImageJ 1.44
97 software (Abràmoff et al. 2004) to use in gas exchange calculations. To determine gas exchange
98 rates we used a CIRAS-2 portable photosynthesis system (PP systems, Haverhill, MA, USA)

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

101 *Total % Nitrogen:* We collected new growth needles and stems from 2012 growth for *A.*
102 *tsugae*-infested (n=15) and control trees (n=15) in September and October. These needles and
103 stems were oven dried to constant weight at 70°C and then finely ground. We determined total
104 %N through analysing ~2-3 mg of each sample by dry combustion with a CHNOS analyzer
105 (vario Micro cube, Elementar Americas, Mt. Laurel, NJ).

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

117 **Results**

118 *Hemlock Growth:* *Adelges tsugae* infestation had a significant effect on hemlock growth
119 (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,
120 2D). By the end of the growing season, terminal and side branches on control trees were 41%
121 and 57% longer, respectively, than on *A. tsugae*-infested trees. There was a significant

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

129 *Water Potential:* Water potential was 45% higher in control branches than *A. tsugae*-
130 infested branches ($F_{1,20}=11.36$, $P=0.003$; Fig. 3). Water potential changed over time ($F_{1,20}=7.93$,
131 $P=0.011$), but there was no time*treatment interaction ($F_{1,20}=0.02$, $P=0.900$). On both dates,
132 there was a significant negative correlation between *A. tsugae* density and water potential
133 (September: $F_{1,35}=9.55$, $P=0.006$, $R_2=0.313$; October: $F_{1,21}=7.38$, $P=0.010$, $R_2=0.123$).

134 *Gas Exchange:* Net photosynthetic rates and stomatal conductance were lower in *A.*
135 *tsugae*-infested trees ($F_{1,22}=7.70$ and 14.75 , $P=0.011$ and <0.001 respectively; Figs 4A,B)
136 compared to control trees. Photosynthesis and stomatal conductance were greater in October
137 ($F_{1,22}=37.89$ and 111.04 , respectively, both $P<0.001$). There was a significant time*treatment
138 interaction for both gas exchange variables ($F_{1,22}=9.43$ and 11.35 , $P=0.006$ and 0.003 ,
139 respectively). There was a negative correlation between *A. tsugae* density and stomatal
140 conductance for both September ($F_{1,21}=6.38$, $P=0.020$, $R_2=0.233$) and October ($F_{1,22}=7.18$,
141 $P=0.013$, $R_2=0.238$). There was no relationship between *A. tsugae* density and photosynthesis
142 for September ($F_{1,21}=1.06$, $P=0.314$) but was marginally significant for October ($F_{1,22}=4.02$,
143 $P=0.057$).

144 *% Nitrogen*: Regardless of insect treatment, %N was higher in needles compared to stems
145 (F_{1,51}=200.87, P<0.001; Figs 5A,B). Adelgid-infested foliage had higher %N concentration than
146 control trees (F_{1,51}=8.47, P=0.0053). Interestingly, %N was greater in infested trees in
147 September but not October (time*treatment interaction; F_{1,51}=12.59, P<0.001). When *A. tsugae*
148 were in aestivation (September), %N content was 13% higher in *A. tsugae* -infested needles and
149 27% higher in *A. tsugae*-infested stems. However, when *A. tsugae* were actively feeding in
150 October the %N content differed by less than 1% for both needles and stems compared to control
151 trees.

152 **Discussion**

153 Our study investigated the effects of an insect's presence on various plant performance
154 traits. We found that *A. tsugae* had significant impacts on hemlock growth. The progrediens
155 generation settles and begins to feed immediately before the start of the hemlock growing
156 season. By the end of the growing season, terminal branches on control trees were 41% longer
157 and had 56% more new buds than *A. tsugae*-infested trees. The effect of *A. tsugae* was even
158 more pronounced on side branches. Side branches on uninfested trees grew 56% more and had
159 120% more new buds than *A. tsugae*-infested trees. The significant treatment*time interactions
160 for both terminal and side branches suggests that *A. tsugae* has a greater negative impact later in
161 the growing season. While other studies have shown that *A. tsugae* has a detrimental impact on
162 hemlock growth (McClure 1991; Miller-Pierce et al. 2010), we showed that *A. tsugae*-infested
163 trees have significantly less lateral growth and buds than uninfested trees. If these trends
164 continue, such difference in branch architecture could have profound effects on small insects
165 with limited active dispersal such as *A. tsugae*. Lateral and increased branching can promote

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

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

178 The impact of *A. tsugae* may be caused by physical injury or chemical cues associated
179 with the initial stylet insertion. Stylet insertion by other sucking insects has been shown to cause
180 long-lasting anatomical and physiological changes (Ecale & Backus 1995; Ladd & Rawlins
181 1965; Shackel *et al.* 2005). *Adelges tsugae* secretes a salivary sheath when feeding at the base of
182 hemlock needles that Young *et al.* (1995) suggests may be 'toxic' and responsible for the
183 disproportional negative impact on hemlock growth caused by *A. tsugae* feeding. In support of
184 the 'toxic' saliva hypothesis, Radville *et al.* (2011) found that *A. tsugae* elicits a local and
185 systemic hypersensitive response in hemlock. Alternatively, the adelgid's impact on
186 photosynthesis and stomatal conductance might be explained by long-lasting changes in nutrient
187 allocation originated when *A. tsugae* feeding.

188 Sap-feeding insects often act as resource sinks (Inbar et al. 1995; Kaplan et al. 2011) that
189 can compete with natural plant sinks (i.e. actively growing tissues). This appears to be true when
190 *A. tsugae* is actively feeding but not when in diapause, as indicated by a 13% decrease in %N
191 after *A. tsugae* resumes feeding in October. During diapause, *A. tsugae*-infested needles and
192 stems have higher %N than control trees, but once *A. tsugae* begin feeding %N does not differ
193 between *A. tsugae*-infested and controls. *Adelges tsugae* feeding is known to alter local and
194 systemic foliar nitrogen content (Gómez et al. 2012; Miller-Pierce et al. 2010; Stadler et al.
195 2005). Nitrogen sink competition between feeding herbivores and new foliage alters leaf nutrient
196 status, resulting in decreased photosynthesis (Larson 1998). Since *A. tsugae* feed on
197 photosynthate from xylem ray parenchyma cells (Young et al. 1995) that transfer and store
198 nutrients it is likely *A. tsugae* induce greater sinks when feeding than when in diapause.
199 Although the increase in %N of *A. tsugae*-infested trees might increase photosynthesis since
200 most leaf N is incorporated into photosynthetic enzymes and pigments (Evans 1989), we instead
201 documented consistent decreases in photosynthesis. *Adelges tsugae* feeding increases free amino
202 acids (Gómez et al. 2012), a finding that would explain the increase in total %N found in our
203 study.

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

211 consist of thick-walled xylem cells and which may hinder water transport efficiency (Mitchell
212 1967). If reductions in water potential are driven by false ring formation, this permanent change
213 in wood anatomy would have a long-lasting impact on water relations and may explain why *A.*
214 *tsugae* feeding impacts tree physiology.

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

222 .

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354 **Figures**

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

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

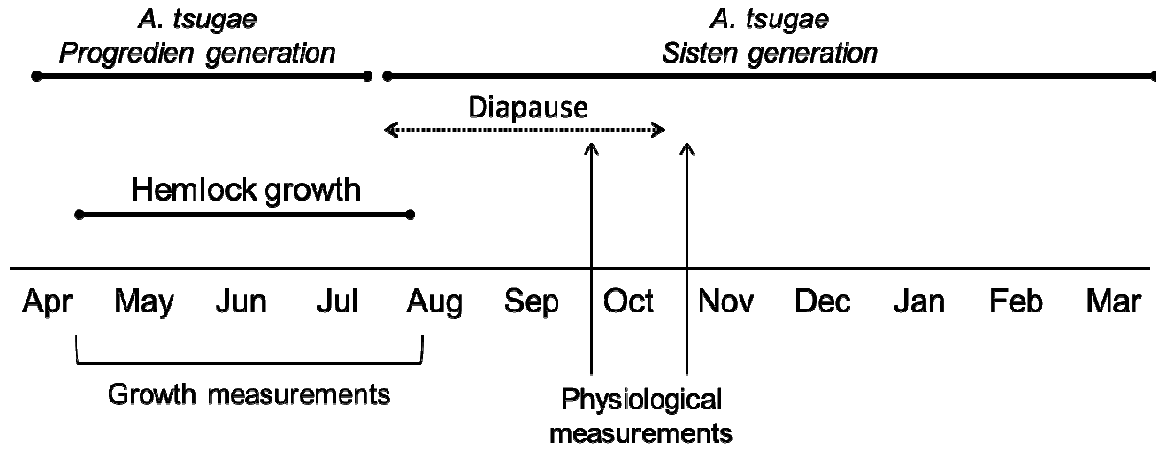
360 Figure 3. Average (\pm SE) water potential measurements in *A. tsugae*-infested (dark gray) and
361 control trees (light gray). Figure 4 . Average (\pm SE) photosynthesis (A) and stomatal
362 conductance (B) in control (light gray) and *A. tsugae*-infested plants (dark gray).

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

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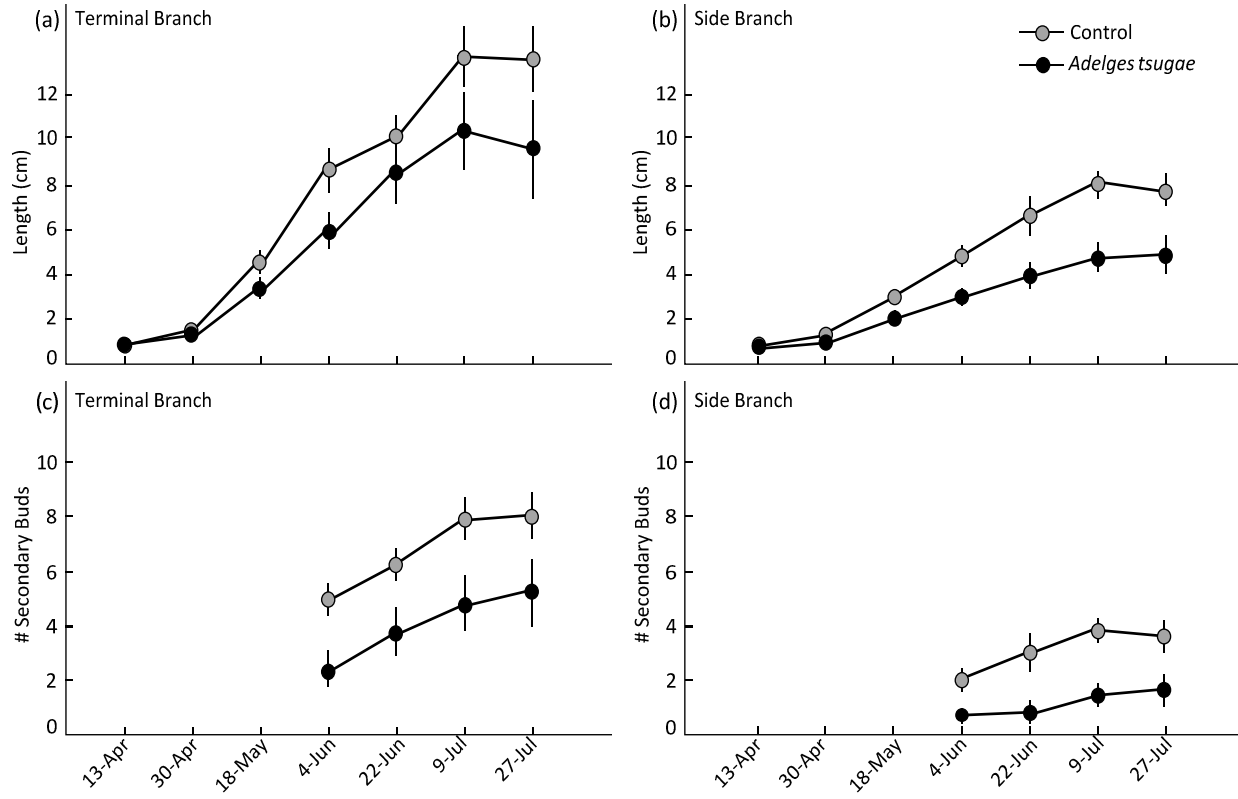
Figure 1



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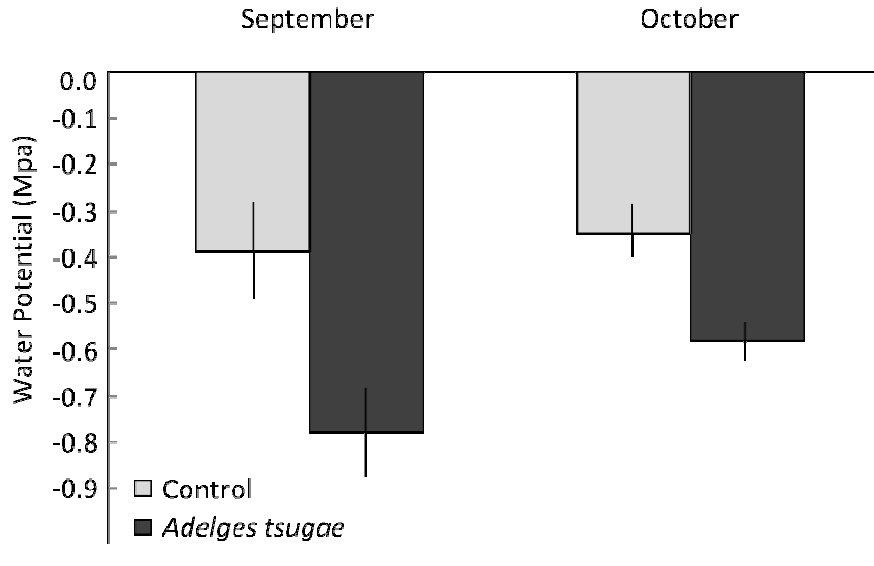
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Figure 2



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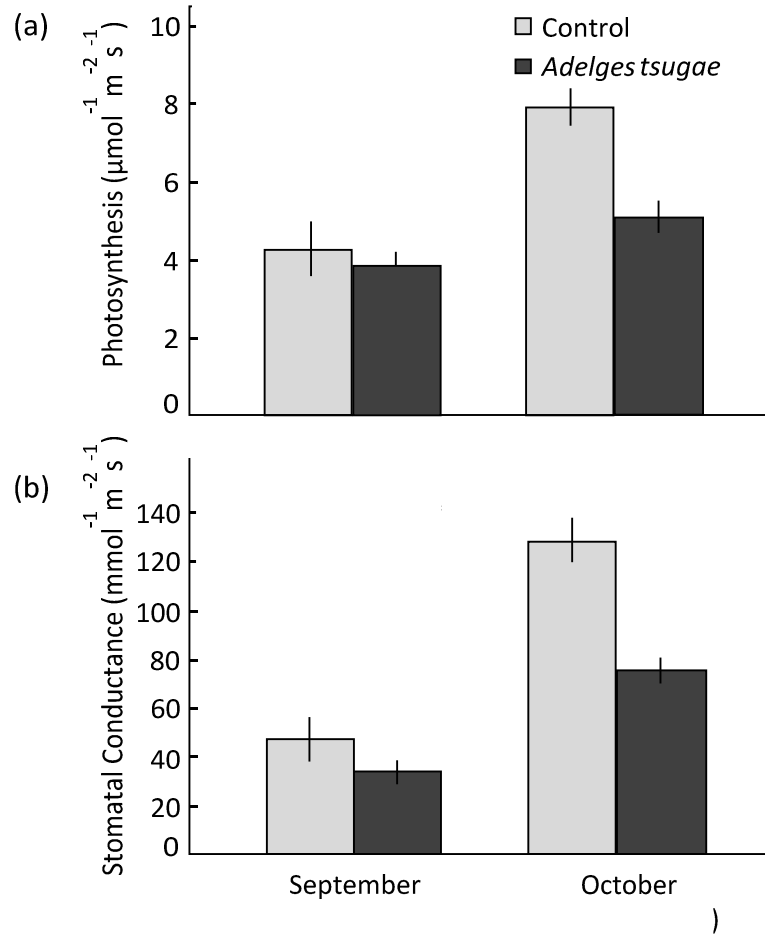
Figure 3



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Figure 4



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Figure 5

