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The Effects of Mite Galling on the Ecophysiology of Two Arctic Willows

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Abstract  
Deciduous plants in the Arctic are increasing in abundance due to warming trends, and this increase will likely contribute to changes in regional carbon dynamics. One of the dominant deciduous-shrub genera, Salix, is highly susceptible to leaf galls, but the influence of arthropod herbivores on plant-level carbon uptake in the Arctic remains poorly studied. We examined the impacts of galling by two eriophyoid mites on a suite of ecophysiological traits in leaves of two species of willows (Salix pulchra Cham. and Salix glauca L.) in Alaskan arctic tundra. Galled leaves showed significant declines in maximum photosynthetic capacity ($A_{\text{max}}$), photosystem II efficiency ($F_{\text{v}}/F_{\text{m}}$), stomatal conductance ($g_s$), and instantaneous water-use efficiency (WUE) in S. pulchra leaves and in $A_{\text{max}}$ and $F_{\text{v}}/F_{\text{m}}$ of S. glauca leaves. Neighboring gall-free leaves on the same shoot as galled leaves had higher $A_{\text{max}}$ and $g_s$ than nearby controls suggesting compensatory responses. Gall-infested tissue had significantly higher concentrations of glucose and fructose compared to gall-free leaves, suggesting a possible preference for these metabolites. Alternatively, this variation in metabolite concentrations in the area of wounding may be associated with the production of defense compounds. To unravel the specific variation in metabolic concentrations related to gall infestation, additional studies are needed. Our findings do suggest that galling mites—ubiquitous but poorly examined in the tundra—have significant impacts on photosynthetic processes that are likely to affect whole-plant functioning in arctic willows.

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Introduction

Current climate warming in the Arctic is expected to modify regional plant communities. Warmer and longer growing seasons are leading to an overall increase in the abundance of deciduous woody shrub species such as dwarf birch (Betula nana) and willows (Salix spp.) (Sturm et al., 2001; Tape et al., 2006; Forbes et al., 2010). The contribution of deciduous shrubs to regional carbon dynamics is thus likely to gain prominence, as increases in their abundances are predicted to lead to an overall increase in carbon storage in northern ecosystems (Oechel et al., 2000). However, the influence of herbivores on leaf and plant-level carbon uptake in the Arctic remains poorly studied. Previous studies on interactions between herbivores and shrubs in the Arctic have been restricted to the influence of generalist vertebrate herbivores on shrub expansions (Olofsson et al., 2009). Ecophysiologlcal studies have not accounted for impacts of common arthropod herbivores on photosynthetic functioning in these species (Starr et al., 2008). Hence, significant impacts of natural enemies on fundamental processes such as (leaf-level) carbon gain and metabolite allocation have been overlooked.

In the Alaskan arctic tundra, Salix forms one of the dominant genera of deciduous plants with 32 known species in interior Alaska alone (Hulten, 1968). Typical light-saturated carbon assimilation rates during the peak growing season among species of Salix range from ~8 to 14 μmol m$^{-2}$ s$^{-1}$ (Oberbauer and Oechel, 1989; Starr et al., 2008). Interestingly, experimental soil warming plus lengthening of growing season do not appear to have an effect on maximum light-saturated assimilation rates in S. pulchra in moist acidic tussock tundra ecosystems, implying that internal physiological constraints arguably prevent exploitation of more favorable growing conditions (Starr et al., 2008).

Salix species in the Arctic are attacked by a diverse group of specialist and generalist herbivores. Vertebrate herbivores include mammalian species, some of which have been implicated in slowing Salix expansion in the Arctic (Olofsson et al., 2009), and avian species such as willow ptarmigan (Lagopus lagopus) that live on dormant willow buds during the winter. Specialist invertebrate herbivores include gall-inducing insects and mites. Among the insects, gall inducers are most commonly members of the Cecidomyiid (Order Diptera) or Tenthredinid (Order Hymenoptera) families; gall-inducing mites belong to the superfamily Errophyioidea. To date, only a handful of studies have examined interactions between Salix species and associated gall inducers. Nematine sawflies (Hymenoptera: Tenthredinida) that induce leaf galls on a number of Salix species in the Arctic have received the most attention. Sawflies in six arctic Salix species induced galls with significantly lower levels of host defensive phenolics in comparison to surrounding leaf tissues and neighboring gall-free leaves (Nyman and Julkunen-Titto, 2000). This resulted in gall tissues being a highly palatable source for inducer larvae within galls. Further, leaf defensive tannins, while higher in galls, were concentrated in the outer cortex of galls, providing a protective layer for gall inhabitants against potential inquiline arthropod or fungal attack. In one species of willow (S. cinerea L.), number of galls per shoot was positively correlated with mean shoot length but negatively correlated with plant (ramet) age, implying that at the whole-plant level, sawflies...
tended to gall the most vigorously growing individuals (plant vigor hypothesis) and on younger shoots that are likely to be more susceptible to arthropod herbivory (Price et al., 1987). Mortality and survival of galling sawflies on arctic willows have also been known to vary geographically, with species in northernmost sites having fewer inquiline parasitoid species but higher proportions of mortality (Roininen et al., 2002). However, the specific impacts of gall inducers on Salix leaf physiological traits remain to be examined.

In this study we examine leaf-level physiological parameters, metabolite allocation, phytochemical composition, and nitrogen concentration in response to mite-induced galling to determine the overall effects on leaf-level health and functioning in two Salix species in the arctic tundra. Salix pulchra is common in moist tussock tundra ecosystems and, along with dwarf birch, comprises the predominant deciduous vegetation in these communities (Fig. 1, part a). Salix glauca is found in drier sites and better-drained sites as a shrub (Fig. 1, part b). Leaves of S. pulchra are galled by an unidentified species of eriophyid mite (F. B. Beaulieu, personal communication; Fig. 1, part c), while S. glauca leaves are galled by the eriophyid mite Acarus tetanothrix (Nalepa) (Amrine and Stasny, 1994; Fig. 1, part d). Specifically, we hypothesize that mite gallings will cause a significant reduction in physiological activity and associated protective leaf pigments in leaves within infestations on S. pulchra and S. glauca. These reductions in physiological capacity associated with galling will directly limit the production of metabolites in infested leaves.

Materials and Methods

STUDY SITE AND DESIGN

This study was conducted at Toolik Field Station (68°38′N, 149°36′W), in the north-facing foothills of the Brooks Range in arctic Alaska during the growing season (22–29 July) of 2011. The study focused on two species of willow, S. pulchra and S. glauca, when these plants are known to be at the height of their physiological activity (Starr et al., 2008). In tussock tundra S. pulchra is a low-growing shrub, growing up to 12–15 cm (Fig. 1, part a), although along streams and water tracks plants may reach 1.5 m. S. glauca grows up to ~1.2 m (Fig. 1, part b). Both shrubs are found across the arctic tundra, with S. glauca reaching into the southwestern United States. In our study site, we found S. pulchra in moist acidic tussock tundra. S. glauca was found in dry health

FIGURE 1. Two Salix species from Toolik Field Station, Alaska. (a) S. pulchra is a dominant deciduous shrub species with a low canopy profile in moist tussock sites, while (b) S. glauca is commonly found as a taller shrub in dry heath sites. Eriophyid mite-induced galls on leaves of (c) S. pulchra and (d) S. glauca. Arrows show ungalled leaves on the same shoot as galled leaves.
areas, as well as on the banks of Toolik Lake. The measurements were divided into three treatments: (a) control leaf from a healthy, un-galled plant; (b) galled leaf from an infected plant; and (c) an adjacent un-galled leaf on the same shoot as the galled leaf. We selected leaves for this study that were sun-exposed and on the outer ‘canopy’ of the plants to minimize variation in physiology associated with differences due to shading or to timing of bud break. The un-galled adjacent leaf was included to test whether galling resulted in compensatory increases in photosynthesis in non-galled neighboring leaves. For *S. pulchra*, 20 plants per treatment were selected for measurements. Galled *S. glauca* was less common in the study location and hence sample sizes were restricted to 12 plants per treatment for this species. For both galled and un-galled leaves, samples were taken only from plants that were free from other forms of herbivory or other physical damage.

**PHYSIOLOGICAL MEASUREMENTS**

Leaves were dark-adapted for a minimum of 10 min and \( F_{v}/F_{m} \) (representing potential dark-adapted quantum efficiency of photosystem II [PSII]) was measured using an OS5-FL modulated-fluorometer (Opti-Sciences, Tyngsboro, Massachusetts). Immediately afterward maximum light-saturated photosynthetic capacity (\( A_{\text{max}} \)), stomatal conductance (\( g_{s} \)), and transpiration (\( E \)) were measured using an infrared gas analyzer (Li-6400 XT portable photosynthesis system; LI-COR, Lincoln, Nebraska). Instantaneous water use efficiency (WUE) was calculated based on \( A_{\text{max}}/E \). Leaves were collected and leaf reflectance was measured immediately upon return to the lab (Uni-Spec SC spectroradiometer; PP Systems, Amesbury, Massachusetts). Leaf reflectance was taken separately on galled and non-galled areas in infected leaves to calculate differences in pigment (total chlorophyll, anthocyanin) concentrations in the infected versus non-infected tissue (see below). Total leaf area was measured using a leaf area meter (Li-3100 area meter; LI-COR, Lincoln, Nebraska). In addition, total number of galls and total galled area were calculated for each galled leaf.

**LEAF METABOLITES AND NUTRIENTS**

Galled, neighboring ungalled, and control leaves were collected from plants (\( n = 10 \)/treatment), separate from those used for physiological measurements (as extra leaf tissue material was required for metabolite and nutrient analyses), and immediately stored in a cooler maintained below \( -20 \) °C to stop physiological activity. They were then transferred to a \( -80 \) °C freezer. Samples were then freeze-dried using a Labconco freeze-drying system (Labconco Co., Kansas City, Missouri) for a minimum of 48 h, ground and extracted with 80% ethanol, and analyzed enzymatically for soluble sugars (glucose, fructose, sucrose) and starch concentrations following methods outlined in Boehringer (1984). For carbon (C) and nitrogen (N) analyses, tissue samples were ground in liquid N, and leaf tissue samples were examined for C and N content using an ECS 4010 analyzer (Costech Analytical Technologies, Valencia, California). For both metabolites and nutrient analyses, galls were excised and processed separately from remaining (ungalled) leaf tissue to examine differences between galled and ungalled tissues within leaves. Gll tissue and remaining leaf tissue were bulked from two or more leaves to obtain adequate tissue material for the above analyses (as individual galls occupy a small fraction of leaf areas).

**LEAF PIGMENTS**

Chlorophyll and anthocyanin concentrations (expressed on a \( \mu \)mol m\(^{-2} \) and nmol cm\(^{-2} \) basis) were calculated from leaf spectral reflectance (from the same leaves following gas-exchange measurements) with equations from Gitelson et al. (2003) and Gitelson et al. (2009), respectively. This approach enabled a rapid, non-destructive method for quantifying pigment concentrations. As with metabolites and nutrients, reflectance was measured separately on galled and ungalled portions of infected leaves.

**STATISTICAL ANALYSES**

A complete randomized block design was used to examine differences in gas-exchange parameters, metabolites, leaf nutrients, and pigments between treatments (galled, neighboring ungalled, and control leaf from gall-free plant) separately for the two species of *Salix* (Zar, 1984). Blocking was used to control for sources of variation between galled plants and their paired controls. Briefly, two leaves (galled and neighboring gall-free) from the same plant plus a control leaf from a nearby gall-free plant were grouped in a block. Tukey’s multiple comparison tests were then used to determine significant differences among groups. An additional ‘treatment’ category was included in the analyses for leaf metabolites, nutrients, and pigments; leaf tissue remaining after galls were excised to determine if these traits were altered in ungalled portions of galled leaves. Data were analyzed using the JMP statistical package (SAS Institute, Carey, North Carolina).

**Results**

**LEAF-LEVEL GAS EXCHANGE AND FLUORESCENCE**

Galled leaves in both willow species showed significant declines in \( A_{\text{max}} \) in comparison to both neighboring gall-free leaves and leaves from ungalled ‘control’ plants (\( S. pulchra: F_{2,57} = 29.63, P < 0.001; S. glauca: F_{2,33} = 6.84, P < 0.01 \); Fig. 2, parts a and e). Further, neighboring gall-free leaves had higher \( A_{\text{max}} \) values than control leaves, but this difference was only significant in *S. pulchra*. Stomatal conductance rates were highest in neighboring leaves and lowest in galled leaves, but this difference was not significant among the three groups (\( S. pulchra: F_{2,57} = 6.36, P < 0.01; S. glauca: F_{2,33} = 1.94, P = 0.17 \); Fig. 2, parts b and f). Transpiration was markedly higher in control leaves of both species compared to galled leaves (\( S. pulchra: F_{2,57} = 6.0, P = 0.005; S. glauca: F_{2,33} = 4.93, P = 0.02 \); Fig. 2, parts d and h). As a consequence, instantaneous WUE was lower in galled leaves in both species (Fig. 2, parts c and g; *S. pulchra*: \( F_{2,57} = 15.29, P < 0.001; S. glauca: F_{2,33} = 4.15, P = 0.03 \). Dark-adapted quantum efficiency of PSII (\( F_{v}/F_{m} \)) was lowest in galled leaves of both species, and significantly so in *S. pulchra* (\( S. pulchra: F_{2,57} = 4.42, P = 0.02; S. glauca: F_{2,33} = 2.43, P = 0.11 \); Fig. 3, parts c and f).

**LEAF METABOLITES AND NUTRIENTS**

Galls had significantly higher levels of simple sugars (glucose, fructose) compared to remaining leaf tissues, neighboring (gall-
free) leaves, and leaves from control plants (S. pulchra: $F_{3,35} = 6.42, P < 0.01$; S. glauca: $F_{3,29} = 10.55, P < 0.001$; Table 1). Sucrose levels did not differ among the different tissue types in S. pulchra ($F_{3,35} = 1.52, P = 0.23$) but were lower in galls of S. glauca compared to neighboring and control leaves ($F_{3,29} = 8.98, P < 0.001$; Table 1). Starch concentration was significantly lower in galls on both host species compared to all other tissues examined (S. pulchra: $F_{3,35} = 8.94, P < 0.001$; S. glauca: $F_{3,29} = 5.81, P < 0.01$; Table 1).

Nitrogen (% dry weight) and carbon (% dry weight) were lower in galled tissue compared to the other tissues on both Salix hosts. This difference was significant in most cases (Table 1). The C:N ratio in galled regions of the leaves was significantly higher compared to remaining leaf tissue in S. pulchra ($F_{3,50} = 5.22, P < 0.01$); in S. glauca, galls had significantly higher C:N compared to neighboring and control leaves ($F_{3,39} = 7.56, P < 0.001$).

LEAF PIGMENTS

Galls had significantly lower concentrations of total chlorophyll compared to remaining (ungalled) leaf tissue, neighboring un-galled leaves from the same shoot, and control leaves from gall-free plants (S. pulchra: $F_{3,75} = 15.75, P < 0.001$; S. glauca: $F_{3,47} = 14.24, P < 0.001$; Fig. 3, parts a and d). Further, in S. glauca, the un-galled portion of affected leaves had lower chlorophyll concentrations than leaves from gall-free, control plants (Fig. 3, part d). Conversely, galls on both species had significantly higher concentrations of anthocyanin compared to the other groups (S. pulchra: $F_{3,35} = 6.21, P < 0.01$; S. glauca: $F_{3,29} = 8.98, P < 0.001$; Table 1).
FIGURE 3. Total pigment ([a, d] chlorophyll and [b, e] anthocyanin) concentrations and (c, f) chlorophyll fluorescence $F_{V}/F_{M}$ measured in galled, neighboring ungalled, and ‘control’ leaves on two species of Salix from Toolik Field Station, Alaska. Pigment concentrations were measured separately for galled and remaining ungalled leaf tissues in both species. Different letters indicate a significant difference between groups at $P < 0.05$ (ANOVA followed by Tukey’s multiple comparison tests). Bars are mean ± 1 SEM.

TABLE 1

Starch, soluble sugar concentrations (g/100 g dry mass), percent carbon (C) and nitrogen (N), and C:N ratio in galls, remaining leaf tissue, neighboring gall-free leaves, and leaves from gall-free control plants on Salix pulchra and S. glauca from Toolik Lake, Alaska. Values are mean (SD). Values with the same letter are not different at $P = 0.05$ level of significance. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

<table>
<thead>
<tr>
<th></th>
<th>Starch</th>
<th>Glucose + Fructose</th>
<th>Sucrose</th>
<th>%N</th>
<th>%C</th>
<th>C:N</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Salix pulchra</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galls</td>
<td>0.036 (0.01) b</td>
<td>0.402 (0.07) a</td>
<td>0.447 (0.08) a</td>
<td>1.77 (0.28) b</td>
<td>47.56 (0.66) b ***</td>
<td>27.57 (3.93) a</td>
</tr>
<tr>
<td>Remaining leaf tissue</td>
<td>0.067 (0.01) a **</td>
<td>0.251 (0.13) b **</td>
<td>0.496 (0.06) a</td>
<td>1.98 (0.15) a b</td>
<td>47.99 (1.10) b ***</td>
<td>23.43 (2.79) b **</td>
</tr>
<tr>
<td>Neighboring ungalled leaf</td>
<td>0.072 (0.03) a **</td>
<td>0.266 (0.12) b **</td>
<td>0.428 (0.07) a</td>
<td>2.08 (0.25) a *</td>
<td>49.48 (1.06) a</td>
<td>25.18 (2.09) a b</td>
</tr>
<tr>
<td>Leaf from control plant</td>
<td>0.063 (0.02) a ***</td>
<td>0.239 (0.07) b **</td>
<td>0.512 (0.13) a</td>
<td>2.05 (0.25) a *</td>
<td>49.88 (0.48) a</td>
<td>24.59 (2.95) a b</td>
</tr>
<tr>
<td><strong>Salix glauca</strong></td>
<td></td>
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</tr>
<tr>
<td>Galls</td>
<td>0.053 (0.01) b</td>
<td>0.313 (0.08) a</td>
<td>0.377 (0.06) b</td>
<td>1.80 (0.34) b</td>
<td>46.49 (1.01) b a</td>
<td>26.52 (4.43) a</td>
</tr>
<tr>
<td>Remaining leaf tissue</td>
<td>0.098 (0.02) a b</td>
<td>0.188 (0.04) b **</td>
<td>0.469 (0.13) a b</td>
<td>2.13 (0.27) a b</td>
<td>46.73 (0.53) a b</td>
<td>22.21 (2.89) a</td>
</tr>
<tr>
<td>Neighboring ungalled leaf</td>
<td>0.122 (0.05) a **</td>
<td>0.165 (0.08) b **</td>
<td>0.564 (0.11) a **</td>
<td>2.21 (0.27) a *</td>
<td>46.81 (1.36) a b</td>
<td>21.53 (3.38) b *</td>
</tr>
<tr>
<td>Leaf from control plant</td>
<td>0.116 (0.03) a **</td>
<td>0.151 (0.03) b ***</td>
<td>0.569 (0.06) a ***</td>
<td>2.37 (0.29) a ***</td>
<td>47.77 (0.77) a **</td>
<td>20.48 (2.44) b ***</td>
</tr>
</tbody>
</table>


**Discussion**

**LEAF PHYSIOLOGY**

During this study mite galling was shown to affect leaf-level physiology in both willow species, with significant reductions in $A_{\text{max}}$, WUE, and photosystem II efficiency. These findings give support to our hypothesis that mite galling causes a significant reduction in physiological activity and associated protective leaf pigments in leaves with infestations on *S. pulchra* and *S. glauca*. $A_{\text{max}}$ in galled leaves decreased by 40% and 35% compared to control leaves from gall-free plants of *S. pulchra* and *S. glauca*, respectively. This decrease was even larger in comparison to neighboring gall-free leaves on the same shoot (55% and 38%), suggesting compensation by neighboring leaves on the same shoot, at least in *S. pulchra*. However, we did not see a significant response in compensation by neighboring leaves in *S. glauca*.

Stomatal water loss and transpiration showed reductions in galled leaves, although these were not significantly lower. However, instantaneous WUE was markedly reduced in galled leaves of *S. pulchra* compared to both control and neighboring gall-free leaves. Gall tissues had reduced PS II efficiency compared to ungalled tissues in neighboring and control leaves, as seen by lowered $F_{v}/F_{m}$ values in galls on both plant species. Depression in PS II efficiency in response to galling has been observed in other gall infested plants (Aldea et al., 2006; Huang et al., 2011) and is indicative of damage to PS II reaction centers (Oxborough, 2004) and chlorophyll-protein complexes within galls (Yang et al., 2003). Thus the damage inflicted by mite galling on *Salix* in this study is not confined to just suppression of stomatal activity but also appears to affect photochemical pathways within the photosynthetic apparatus of host leaves.

Whether galling results in decreases or compensatory increases in gas-exchange appears to be a function of either the type of gall induced (i.e. via specialized nutritive tissue layers, vascular disruption, etc.) and/or the complexity of galls themselves. In contrast, galling leads to significant declines in net photosynthetic rates ($A_{\text{max}}$) in other mite- and insect-galled vegetation (Crawley, 1983). It is worth noting, however, that in all these cases, increased photosynthetic rates in leaves were a result of gall induction on apical meristems (Fay et al., 1993; Dorchin et al., 2006), supporting the idea for plant compensation against herbivory (Crawley, 1983). It is worth noting, however, that in all these cases, increased photosynthetic rates in leaves were a result of gall induction on apical meristems (Fay et al., 1993; Dorchin et al., 2006), supporting the idea for plant compensation against herbivory (Crawley, 1983). However, we did not see a significant response in compensation by neighboring leaves in *S. glauca*.

Whether galling results in decreases or compensatory increases in gas-exchange appears to be a function of either the type of gall induced (i.e. via specialized nutritive tissue layers, vascular disruption, etc.) and/or the complexity of galls themselves (reviewed by Welter, 1989). Studies on wasp-induced gall plants have shown increases in $A_{\text{max}}$ (Fay et al., 1993; Dorchin et al., 2006), supporting the idea for plant compensation against herbivory (Crawley, 1983). However, instantaneous WUE was markedly reduced in galled leaves of *S. pulchra* compared to both control and neighboring gall-free leaves. Gall tissues had reduced PS II efficiency compared to ungalled tissues in neighboring and control leaves, as seen by lowered $F_{v}/F_{m}$ values in galls on both plant species. Depression in PS II efficiency in response to galling has been observed in other gall infested plants (Aldea et al., 2006; Huang et al., 2011) and is indicative of damage to PS II reaction centers (Oxborough, 2004) and chlorophyll-protein complexes within galls (Yang et al., 2003). Thus the damage inflicted by mite galling on *Salix* in this study is not confined to just suppression of stomatal activity but also appears to affect photochemical pathways within the photosynthetic apparatus of host leaves.

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**METABOLITE AND NUTRIENT ALLOCATION**

The eriophyid gall-inducers on both *Salix* hosts resulted in significantly higher glucose and fructose concentrations in gall tissues (Table 1). These findings are opposite to our initial hypothesis that reductions in physiological capacity associated with galling will directly limit the production of metabolites in infested leaves. Sucrose concentration, in contrast, was unaltered or even lower in galls. Starch concentrations were consistently lower in gall tissues, similar to prior findings (Harris and Shorthouse, 1996; Motta et al., 2005; Dantas Moura and Almeida Alves, 2009). Previous studies that have measured soluble sugars in galls to understand allocation have generally reported values as total non-structural carbohydrates (starch and sugars) or total sugar concentrations, without distinguishing between different sugar types. Galls induced by an unidentified lepidopteran species on leaves of *Tibouchina pulchra* (Family Melastomataceae) had higher levels of total carbohydrates than either surrounding leaf tissues or neighboring gall-free leaves (Motta et al., 2005). Similarly, Hartley (1998) found increases in total soluble carbohydrates in gall tissues of four eriophyid mite species, and a decrease in the gall tissue of one species, although neither of these was significant. These alterations lead to the belief that gall-inducers can be considered phloem parasites (Larson and Whitham, 1997) and that they could completely control redirection of growth and physiology of attacked plant organs to their own benefit (Shorthouse et al., 2005). Our findings show the same non-significant patterns when total non-structural carbohydrates are reported (not shown). However, measured separately, both gall-inducers appear to show an unambiguous preference for simple sugars. However, we cannot draw conclusions based on our experiment if galling mites infesting *S. pulchra* and *S. glauca* have control over metabolite preference and growth of the host plant.

Alternatively, the metabolite concentration difference found across and within the leaves of *Salix* during this study may be attributed to plant defense strategies. Plants will translocate sugars to locations where infestation has occurred specifically for the synthesis of defense-related components such as ethylene (Samson et al., 2012). Ethylene is known as an endogenous signal molecule necessary for control of numerous herbivore-related defenses such as the production of peroxidase and polyphenol oxidase (Chakrabarti et al., 2011).

Only further experimental studies in the area of plant metabolic function and defense will confirm whether specific sugars are indeed a preference by the inducer or, alternatively, whether the synthesis of starch (from simple sugars) has been reduced or starch has been broken down to sucrose and then further to simple sugars in larger quantities within galls or whether translocated su-
crores from neighboring leaves are broken down into simple sugars but not consumed by primary metabolism within galls. Or, alternatively, this response is directly related to specific defense mechanisms by the plant that are induced by the galling infestation.

Galls on both plant species had significantly lower nitrogen and, consequently, higher C:N ratios than in neighboring leaves on the same shoot or leaves from control plants. This increase in gall C:N ratios was significant despite lower carbon levels in these tissues. Reduced nitrogen in galls is consistent with patterns seen in other eriophid and insect gall systems (Hartley, 1998; Patankar et al., 2011). On the basis of measures of total nitrogen, gall inducers may not have a strong preference for increased nitrogen in galls. Alternatively, it is possible that parameters such as total nitrogen are too simplistic to allow detection of actual nutritional consumption in gall-inducing arthropods within galls. Nevertheless, it appears that, at least in mite-induced galls, nitrogen is either (a) not available to the gall inducer, or (b) discriminated against by the gall inducer. Total carbon was lower in galls, suggesting that galls did not act as sinks for photo assimilates, but instead simple sugars were translocated into the gall, as seen by elevated glucose and fructose concentrations.

**FOLIAR PIGMENTS**

Reduced chlorophyll and increased anthocyanin concentrations in galls on both *Salix* species showed a pattern consistent with arthropod gall inducers in general (Yang et al., 2003; Huang et al., 2011). The adaptive significance of increased anthocyanin levels in galls is still unclear, but anthocyanins arguably serve a protective role from other forms of herbivory (Gould, 2004) and light-related photo inhibition and oxidation (Close and Beadle, 2003; Oberbauer and Starr, 2002). Nevertheless, increased anthocyanin (along with decreased chlorophyll) likely contributes to decreases in photosynthetic activity seen in infected *Salix* leaves on the two species examined.

**CONCLUSION**

Arctic deciduous plants are subject to various forms of external stresses that include both biotic and abiotic factors. Even so, the role of biotic agents has been little studied. Diminutive plant parasites such as eriophyid gall mites significantly influence fundamental physiological processes in leaves of their host plants. Further, these influences are likely to have an effect beyond the leaf level, given established populations on host plants. In other ecosystems, eriophyid gall-inducing mites are suggested to be responsible for reduced growth rates in hosts, indicating their effect on host functioning beyond the phylloplane (Patankar et al., 2011). The *Salix* species in this study represent a functional group of plants (deciduous shrubs) that have increased (Myers-Smith et al., 2011) and are likely to continue to increase in prominence within arctic ecosystems on the basis of current climate change scenarios (Sturm et al., 2001). Along with deciduous shrub expansion, certain ‘outbreak’ species have been implicated to play an increasingly important role in the region (Jepsen et al., 2008), even leading to shifts in watershed-scale carbon balance in some areas (Christensen et al., 2007). Nevertheless, the influences of ‘chronically associated’ specialist herbivores such as gall-inducing arthropods in this region have not been examined.

The present study provides a foundation for future work to incorporate herbivory-mediated influences on plant carbon dynamics in the tundra. Nevertheless, further questions remain to be answered in order to fully grasp the overall impacts of these ubiquitous herbivores: How common are gall inducers on *Salix* (and other deciduous species) in the arctic tundra? What are their densities at the leaf and whole-plant level? How do impacts at the leaf level translate to effects at the whole-plant level? Based on our results here, we suggest that ‘chronically associated’ plant parasites such as gall-inducing mites are an important component of host plant functioning that are likely to impact hosts beyond the leaf to influence whole-plant performance in arctic tundra deciduous species.

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