EFFECTS OF DEFOLIATION ON SANDBAR WILLOW (*SALIX INTERIOR)* CHEMISTRY,

PRODUCTION, AND SUBSEQUENT OVERWINTER BROWSING BY MAMMALIAN

HERBIVORES

By

Brian Patrick Allman

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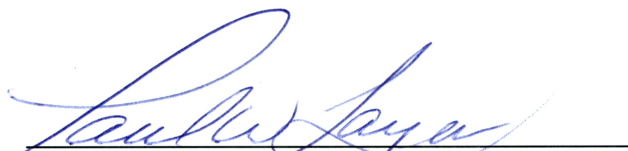_____
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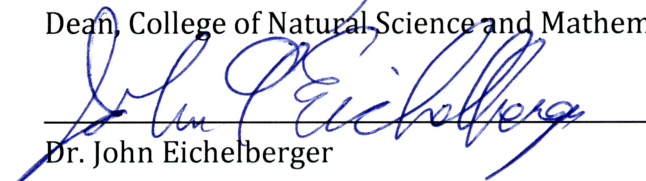
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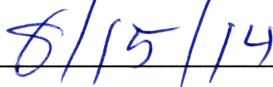Dr. Diane Wagner, Chair, Department of Biology and Wildlife

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EFFECTS OF DEFOLIATION ON SANDBAR WILLOW (*SALIX INTERIOR*)

CHEMISTRY, PRODUCTION, AND SUBSEQUENT OVERWINTER

BROWSING BY MAMMALIAN HERBIVORES

A

THESIS

Presented to the Faculty

of the University of Alaska Fairbanks

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for the Degree of

MASTER OF SCIENCE

By

Brian Patrick Allman, B.A.

Fairbanks, Alaska

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Abstract

Herbivory can cause changes in plant characteristics, allowing temporally isolated herbivores to indirectly affect one another through their effects on shared host plants. The objective of this thesis was to test how defoliation of the willow *Salix interior* affects current annual stem production and chemistry, and how changes in these traits may indirectly affect mammalian herbivores. I studied the effect of manual defoliation on *S. interior* leaf and stem chemistry, and the effect of insect folivory on *S. interior* stem chemistry, production, and mammal herbivore offtake. Manual defoliation of *S. interior* affected stem chemistry by significantly increasing stem N concentration and decreasing stem C:N ratio, but did not alter leaf chemistry. Neither stem nor leaf protein precipitation capacity (PPC), a measure of tannin activity, were affected by manual defoliation. In a second field experiment I investigated the effects of natural levels of insect folivory on *S. interior* stem characteristics, testing the effects of insect herbivore suppression on stem production, chemical composition, protein precipitation capacity, and overwinter mammal browsing. Insect folivory did not significantly alter stem chemistry, but significantly reduced stem production by reducing mean stem diameter the following year. These findings indicate that defoliation of *S. interior* can improve nutritional quality and reduce availability of stems for mammal herbivores foraging over the subsequent winter.

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Introduction

      Herbivory can alter many plant characteristics, including nutritional value, chemical defenses, and patterns of growth or reproduction (Bowsher 2008, Karban and Baldwin 1997, Palo and Robbins 1991, Werner and Peacor 2003). Changes in plant chemical composition or induction of plant defenses by herbivory can affect both current and subsequently grown tissues (Bowsher 2008, Karban and Baldwin 1997, Ohgushi 2005, Werner and Peacor 2003). Plant responses to herbivory can alter nutritional quality of forage both positively and negatively. For example, defoliation of red alder (*Alnus Rubra*) by the western tent caterpillar (*Malocosoma californicum pluviale*) reduced foliar nitrogen concentrations after multiple seasons of herbivory, reducing the nutritional quality of red alder leaves (Myers and Williams 1987). By contrast, winter browsing by moose (*Alces alces*) on birch (*Betula spp.)* increased leaf nitrogen and digestible protein concentrations the following summer (Danell and Huss-Danell 1985), thereby improving the nutritional quality of leaves for subsequent herbivores.

      The effects of defoliation on plant chemical defenses are variable, and can be influenced by many plant traits, including plant species, plant ontogeny, nutrient availability, nutrient storage, herbivores species, and the timing of the defoliation event (Barbehenn and Constabel 2011, Fields and Orians 2006, Karban and Baldwin 1997, Lindroth et al. 2007, Stamp 2003). In silver birch (*Betula pendula*), both artificial damage and insect folivory by leaf grazers and leaf miners increased concentrations of total phenolics in leaves later in the season (Hartley and Lawton 1987). These phenolics are believed to act as plant defenses, reducing plant digestibility and palatability (Bowsher 2008, Hartley and Lawton 1987, Barbehenn and Constabel 2011). Induced plant defenses

are not necessarily ubiquitously induced. Induction of plant defenses may vary in response to the source, location, and severity of damage (Barbehenn and Constabel 2011, Fields and Orians 2006, Hartley and Lawton 1987). In the willow *Salix sericea*, for example, folivory by three different beetle species induced greater concentrations of the phenolic glycoside salicortin in young leaves, but did not significantly alter salicortin concentrations in mature leaves (Fields and Orians 2006).

Herbivory and damage to plant tissues can alter other plant characteristics in addition to chemical defenses and composition, including photosynthesis, production, and reproduction (Bowsher 2008, Danell and Huss-Danell 1985, Ohgushi 2005, Palo and Robbins 1991, Schwenk and Strong 2011). For example, both insect herbivory during the growing season and moose herbivory over winter of striped maple (*Acer pensylvanicum*) decreased leaf number the following year (Schwenk and Strong 2011). By comparison, moose winter browsing of birches increased leaf size the following growing season, though it did not have a significant effect on leaf number (Danell and Huss-Danell 1985). In another study, oviposition by the spittlebug *Aphrophora pectoralis* on the willows *S. miyabeana* and *S. sachalinensis* increased the number of dead shoots the year of oviposition, but increased bud number and shoot length the following year (Nozawa and Ohgushi 2002). Changes in plant chemical composition, including chemical defenses, can also alter plant traits through possible resource costs of producing defenses, or altering growth patterns (Baldwin 1990, Orians et al. 2010, Stamp 2003). In one study, willow seedling biomass and proportion of mass in root tissues were negatively correlated with total phenolic concentrations, though this correlation was not found in older plants (Orians et al. 2010). Similarly, seed production in tobacco plants (*Nicotiana sylvestris*) was reduced

in plants with induced alkaloid defenses in response to artificial leaf damage (Baldwin 1990). These findings show that herbivore-induced changes in plants may be interrelated, with a change in one characteristic having additional secondary effects on other traits. Consequently, measuring the interrelated effects of defoliation on plant characteristics can be challenging.

Changes in plant traits in response to defoliation may be induced in both the short- and long-term (Bryant et al. 1991, Clausen et al. 1989 and 1991, Tuomi et al. 1984). While short-term induction can occur within mere minutes or hours of damage, induction can also occur over days or weeks within the same season as damage occurs. In grey alder, damage by the chrysomelid beetle *Agelastica alni* induced increased trichome densities, a form of physical defense, within 2-3 weeks of insect herbivory (Baur et al. 1991). Long-term induction can also occur, with plant characteristics altered for multiple months or even years after damage occurs (Bryant et al. 1991 and references within, Clausen et al. 1991 and references within, Myers and Williams 1987). For example, damaging the roots of mountain birch significantly increased leaf phenolic concentrations for at least 3 years after initial damage occurred (Tuomi et al. 1984).

Because defoliation and damage to plants can significantly alter plant chemical defenses, chemical or nutritional composition, production, and mortality in both the short- and-long-term, temporally separated herbivores can indirectly affect others of the same or different species (Nozawa and Ohgushi 2002, Ohgushi 2005, Schwenk and Strong 2011). Interactions between species through induced changes in plants are classified as trait- mediated indirect interactions (Ohgushi 2005, Werner and Peacor 2003). Indirect interactions between herbivores through changes in shared food species can occur within a

season, or over multiple seasons (Hartley and Lawton 1987, Myers and Williams 1987, Ohgushi 2005, Schwenk and Strong 2011, Werner and Peacor 2003). For example, overwinter browsing of striped maple by moose improved the quality of leaves for insect herbivores feeding the following season and consequently increased overall insect herbivory on previously browsed plants the following year (Schwenk and Strong 2011). Another study on the effect of moose on insect herbivores found that moose herbivory of birches increased the number of individuals of multiple different insect species found on browsed trees the following year (Danell and Huss-Danell 1985). Previous studies on indirect herbivore interactions have tended to examine how mammalian herbivores may affect insects, but few published studies examine how insect herbivores feeding in summer may affect mammal herbivores feeding on the same plants overwinter.

Sandbar willow (*Salix interior*), the focal species of this study, is an important species for moose and several other mammalian herbivores throughout interior Alaska (Newsholme 2003, Risenhoover 1989, Seaton 2002). *S. interior* is a common willow on floodplains and moist alluvial soils throughout northern and western United States and Canada (Newsholme 2003). Current annual stems of many deciduous species comprise the majority of moose winter diets (Oldemeyer et al. 1977, Risenhoover 1989, Seaton 2002) and *S. interior* is one of the most highly preferred forage species (Milke 1969, Risenhoover 1989, Seaton 2002).

The insect folivore the willow leaf blotch miner, *Micrurapteryx salicifoliella*, also feeds heavily on *S. interior,* though only during the growing season, as opposed to throughout the year like moose (Furniss et al. 2001). The willow leaf blotch miner has been present in interior Alaska at outbreak levels on and off since the 1990s, and as recently as

the early 2010s (FS-R10-FHP 2013, FS-R10-TP-123 2004, Furniss et al. 2001, Holsten et al. 2009). Larval mining by the willow leaf blotch miner has been shown to decrease annual production in several interior Alaskan willows, as measured by stem elongation (Wagner and Doak, unpublished data). Research on the effects of insect herbivores such as the willow leaf blotch miner on plant characteristics has tended to examine changes in leaf characteristics, but little research has examined the effects of insect herbivory on stems (Lindroth et al. 2007).

S. interior produces condensed tannins, also known as proanthocyanidins, as a chemical defense against many herbivore species (Matsuki 1992) and no other known defensive phenolics have been identified in *S. interior* . Condensed tannins reduce herbivory of many mammal species, though their effectiveness against insect herbivores has been contested and debated in literature (Barbehenn and Constabel 2011, Feeny 1970, Palo and Robbins 1991). Tannins are believed to have a protein-binding effect, reducing the nutritional value of plant tissues with high tannin content (Bowsher 2008, Barbehenn and Constabel 2011, Palo and Robbins 1991). Mammal herbivore diet selection is dependent on both chemical defenses and nutritional value of plant tissues, with greater emphasis on the chemical defenses of plants than on their nutritional value (Bryant and Kuropat 1980, Dearing et al. 2000, Iason and Villalba 2006, Shipley and Spalinger 1992). If the concentration or activity of tannins in *S. interior* is altered by previous insect herbivory, this may then affect the nutritional value of *S. interior* stems for moose feeding overwinter, or their dietary preferences.

My thesis examined the effects of defoliation on *S. interior* chemical composition, chemical defenses, and nutritional value. The effects of both artificial and natural

defoliation of *S. interior* were studied in both the short and long-term. In Chapter 1 I examined how artificial defoliation of *S. interior* affects tannin activity, nutritional value, and chemical composition of leaf tissues within season and stems both within season and the following autumn. Chapter 2 focused on the effect of ambient insect folivory on *S. interior* stem production and stem tannin activity, nutritional value, and chemical composition the following winter. The second chapter also examined the effect of insect folivory and its attendant changes in plant traits on mammal browsing during winter.

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Chapter 1: Effects of defoliation on sandbar willow (*Salix interior*) leaf and stem chemistry [1]

1.1 Abstract

Defoliation can alter a number of plant characteristics, including chemical composition, defenses, or resource allocation. Alterations to plant characteristics in response to defoliation can occur within a season, and may persist for multiple seasons thereafter. Previous research on plant response to defoliation has focused on the effects of defoliation on leaves, while the effect of defoliation on stems has been largely unstudied. The effects of defoliation on stems may be ecologically important, as many mammalian herbivores in Alaska feed primarily on dormant stems during winter. In this study, we examined the effect of defoliation on *Salix interior* stem and leaf nutritional composition and chemical defenses within the growing season, as well as stem nutritional composition and chemical defenses in dormant tissues the following autumn. To measure the effect of defoliation on *S. interior*, we compared 25% and 75% manually defoliated plants against controls. Controls were protected from ambient insect folivory by applying insecticide at the beginning of the experiment. Defoliation significantly increased autumn stem N concentration in heavily defoliated plants. Defoliation did not significantly affect subsequent insect folivory, leaf N concentration, stem or leaf C concentration, stem or leaf C:N ratio, or stem or leaf protein precipitation capacity. Stems in autumn contained significantly higher N and lower C concentrations than in summer across treatments. These

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results indicate that defoliation during the growing season can affect stem chemical composition the following season, and suggest that defoliation during the growing season may alter patterns of *S. interior* N storage the following winter.

1.2 Introduction

Defoliation can alter plant characteristics by inducing chemical defenses, reducing photosynthesis, and reducing growth (Bryant et al. 1991, Karban and Baldwin 1997, Ohgushi 2005, Stamp 2003). Plant response to defoliation can be rapid, altering chemical defenses in leaves within 24 hours (Clausen et al. 1989). In addition to short-term induction, previous defoliation can alter plant traits in subsequent seasons and years (Bryant et al. 1991, Clausen et al. 1991, Myers and Williams 1987). Plant responses to defoliation may be correlated with the severity of a defoliation event (Bryant et al. 1991, Palo and Robbins 1991, Strauss et al 2002), though this is not always the case (Fields and Orians 2006).

Many studies have examined how defoliation can affect leaf characteristics, while comparatively few studies have examined the effects of defoliation on plant stems. Generally, defoliation tends to increase leaf chemical defense concentrations and reduce photosynthesis and leaf production. The effects of defoliation on stems, while relatively unknown, may be ecologically significant. Changes in stem chemistry, production, or defenses may indirectly affect mammalian herbivores, because many mammalian herbivores rely on current year's growth of woody stems as a dietary staple overwinter (Risenhoover 1989, Milke 1969, Seaton 2002) and changes to stem chemical defenses or nutritional composition can alter mammal feeding behaviors (Bryant and Kuropat 1980,

Dearing et al. 2000). The few studies examining the effect of defoliation on dormant winter

stems have found evidence that defoliation can affect stem chemistry and production

(Hjalten et al. 1994, Lindroth et al. 2007). In quaking aspen (*Populus tremuloides)*, previous

defoliation lowered stem chemical defenses and increased stem nitrogen concentrations

the following winter, which the authors suggested may indicate changes in nitrogen

storage patterns in defoliated plants (Lindroth et al. 2007). This may also affect N content

of leaves, in addition to affecting stem N content. In birch (*Betula pubescens)* herbivory of

stems and leaves had no effect on stem chemical defenses, but reduced stem production

(Hjalten et al. 1994). It is currently unknown how stems of willows (*Salix spp.*) are affected

by defoliation.

Multiple willow species increase leaf chemical defense concentrations in response

to defoliation (Fields and Orians 2006, Matsuki 1992, Ruuhola and Julkunen-Tiitto 2003).

Salix interior, the focal species of this study, contains condensed tannins as a chemical

defense against herbivory (Matsuki 1992, Palo 1984). Tannins are inducible by defoliation,

but inducibility varies among species, as well as by inductive causes, and in response to a

variety of environmental variables (Barbehenn and Constabel 2011, Kraus et al. 2003). It is

unknown if tannin activity is inducible by defoliation in either leaves or stems of *S. interior*.

The objective of this study was to examine the effects of defoliation on *S. interior*

stem and leaf chemistry within-season and stem chemistry the following autumn. To

understand how willow stem chemical defenses and composition are affected by

defoliation, we manually defoliated *S. interior* plants and measured leaf and stem chemical

composition one month later, and stem chemistry again in late autumn after leaves had

senesced. We predicted that defoliation would reduce leaf area and thus carbon fixation,

which would likely decrease plant C:N ratio. Further, we predicted there would be an induced response in leaf tissues in response to defoliation, and expected that the effects of defoliation would likely affect *S. interior* similarly to confamilial quaking aspen. We thus predicted that defoliation of *S. interior* would: (1) decrease leaf N concentrations, (2) increase stem N concentrations, (3) increase leaf protein precipitation capacity (PPC), and (4) reduce stem PPC.

1.3 Methods

1.3.1 Study area

The study was conducted on the floodplain of the Tanana River, southwest of Fairbanks, Alaska. Three study plots were established in early successional sites dominated by *S. interior* near the Fairbanks International Airport (64 47'26.94° N, 147 53'43.90°W).

1.3.2 Experimental design

To experimentally test the effects of defoliation on *S. interior* leaves and stems we used a completely randomized block design. In May 2013, three ~20x20 meter study plots were established approximately 20 meters apart on the Tanana river floodplain east of the Fairbanks International Airport in Fairbanks, Alaska (64 47'26.94N, 147 53'43.90W).

In each of the three plots, 24 plants at least 30 cm tall were randomly selected for use in the study, for a total of 72 plants. Plants were on average 58 cm tall (SD=13.8 cm). Eight plants in each plot were randomly assigned to control, low-defoliation, and high-defoliation treatments. In order to prevent ambient insect folivory and possible induction of defenses in control plants, we sprayed controls with Conserve (Dow Agrosciences,

Indianapolis, Indiana), an insecticide known to be effective against leaf miner larvae with relatively quick rate of degradation and few human health concerns (EPA US 1999). Plants assigned to low or high defoliation treatments were sprayed only with water.

Defoliation treatments were imposed on 19 June 2013. We removed 25% of leaf are from plants in the low-defoliation treatment, and 75% of leaf area from plants in the high-defoliation treatment, clipping laterally across the leaf blade. Control plants were handled in a similar manner, but no leaf area was removed.

In order to determine whether the defoliation treatments induced short-term resistance to herbivory, on 22 July 2013 we assessed damage to leaf tissues grown after defoliation. We randomly selected two shoots per plant, and estimated leaf damage on the five most distal leaves per selected shoot. We visually estimated leaf damage as a percentage of total leaf area affected by various forms of invertebrate folivory, including leaf mining, skeletonization, or leaf area removed. Data were collected by a single observer who was trained and tested prior to data collection to ensure visual estimations correlated with measurements of leaf damage previously assessed with image analysis software ($R^2>0.90$, N>55 leaves: ImageJ software, National Institutes of Health). Leaf damage was averaged within each plant.

To measure the effect of defoliation on summer leaf and stem chemistry, we randomly selected and destructively sampled half of the study plants in each plot in July 2013 by removing three current annual stems, along with their leaves, from each selected plant. Leaf and stem samples each pooled separately within plants for further analyses. Samples were transported on ice back to the university where they were stored at -80° C.

To measure the effects of defoliation on the chemical composition of dormant stems, we collected samples from the remaining 12 study plants within each block during the first week of October 2013, after leaf senescence. We removed 3 current annual stems from each plant, placed the collected samples on ice, and transported them back to the university where they were stored at -80° C.

1.3.3 Chemical analyses

We analyzed the elemental composition and tannin activity of summer leaf tissues and both summer and autumn stems. Stem and leaf samples were lyophilized using a FreeZone Triad Freeze Dry System (Labconco, Kansas City, MO, USA) for 48-72 hours. Dried samples were ground using a Wiley mill over a 40-mesh screen and stored in airtight containers at room temperature. Elemental composition (C and N concentrations) was determined on duplicate 0.1 gram samples of ground tissue using a LECO 2000 CNS Analyzer (LECO Instruments, St. Joseph, MI, USA).

To assess tannin activity within leaves and stems, we used a modification of the Robbins et al. (1987) protocol to calculate tannin protein precipitation capacity (PPC). Three replicates of each stem and leaf sample were analyzed per plant for PPC, which was used as a measure of tannin activity. We soaked 0.50 grams of each sample in 20.0 mL of 50% methanol for 5 minutes. Soaked samples were sonicated for 15 minutes and re-sealed in airtight containers to incubate at room temperature for 30 minutes. After incubation, 2.0 mL of the sonicated solution was pipetted in 2.0 mL microcentrifuge tubes and centrifuged for 15 minutes at 5000 RPM. 35.0 μL of the supernatant was then pipetted from the microcentrifuge tubes and combined with 140.0 μL of 5mg/mL BSA in 0.2 M acetic acid

acetate buffer with 0.17 M NaCl in a 96-well centrifugable microplate. Microplates were

centrifuged for 10 minutes at 6000 RPM, after which 5.0 μL of the supernatant from each

well was pipetted and combined with 250.0 μL of Bio-Rad Quick Start Bradford Protein

Assay Reagent (Bio-Rad Laboratories, Hercules, CA, USA). Solutions were vortexed for 30

seconds, and then incubated at room temperature for 6 minutes in airtight containers. After

incubation, sample absorbance was read on a microplate reader at 590 nm using a blank of

250.0 μL of Bio-Rad Bradford Protein Assay Reagent and 5.0μL of 0.2 M acetic acid acetate

buffer with 0.17 M NaCl. We made standards at 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, and 5.0 mg/mL

BSA concentrations. The mass of the remaining, soluble protein per sample well was

calculated from the standard curve. We calculated mass of protein precipitated by

subtracting the soluble protein mass from initial mass of BSA placed in the well. PPC was

calculated by dividing the mass of protein precipitated by the mass of plant sample in each

well, and is reported as mg BSA precipitated per g of sample.

1.4 Statistical analysis

We used univariate ANOVA models to test the fixed effect of defoliation on

subsequent leaf damage sustained by *S. interior* and on measurements of plant composition

and tannin activity. Plant height was originally included in analyses as a covariate, but did

not account for a significant portion of variation and was removed. In all statistical models,

the random effect of site was included as a blocking factor in order to account for possible

spatial variation in *S. interior* characteristics. Models of defoliation effects on stem

chemistry included the season of sample collection and the interaction of defoliation

treatment and season of collection as main effects. Following a significant effect of

defoliation treatment or a significant season by defoliation interaction, means were compared with Tukey HSD tests. Data are presented as least square means, hereafter referred to as "means."

All analyses were conducted using JMP 9 software (SAS Institute, Cary, NC).

1.5 Results

1.5.1 Effect of defoliation on subsequent folivory

The average proportion of leaf area damaged by herbivory after application of treatment was lower in defoliated than control plants, but this difference was not statistically significant ($F_{2, 57.28}$ = 2.21, P = 0.1184; Fig 1.1). Across treatments, plants had an average of 3% (SE=0.6%) leaf area damaged.

1.5.2 Effect of defoliation on leaf chemistry

Defoliation treatment did not significantly affect chemistry of *S. interior* leaves grown after defoliation in terms of any measured factors: leaf N concentration, C concentration, C:N ratio, or PPC (Table 1.1).

1.5.3 Effect of defoliation on stem chemistry

The concentration of N in stems of *S. interior* was higher in defoliated plants than controls, but only control plants and high-defoliation treated plants were significantly different from one another ($F_{2, 46.91}$ = 4.99, P = 0.0109; Fig 1.2A). Stem N concentration was significantly greater in autumn than summer ($F_{1, 47.64}$ = 4.56, P = 0.0380; Fig 1.2B). The interaction between season and defoliation was not significant ($F_{2, 47.38}$ = 1.92, P = 0.1580).

The concentration of C in stems of *S. interior* was not significantly affected by defoliation ($F_{2, 46.29}$= 1.80, P = 0.1773; Fig 1.2C). Stem C concentration, however, did vary significantly between seasons, with stems collected in summer having lower average C concentrations than plants collected in autumn ($F_{1, 46.73}$ = 45.54, P < 0.0001; Fig 1.2D). The interaction between season and defoliation treatment on stem C concentration was not significant ($F_{2, 47.1}$= 1.00, P = 0.3739).

Defoliation significantly reduced *S. interior* stem C:N ratio ($F_{2, 46.63}$ = 6.67, P = 0.0028; Fig 1.2E). The difference in stem C:N ratio was marginally significant between seasons ($F_{1, 47.23}$= 3.04, P = 0.0880; Fig 1.2F), as was the interaction between season and defoliation ($F_{2, 47.37}$ = 2.79, P = 0.0714).

PPC of stems was not significantly affected by defoliation ($F_{2, 53.72}$ = 2.05, P = 0.1392) or season of collection ($F_{1, 53.44}$ = 1.20, P = 0.2777). The interaction between season and defoliation also had no significant effect on stem PPC ($F_{2, 54.44}$ = 1.47, P = 0. 2388). Across treatments, mean stem PPC was 514 mg BSA/g DM (SE=12.27mg BSA/g DM).

1.6 Discussion

Previous research on the effect of defoliation on leaf chemistry has found increased chemical defense concentrations in response to defoliation in many plant species. Tannin inducibility varies among plant species, as well as in response to a number of environmental variables (Barbehenn and Constabel 2011, Kraus et al. 2003). Although previous studies have found induction of leaf tannin concentrations in response to defoliation in multiple other willow species, (Bryant 2003, Fields and Orians 2006, Raupp and Sadof 1991), we found no evidence of tannin induction by *S. interior* (Table 1.1).

Likewise, we found no significant effect of defoliation on leaf N concentration, C concentration, or C:N ratio. By comparison, in confamilial quaking aspen, leaf nitrogen concentration was not affected by defoliation (Osier and Lindroth 2001), while in white birch it was reduced (Tuomi et al. 1984), and in red alder, western tent caterpillar herbivory significantly reduced leaf nitrogen concentrations, though only after extended periods of herbivory (Myers and Williams 1987). Overall, our findings indicate that a single bout of defoliation of *S. interior* does not significantly affect leaf C, N, or C:N ratio.

We found no clear effect of defoliation early in the season on insect folivory later in the season. Our data show a trend towards lower insect folivory on previously defoliated plants, though this trend is not statistically significant. This trend suggests it may be informative to re-examine short-term resistance to insect folivory in *S. interior*, despite the absence of induced tannins*.*

Although much research has examined the effect of defoliation on subsequent leaf nutritional value for and chemical defenses against herbivores, very little research has studied the effect of defoliation on dormant woody tissues, either within or across seasons (Lindroth et al. 2007). In this study, previous defoliation increased the dormant stem N content (Fig 1.2A), reduced tissue C:N ratio (Fig 1.2E), and did not significantly affect stem tannin activity. A possible mechanism for this result is that defoliation reduced the overall rate of carbon fixation in defoliated plants. If nutrient uptake by roots was not proportionally reduced by defoliation, the amount of nitrogen relative to carbon uptake would likely increase, thereby reducing the C:N ratio, and resulting in a greater proportion of nitrogen in dormant stems overwinter. Previous research on the effect of defoliation on stem chemistry matches our findings. Working with aspen, Lindroth et al (2007) also found

that defoliation increased subsequent stem nitrogen concentration, much as we did. Furthermore, Lindroth et al (2007) reported significant variation in some, though not all, plant genotypes in response to defoliation. The only other study we were able to find on the effect of defoliation on winter stem chemistry (Hjalten et al. 1994) reported that previous defoliation did not induce increased stem total phenolic concentrations; stem nitrogen concentrations were not described.

A potential source of criticism in this study is that we examined the effect of artificial, rather than natural defoliation. This may affect the applicability of our findings to natural systems, as artificial wounding does not always affect plants in the same manner as herbivory, and may not induce defenses in the same manner or to the same degree as herbivory (Karban and Meyers 1989, Tallamy and Raupp 1991). However, this concern may not be warranted, as multiple other studies have found that artificial defoliation and natural herbivory can elicit the same responses in a variety of species (Clausen et al. 1989, Fowler and Lawton 1985 and references).

The results of this study may have ecological significance. While insect herbivores only infrequently defoliate plants to the degree our defoliation treatments did, during insect outbreaks, levels of defoliation can exceed those applied in here (Volney and Fleming 2000). In Fairbanks, Alaska, where this study took place, there have been multiple outbreaks of the willow leaf blotch miner (*Micrurapteryx salicifoliella*) since the late 1990s through the early 2010s (Furniss et al. 2001, FS-R10-FHP 2013, FS-R10-TP-123 2004, Holsten et al. 2009). This leaf mining folivore is known to affect *S. interior* and many other common local willow species (Furniss et al. 2001, Holsten et al. 2009), and during severe

outbreaks may damage willows to a degree comparable with the higher level of defoliation employed in this study.

Our results suggest that high levels of defoliation, such as those during insect outbreaks, may significantly increase *S. interior* stem nitrogen concentrations in subsequent seasons. Many mammalian herbivores in interior Alaska depend on dormant stems of willows and other deciduous forage species in winter (Belovsky 1981, Risenhoover 1989, Seaton 2002). These herbivores select browse based upon both plant nutritional value and chemical defenses (Bryant and Kuropat 1980, Dearing et al. 2000, Oldemeyer et al. 1977). Our data, showing an increase in stem nitrogen in response to defoliation but no change in tannin activity, suggest that prior defoliation of *S. interior* can improve the nutritional quality of *S. interior* stems for mammalian herbivores foraging overwinter.

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Table 1.1: Results of leaf chemical analysis ANOVA models. ANOVA models testing the effect of defoliation treatments on mean leaf tissue chemical and nutritional composition in *S. interior.*

Variable	Mean	SE	d.f.	*F*-value	*P*-value
N (%)	1.4	0.025	2, 23.38	1.46	0.2484
C (%)	45.9	0.203	2, 27.57	0.06	0.9398
C:N Ratio	33.6	0.765	2, 28.17	1.07	0.3559
PPC (mg BSA/g DM)	632	20.482	2, 26.33	0.16	0.8518
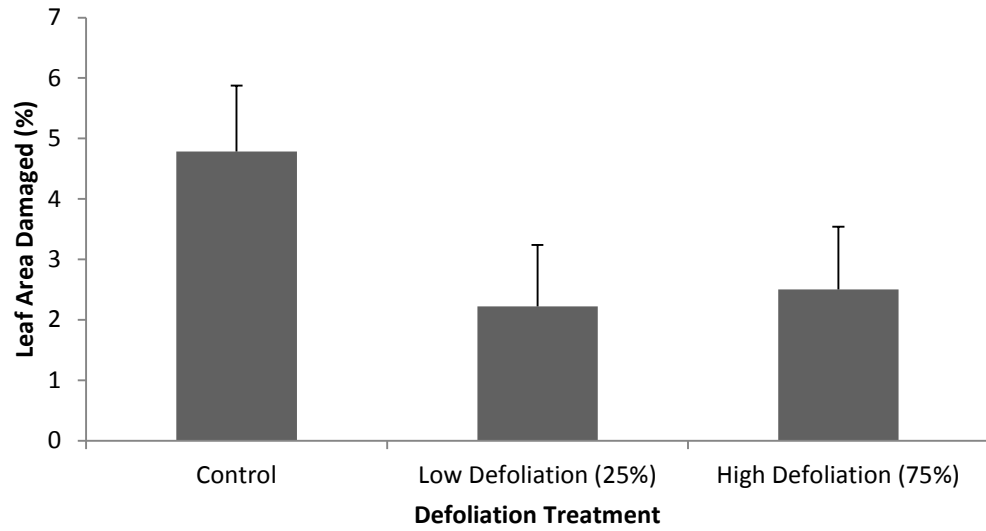
Figure 1.1: Effect of defoliation treatment on mean leaf area damaged per plant following defoliation treatments. Data shown are least square means ±SE, n=62.
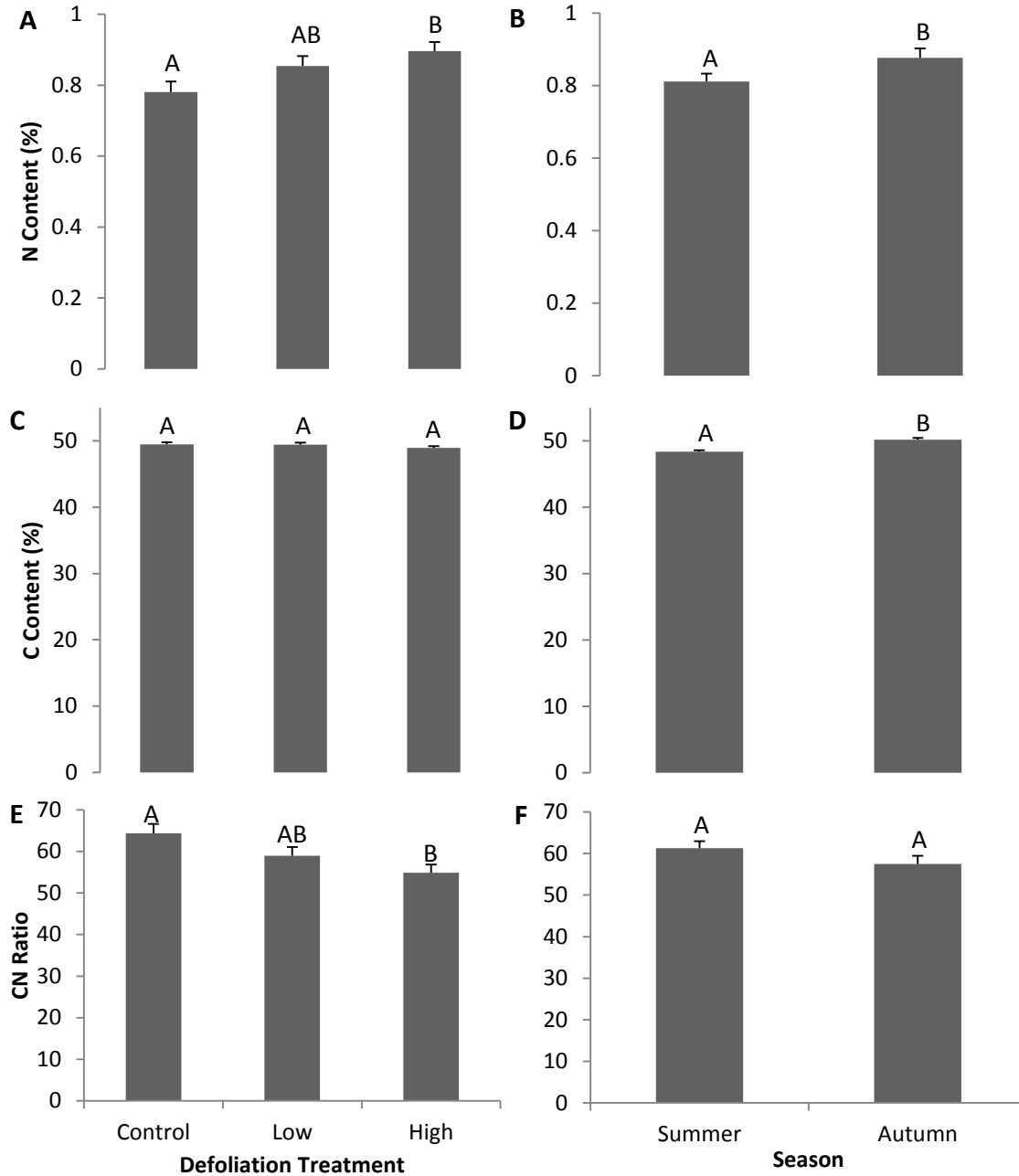
Figure 1.2: Effect of defoliation and season on stem chemistry. 1.2A and 1.2B show %N by treatment and season, respectively, 1.2C and 1.2D show %C by treatment and season, respectively, and 1.2E and 1.2F show C:N ratio by treatment and season, respectively. Data shown are least square means ±SE, n=54. Treatments not connected by the same letter are significantly different (Tukey HSD, α=0.05).

Chapter 2: The effect of summer insect folivory on *S. interior* winter forage quantity, quality, and consumption by mammalian herbivores[1]

2.1 Abstract

Plant production, elemental composition, and chemical defenses can be altered by insect herbivory. Changes in plant characteristics due to insect herbivory can occur rapidly after damage, and can persist for multiple years thereafter. Consequently, mammalian herbivores browsing on plants previously affected by insect herbivores may be indirectly affected if insect herbivory impacts the quantity or nutritional quality of plant tissues. In interior Alaska, insect herbivores feed during the growing season, while mammalian herbivores feed on leaves and stems of many of the same species of plants throughout the entire year. The indirect effects of insect herbivory on mammalian herbivore winter diet quality and quantity have been largely unstudied, but may be ecologically significant. In this study we examined the effects of insect and mammalian herbivore exclusion on current annual stem production, chemical composition, tannin activity, and moose winter browsing in the willow *Salix interior*. We found that reducing insect folivory during the growing season increased *S. interior* stem production in terms average stem diameter, and consequently, stem biomass. We found no significant effect of insect folivory on stem number, tannin activity, chemical and nutritional composition, or overwinter mammalian browsing. We also found no significant effect of mammalian herbivore exclusion on *S. interior* stem N and C concentrations, C:N ratio, or tannin activity. The results of this study

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suggest that low levels of insect folivory may affect the quantity, but not necessarily the quality, of key overwinter dietary plant species such as willows for mammalian herbivores.

2.2 Introduction

Herbivory can affect plant traits including photosynthesis, production, chemical and physical defenses, and nutritional quality (Bowsher 2008, Karban and Baldwin 1997, Ohgushi 2005, Strauss et al. 2002, Werner and Peacor 2003). Changes in plant characteristics can be induced by herbivory within a season and may persist for multiple years thereafter (Myers and Williams 1987, Ohgushi 2005, Schwenk and Strong 2011). For example, herbivory by the spittlebug *Aphrophora pectoralis* on the willow *Salix miyabeana* caused increased shoot growth and leaf number the following year (Nozawa and Ohgushi 2002), and artificial defoliation of quaking aspen (*Populus tremuloides)* reduced plant growth and increased condensed tannin concentrations a full year after defoliation (Osier and Lindroth 2004). Plant characteristics, such as concentrations of chemical defense compounds or protein content, can affect the feeding behaviors of generalist herbivores (Barbehenn and Constabel 2011, Stamp 2003). Due to the potentially long-lasting effects of herbivory on plant characteristics, herbivores feeding on previously damaged plants may be indirectly affected by previous herbivory (Ohgushi 2005, Schwenk and Strong 2011). The indirect effects of one herbivore on another through changes in shared food species characteristics are referred to as trait-mediated indirect effects. Trait-mediated indirect effects of herbivory can have both positive and negative effects on other herbivores feeding later in the season or in subsequent years (Ohgushi 2005).

Previous research on the effects of herbivory on plant traits has primarily examined the effects of herbivory on leaves, while the effects of herbivory on stems has not been thoroughly examined (Lindroth et al. 2007). Yet deciduous stems are an important source of nutrition for mammalian browsers in the far North, and therefore effects of insect herbivores on stem quality and quantity may impact economically and culturally important wildlife species. In interior Alaska, moose rely on current annual growth stems for their diet during winter (Risenhoover 1989, Seaton 2002). Previous studies observing moose feeding behavior in winter have found that willow stems (*Salix spp.*) comprise from 43% (Seaton 2002) to over 90% of moose winter diet (Risenhoover 1989). Mammalian herbivore diet selection is affected by both the concentration of plant chemical defenses and tissue nutritional quality (Bryant and Kuropat 1980, Boeckler et al. 2011, Dearing et al. 2000). For example, moose (*Alces alces*) diet preference is inversely correlated with concentrations of total plant phenolics and condensed tannins (Oldemeyer et al. 1977, Bryant and Kuropat 1980, Risenhoover 1989, Stolter et al. 2005), which reduce browse palatability and/or digestibility (Palo 1984, Robbins et al. 1987). If insect herbivory during the growing season alters willow stem production, nutritional quality, or chemical defenses, moose browsing overwinter may be affected.

Herbivory can alter the chemical composition of multiple willow species (Boeckler et al. 2011, Bryant 2003, Fields and Orians 2006, Matsuki 1992, Nozawa and Ohgushi 2002, Ruuhola and Julkunen-Tiitto 2003), which may in turn affect their nutritional quality for herbivores. In the confamilial species quaking aspen (*Populus tremuloides)*, a mix of insect folivory and manual defoliation increased the nutritional quality of winter dormant twig tissues, decreased wood phenolic glycoside concentrations, and in plants with low nutrient

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availability, increased nitrogen concentrations (Lindroth et al. 2007). While defoliation of quaking aspen decreased tannin concentrations in some genotypes, it did not affect tannin concentrations across all genotypes.

Salix interior, the focal species of this study, is a common willow distributed throughout the northern and western United States and Canada. It is generally found along floodplains, on sandbars, and in moist alluvial soils (Newsholme 2003). *S. interior* produces condensed tannins, also known as proanthocyanidins, as a chemical defense against many herbivores (Matsuki 1992). The protein binding capacity of tannins has been shown to decrease digestible protein content of plant tissues, including in multiple willow species (Barbehenn and Constabel 2011, McArt et al. 2009, Robbins et al. 1987). Despite the presence of tannins, *S. interior* serves as a major food source for both mammal (e.g. moose) and insect herbivores (e.g. the willow leaf blotch miner, *Micrurapteryx salicifoliella*) throughout interior Alaska and Western Canada (Furniss et al. 2001, Risenhoover 1989, Seaton 2002).

Insect folivory may affect *S. interior* traits, which may consequently affect mammalian herbivores. In the willow *Salix sericea*, some types of phenolic glycosides, a chemical defense that can affect herbivore food selection patterns, were induced by insect herbivory (Fields and Orians 2006). In interior Alaska, larval leaf mining by the willow leaf blotch miner reduced the annual production of several *Salix* species, as measured by stem elongation (Wagner and Doak, unpublished data), but the effect of willow leaf blotch miner mining on *S. interior* stem biomass has not been studied. The willow leaf blotch miner has been present at outbreak levels in interior Alaska, where this study took place, off and on since the 1990s and was prevalent as recently as the early 2010s (FS-R10-FHP 2013, FS-

R10-TP-123 2004, Holsten et al. 2009). Changes in *S. interior* chemistry or production caused by the willow leaf blotch miner may negatively affect mammalian herbivores feeding on previously defoliated plants during winter.

To measure the direct effects of insect herbivores on *S. interior* and the indirect effects on moose browsing, we used a field experiment to examine folivory-induced effects on the quality, quantity, and subsequent consumption by moose of *S. interior* winter-dormant stems. We predicted that willows with experimentally reduced insect herbivory would (1) contain lower stem nitrogen content, (2) express greater tannin protein precipitation capacity (PPC), (3) produce greater stem biomass, and (4) be browsed proportionally less heavily by moose overwinter.

2.3 Methods

2.3.1 Study area

The study was conducted on the floodplain of the Tanana River, in the Bonanza Creek LTER sites approximately 20 km southwest of Fairbanks, Alaska. We established six study sites in early successional habitat dominated by willows (*S. interior, S. niphoclada, S. pseudomyrsinites)*, with low densities of balsam poplar (*Populus balsamifera),* and alder (*Alnus tenuifolia)* (Table 2.1).

2.3.2 Experimental design

To test the effects of insect suppression and mammal exclusion on *S. interior* stem chemistry and subsequent mammal browsing, we used a completely randomized two-factor split-plot design. Mammal exclosure was included in the experimental design in

order to isolate the effects of insect herbivores from those of mammals. In May 2012, we established two 9 x 12 m plots on each of six experimental sites. We randomly assigned one of each pair of plots to be a fenced mammal exclosure plot and the other to be an unfenced control plot. Mammal exclosure plots were surrounded by 9 m x 12 m x 2 m tall chain-link fencing. At each site, both plots were divided into two subplots of approximately 4 x 7 m with a 1 m buffer strip on all sides. Subplots were randomly assigned to be insecticide-sprayed or control subplots, with control subplots sprayed only with water (Fig. 2.1).

We applied insecticide in order to reduce ambient levels of insect herbivory, particularly by the willow leaf blotch miner. Insect suppression treatment subplots were sprayed with Conserve (Dow Agrosciences, Indianapolis, Indiana), an insecticide known to be effective against leaf miner larvae with a relatively quick rate of degradation and few human health concerns (EPA US 1999). Insecticide was applied using a backpack sprayer to all treatment subplots during the first week of June 2012. Paired control subplots were sprayed with an equivalent volume of water using the same backpack sprayer model.

In order to test the effectiveness of the insect suppression treatment, we measured leaf damage on fifteen plants per subplot. We used a randomized coordinate system to sample 15 *S. interior* individuals per subplot, tagged them for later identification, and then recorded the height of each plant, ranging from 12-150 cm. We measured leaf damage in late summer 2012 on all tagged plants in a nondestructive manner by randomly selecting two shoots per plant and estimating leaf damage on all leaves on selected shoots. We estimated leaf damage as a percentage of total leaf area affected by leaf mining and leaf chewing (leaf area missing, mined, or skeletonized) and averaged data within plants. Leaf mining on our experimental sites was characteristic of willow leaf blotch miner folivory,

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identified by the characteristic dark blotches left on leaf surfaces after willow leaf blotch miner larvae feed. Many *S. interior* plants were also infected by tar spot fungus (*Rhytisma acerinum*), so we opportunistically measured leaf area infected by tar spot fungus as well. All observers were trained and tested prior to data collection to ensure their visual estimations of leaf damage correlated with measurements made with image analysis software (R^2>0.90 for all observers, N>55 leaves: ImageJ software, National Institutes of Health).

2.3.3 Estimating stem biomass using stem diameters

In order to estimate willow stem production and overwinter mammalian browsing, we derived an allometric equation for the relationship between the diameter of winter dormant stems (n=71) and stem dry mass (Brown 1976, Kielland and Osborne 1998; Fig. 2.2). Stem samples were desiccated for 48-72 hours in a drying oven to constant mass and weighed to the nearest mg.

2.3.4 Stem production and mammalian herbivore browse measurements

In April and May 2013, we quantified stem production and the biomass of mammalian herbivore overwinter browsing. We estimated current annual stem production by counting the total number of new stems initiated in the previous year on six randomly-chosen plants on every subplot. For each plant, we haphazardly chose two branches and measured the diameter at the base of up to ten current annual growth stems per branch using calipers. If fewer than ten current annual stems were available, we measured stem diameter on additional shoots, up to ten total stems per plant. Stem biomass was estimated

from basal stem diameters using the allometric equation. Total biomass produced per plant was calculated by multiplying the average stem biomass by the number of current annual stems present.

To quantify mammalian herbivore overwinter browsing, in April and May 2013 we measured the diameter of stems that had been removed by mammalian herbivores at the point the stem was browsed on six randomly selected plants per unfenced subplot using calipers. All selected plants were a minimum 20 cm in height in order to collect from plants likely visible over the snowline during winter, and thus available to foraging moose. Stem diameters were measured on up to ten browsed stems per plant, and the total numbers of browsed stems per plant were counted. Using the same equation relating *S. interior* biomass to stem diameter, we estimated the amount of biomass removed distal to the point at which each stem was browsed. We calculated total biomass browsed by averaging estimated biomass of browsed stems within a plant and multiplying this value by the number of browsed stems present. For each plant, we calculated the proportion of new growth browsed by mammalian herbivores by dividing the mean browsed stem biomass by the mean produced stem biomass.

2.3.5 Chemical analyses

To assess the effects of invertebrate herbivory on the chemistry of *S. interior* dormant stems, we compared the chemical characteristics of dormant stems collected from insect suppression treatment and control subplots. We collected samples only from plants within mammal exclosures, in order to avoid confounding the effects of insect and mammalian herbivory. We harvested three current annual stems from each of five

randomly selected untagged plants per mammal exclosure subplot. After collection, samples were transported on ice to the University of Alaska Fairbanks and stored at -80^{0}C. Stem samples were lyophilized using a FreeZone Triad Freeze Dry System (Labconco, Kansas City, MO, USA) for 48-72 hours. Samples were pooled within plants and ground using a Wiley mill over a 40-mesh screen. Ground samples were stored at room temperature in airtight containers. C and N composition as percentages of dry mass were determined by analyzing duplicate samples of 0.1 grams of ground sample using a LECO 2000 CNS Analyser (LECO Instruments, St. Joseph, MI, USA).

To assess PPC of current annual stems, we used a modification of the protocol used by Robbins et al. (1987). Samples of 0.50 gram were placed in 20.0 mL of 50% concentration methanol for 5 minutes. After soaking, samples were sonicated for 15 minutes. Samples were re-sealed in airtight containers and allowed to incubate at room temperature for 30 minutes after sonication. After 30 minutes, 2.0 mL of sonicated solution was pipetted in 2.0 mL microcentrifuge tubes and centrifuged for 15 minutes at 5000 RPM. 35.0 µL of the supernatant was pipetted from the microcentrifuge tubes and combined with 140.0 µL of 5mg/mL BSA in 0.2 M acetic acid acetate buffer with 0.17 M NaCl in 96-well centrifugable microplates. Microplates with sample solution were then centrifuged for 10 minutes at 6000 RPM, after which 5.0 µL of the supernatant was pipetted from each well and combined with 250.0 µL of Bio-Rad Quick Start Bradford Protein Assay Reagent (Bio-Rad Laboratories, Hercules, CA, USA). The solution was vortexed for 30 seconds and incubated at room temperature for 6 minutes. Absorbance was read on the microplate reader at 590 nm using a blank of 250.0 µL of Bio-Rad Bradford Protein Assay Reagent and 5.0µL of 0.2 M acetic acid acetate buffer with 0.17 M NaCl. Standards were made at 0.25,

0.5, 1.0, 1.5, 2.0, 3.0, and 5.0 mg/mL BSA concentrations and the resulting standard curve used to calculate protein content of the samples. We calculated BSA precipitated by subtracting the soluble protein mass remaining in a well from the initial mass of BSA mass added to each well. PPC was calculated by dividing the mass of BSA precipitated in samples by the original mass of ground sample in each well, giving PPC in terms of mg BSA precipitated/g DM sample.

Crude protein (N*6.25%) was calculated using N concentrations found in C:N analysis. Total digestible protein was calculated using the following equation from Robbins et al. (1987): Z=-3.87+0.9283X-11.82Y, where

X=Crude protein (%DM), Y=PPC (μg/μl), Z=Digestible Protein (g/100g DM).

2.4 Statistical analysis

We used univariate ANOVA models to test the fixed effects of insect suppression and when relevant, mammalian exclosure, on *S. interior* leaf damage, stem production, stem chemistry, and overwinter mammal browsing. Models used a nested design in order to account for possible non-independence of samples collected from the same sites, plots, or subplots. Models including both mammalian exclosure and insect suppression as main effects included the random effects of site, plot nested within site, and subplot within plot within site. Models lacking the effect of mammalian exclosure included the random effects of site and subplot nested within site. Plant height was included as a covariate in models of stem production, mammal browsing, and proportion of produced current annual stems browsed by moose overwinter. Leaf damage data were square root transformed, and current annual and browsed stem numbers, diameters, maximum diameters per plant, and

total biomass per plant were log (x+1) transformed to fit model assumptions. Following

significant interactions between model effects, means were compared with Tukey HSD

tests. All analyses were conducted using JMP 9 software (SAS Institute, Cary, NC).

2.5 Results

2.5.1 Leaf damage

Total leaf area damaged (total surface area damaged by all forms of insect folivory),

was 3.0-fold greater on plants in control subplots than in pesticide sprayed subplots ($F_{1, 10.05}$ = 15.52, P = 0.0027; Fig. 2.3). Mammal exclusion did not significantly affect total leaf

area damaged ($F_{1, 4.657}$ = 5.0523, P = 0.0784). The interaction between mammal exclusion

and insect suppression treatments was not significant, suggesting that insect suppression

treatments affected plants similarly inside and outside of mammal exclosures ($F_{1, 10.04}$ =

3.38, P = 0.0959).

Leaf damage was further divided into its component sources (leaf mining,

skeletonization and leaf area removed, and fungal damage), and the effects of the

experimental treatments analyzed on each. Leaf mining, characteristic of *Micrurapteryx*

salicifoliella larval mining, was 5.2-fold greater on control subplots than on pesticide

sprayed subplots ($F_{1, 9356}$ = 30.9869, P = 0.0003; Fig. 2.4A), but mammal exclusion did not

significantly affect leaf mining ($F_{1, 5.132}$ = 0.0015, P = 0.9703). There was a marginally-

significant interaction between mammal exclusion and insect suppression on willow leaf

blotch miner damage ($F_{1, 9.554}$ = 4.7363, P = 0.0558; Fig. 2.4A), resulting from a stronger

effect of insect suppression treatment on unfenced plots.

The proportion of leaf area removed or skeletonized was significantly reduced by insect suppression, by 2.8-fold ($F_{1,\ 10.06}$ = 6.77, P = 0.0263; Fig. 2.4B), and by mammal exclusion, by 1.5-fold ($F_{1,\ 4.867}$ = 19.96, P = 0.0070). The interaction between mammal exclusion and insect suppression was not significant ($F_{1,\ 10.04}$ = 1.81, P = 0.2076).

Although fungal infection was not the intended target of chemical treatment, it did suppress leaf damage caused by tar spot fungus (Fig. 2.4C) by 1.8-fold ($F_{1,\ 10.11}$ = 11.05, P = 0.0076). Mammal exclusion did not significantly affect fungal infection damage ($F_{1,\ 5.032}$ = 0.01, P = 0.9077), nor was there a significant interaction between mammal exclusion and insect suppression ($F_{1,\ 10.11}$ = 0.89, P = 0.3672).

2.5.2 Protein precipitation capacity of stems

PPC of *S. interior* stems was on average 401 mg BSA/g DM (SE=15.68). PPC was not significantly affected by insect suppression ($F_{1,\ 5.054}$ = 0.04, P = 0.8523; Fig. 2.5), plant height ($F_{1,\ 52.04}$ = 1.52, P = 0.2225), nor their interaction ($F_{1,\ 51.53}$ = 0.02, P = 0.8892).

2.5.3 Chemical composition of stems

The concentration of stem C, N, C:N ratio, and digestible protein were not significantly affected by insect suppression, plant height, or the interaction between insect suppression and height (Table 2.2) Across treatments, *S. interior* stems had 49.03 %C (SE=0.09%) by mass, 1.11 %N (SE=0.03) by mass, and an average C:N ratio of 46.84 (SE=1.38). The combination of low stem N concentration and high PPC resulted in a negative average value for digestible protein (-2.19g/100g, SE=0.28g).

2.5.4 Production of current annual stems

Suppression of insect herbivory had a positive effect on stem biomass production. Plants on insect suppression subplots produced an average 1.6-fold greater stem biomass ($F_{1, 9.076}$ = 7.09, P = 0.0257; Fig. 2.6). Mammal exclusion did not significantly affect stem production ($F_{1, 4.911}$ = 0.49, P = 0.5146). As expected, larger plants exhibited greater annual production ($F_{1, 132.5}$ = 43.10, P < 0.0001). The interaction between plant height and insect suppression treatments was significant ($F_{1, 124.7}$ =4.51, P = 0.0356), due to a greater effect of insect suppression on stem production in small than large plants. There was no significant interaction between insect and mammal exclusion ($F_{1, 9.74}$ = 0.37, P=0.5568).

Stem production per plant was calculated using stem diameter and stem number. Stem diameter was positively affected by insect suppression, with an average 1.1-fold larger diameter on insect suppression subplots ($F_{1, 9.813}$ = 5.20, P = 0.0462; Fig 2.7), while stem number was not significantly affected by insect suppression ($F_{1, 8.659}$ = 0.62, P = 0.4530).

2.5.5 Overwinter browsing by moose

Moose browse offtake was 2.2-fold greater on plants in insect suppression subplots. However, this difference was not statistically significant ($F_{1, 4.814}$ = 3.14, P = 0.1387; Fig. 2.8). Mammal browsing was not significantly affected by plant height ($F_{1, 62.75}$ = 2.43, P = 0.1242), or the interaction between insect suppression and plant height ($F_{1, 62.94}$ = 0.43, P = 0.5134).

We also examined the proportion of new growth browsed by moose over winter. Moose browsed 1.6-fold more of the available current annual stems on insect suppression

treated subplots, though this was only marginally significant ($F_{1, 4.75}$ = 5.3295, P = 0.0718; Fig 2.9). Plant height did not significantly affect the proportion of current annual stem biomass browsed ($F_{1, 63.67}$ = 0.59, P = 0.4463), nor did the interaction between insect suppression and plant height ($F_{1, 58.98}$ = <0.0001, P = 0.9987).

2.6 Discussion

In this study we investigated the direct effects of insect folivory on *S. interior* stem production and chemistry, as well as trait-mediated indirect effects of insect folivory on mammalian herbivore overwinter browsing. We initially expected that insect folivory of *S. interior* would increase the nutritional quality of stems over winter, decrease stem production, reduce stem PPC, and proportionally increase subsequent mammalian overwinter browsing. Our findings indicate that summer insect folivory reduced *S. interior* stem production, but we did not find evidence that insect folivory altered *S. interior* stem chemical composition. We found some evidence suggesting that insect folivory of *S. interior* may alter mammal winter browsing, though these findings were less well supported than our other significant findings.

We originally anticipated that stem PPC would be lower in *S. interior* plants exposed to folivory than plants in insect suppression subplots, similar to how previous defoliation of *Populus tremuloides* resulted in lower winter stem tannin concentrations (Lindroth et al. 2007). However, we found no significant change in stem PPC in response to defoliation. Previous studies have suggested that there may be an 'all-or-none' induction response in willows, where a certain damage threshold must be reached for induction of chemical defenses to occur (Fields and Orians 2006). While even very relatively low levels of

herbivory can induce chemical defenses in some plant species, other species do not

respond to leaf damage by increasing chemical defenses until a certain threshold level of

damage is reached (Karban and Baldwin 1997). Not including tar-spot fungal damage,

which affected an average of 2.3% leaf area per plant (SE=1.1%), plants in control subplots

averaged 6.7% leaf area damaged (SE=1.8%). This degree of leaf damage is within the

range at which increased chemical defense concentrations were induced in plants in

previous studies (Karban and Baldwin 1997). Our results in this study, as well as those

presented in Chapter 1, suggest that in *S. interior,* condensed tannins are not induced by

defoliation.

We calculated extremely low, and often negative, digestible protein content in *S.*

interior winter stems (Mean=-2.2 g/100g DM, SE=0.28g, n=57). While we had anticipated

relatively low nutritional value from *S. interior* on the floodplain, due to a nutrient-poor,

early successional environment, a negative digestible protein value was unexpected. This is

likely due to the fact that *S. interior* in this study had lower average N concentration and

higher PPC than previously reported plant species whose digestible protein content was

analyzed using the equation we employed, including other willows (Robbins et al. 1987,

McArt et al. 2009), suggesting that the equation used to calculate digestible protein content

in this study is likely inappropriate for *S. interior.* In a study of the protein binding capacity

of tannins in mammal herbivore feed, deciduous plant stems collected in winter ranged

from 6-12% crude protein as a proportion of dry mass (Robbins et al. 1987), while *S.*

interior stems in our study had an average of 6.9% crude protein (SE=0.2%) by mass.

Additionally, in a study on nitrogen availability in moose diet, leaves and stem tips of two

other willow species were tested for PPC. PPC varied over time, but typically ranged

between 120-350 mg BSA precipitated per g DM (McArt et al. 2009). By comparison, in our study, the mean tannin PPC across all treatments was 401 mg BSA precipitated per g DM (SE=16 mg BSA/g DM).

The results indicate that insect folivory may affect mammalian herbivores feeding over winter by reducing the forage availability of shared host species. A 1.6-fold greater amount of stem biomass was produced by *S. interior* in response to a 3-fold reduction by insect folivory. Insect folivory damaged an average of 6.7% (SE=1.8%) leaf area in control plants, which suggests that stem production by *S. interior* can be affected by low levels of folivory. Other studies have reported reductions in plant annual production in response to levels of insect herbivory comparable to those in this study, though production was not reported in terms of stem biomass (Hjalten et al. 1994, Lindroth et al 2007, Ohgushi 2005, Schwenk and Strong 2011). Reduced willow stem production may have a significant ecological impact on mammalian herbivores (Belovsky 1981, Coltrane and Barboza 2010). In interior Alaska, *S. alaxensis*, *S. planifolia*, and *S. interior* are the most highly preferred willow forage species for moose (Milke 1969, Risenhoover 1989, Seaton 2002). If preferred willow species, such as *S. interior,* are negatively affected by insect herbivory, moose may have to browse less palatable or nutritious species.

Overall, our findings indicate that the low natural levels of insect folivory observed in this study tend to reduce the availability of current annual stems for mammalian herbivores overwinter, but at this level of folivory the nutritional quality of the stems were not affected. The trend of reduced stem production was associated with smaller stems which were browsed by moose at a marginally lower rate than those stems protected from insect folivory. The collective results of this study suggest that levels of insect folivory such

as those seen during this study negatively affect the quantity, but not nutritional quality, of

S. interior winter dormant stems.

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Table 2.1: Coordinates and percent ground cover of common plant species found on experimental sites. Species listed by code. Species codes are:
S. niph = Salix niphoclada, S. inte = S. interior, S. psmy = S. pseudomyrsinites, S. alax = S. alaxensis, P. bals = Populus balsamifera, A. tenu = Alnus tenuifolia.

|	**Site Coordinates**		**Mean % Ground Cover by Species**					
			S.	*S.*	*S.*	*S.*	*P.*	*A.*
Site	Latitude	Longitude	*inte*	*niph*	*psmy*	*alax*	*bals*	*tenu*
1	64 42.47° N	148 09.15° W	7.6	0.3	3.5	0.0	2.6	0.4
2	64 42.51° N	148 09.12° W	9.5	1.6	0.9	0.0	0.6	0.5
3	64 42.50° N	148 10.43° W	5.3	11.2	0.3	0.2	0.2	0.1
4	64 42.38° N	148 13.16° W	11.0	2.3	0.4	0.0	1.6	0.2
5	64 40.53° N	148 17.86° W	6.4	5.7	2.8	0.6	1.1	1.5
6	64 40.54° N	148 17.41° W	2.9	2.9	3.9	0.0	0.9	0.0

Table 2.2: Results of chemical analysis ANOVA models on stem chemistry. Models testing effect of insect suppression, plant height, and their interaction on *S. interior* stem chemistry and nutritional composition.

| Dependent Variable	Model Effect	d.f.	*F*-value	*P*-value
N Concentration	Insect Suppression	1, 5.006	2.1416	0.2032
	Plant Height	1, 49.58	1.0484	0.3108
	Insect Suppression*Height	1, 52.34	0.1516	0.6986
C Concentration	Insect Suppression	1, 3.899	6.3597	0.0668
	Plant Height	1, 33.31	0.0100	0.9211
	Insect Suppression*Height	1, 44.3	1.5734	0.2163
C:N Ratio	Insect Suppression	1, 5.044	1.5580	0.2668
	Plant Height	1, 51.15	0.2311	0.6328
	Insect Suppression*Height	1, 52.38	0.0504	0.8232
Digestible Protein	Insect Suppression	1, 4.958	1.4803	0.2785
	Plant Height	1, 49.3	0.0419	0.8387
	Insect Suppression*Height	1, 51.62	0.4064	0.5266
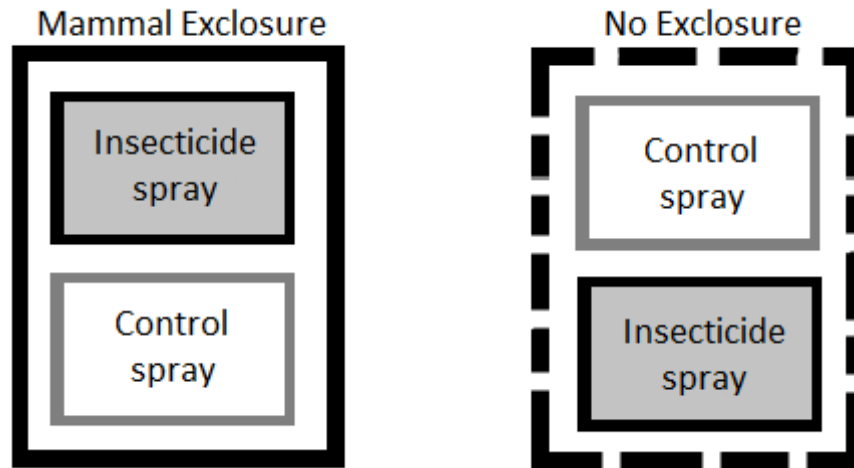
Figure 2.1: Sample experimental site setup. Experimental design, showing paired mammal exclosure and control plots, each containing paired subplots assigned to insect suppression treatment or control. Subplots are isolated from each other and from the plot edge by a 1 m boundary.
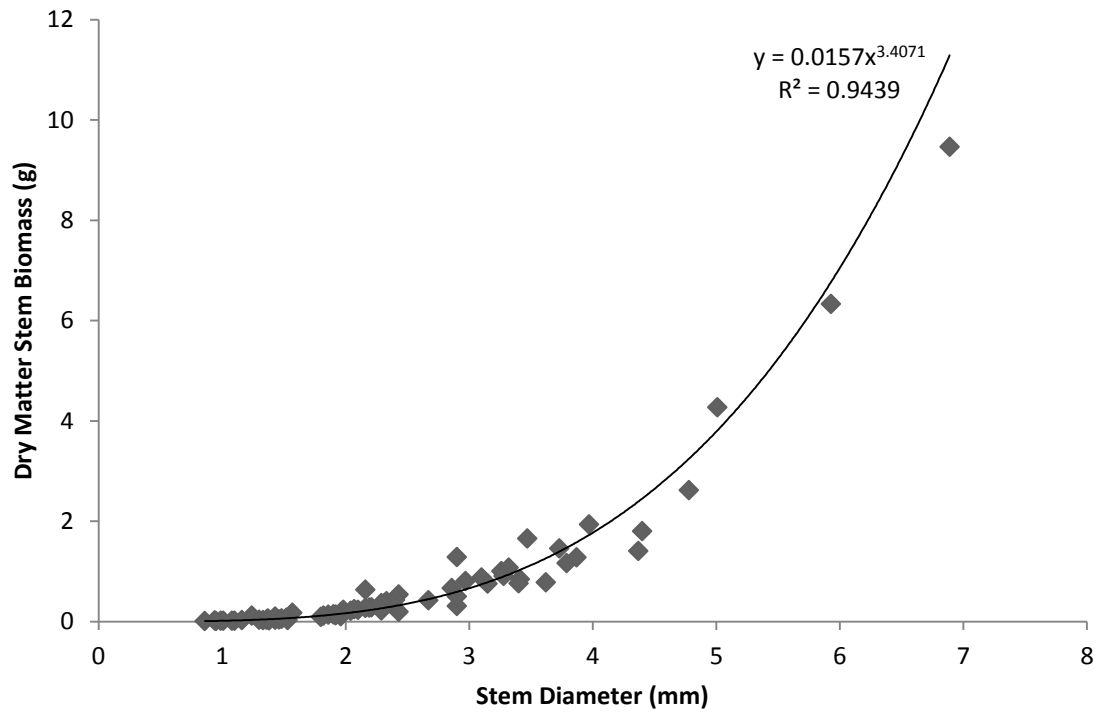
Figure 2.2: Allometric relationship between *S. interior* stem diameter and dry stem biomass.
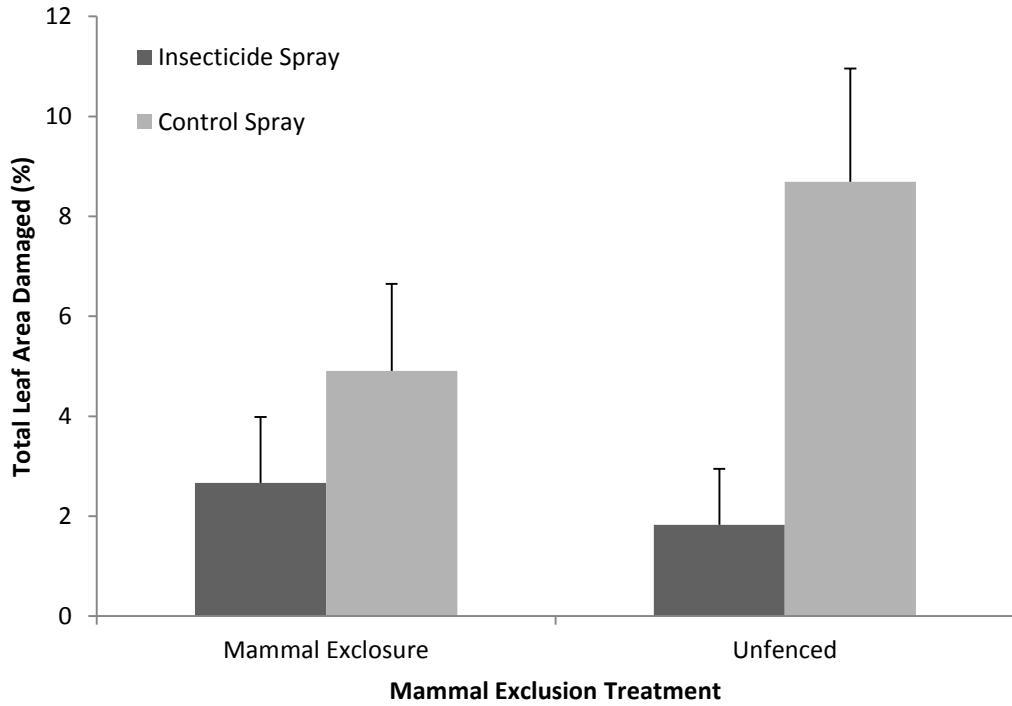
Figure 2.3: Total leaf area damaged by experimental treatments. Bars show the mean leaf area damaged by invertebrate herbivores within insect and mammal exclusion treatments in *S. interior*. Bar color indicates insect suppression treatment. Values are backtransformed least square means ± SE, n=140.
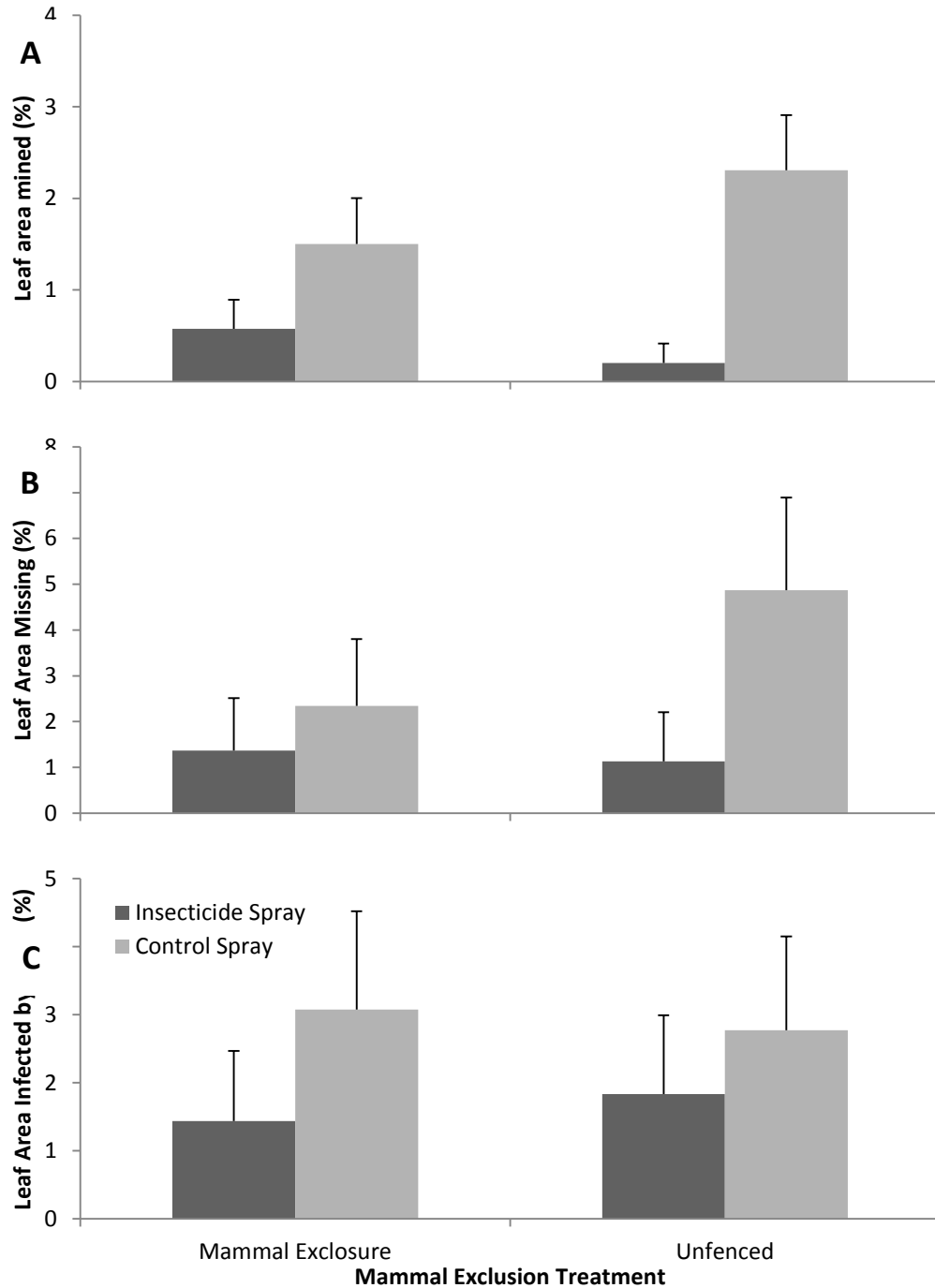
Figure 2.4: Effects of experimental treatments on leaf damage from measured sources. Graphs show the effects of mammal exclusion and insect suppression on *S. interior* leaf area A) mined, B) missing, and C) infected by tar spot fungus. Values are backtransformed least square means ±SE, n=57.
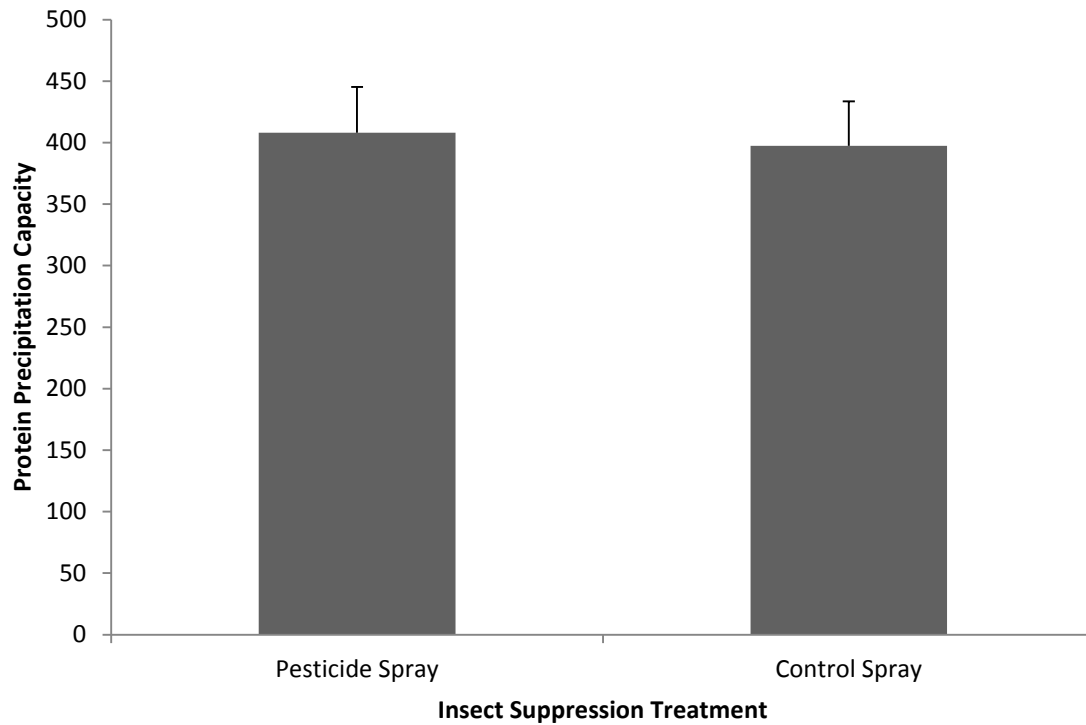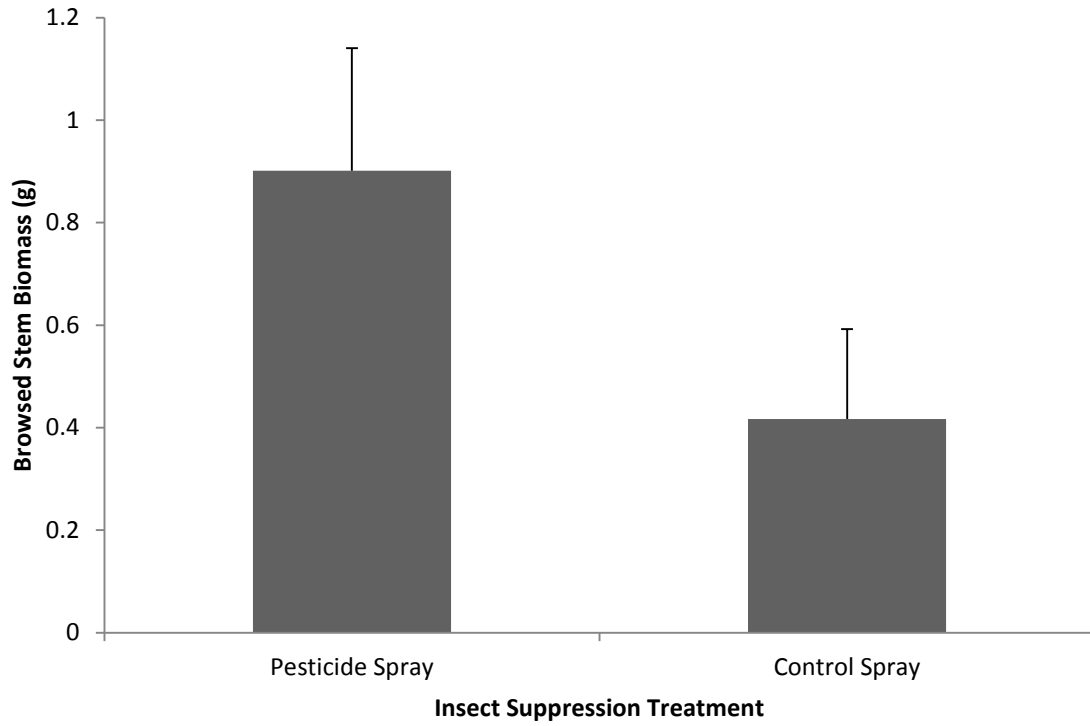
Figure 2.5: Effect of insect suppression on stem PPC (mg BSA precipitated/g DM). Values are least square means ± SE, n=57.
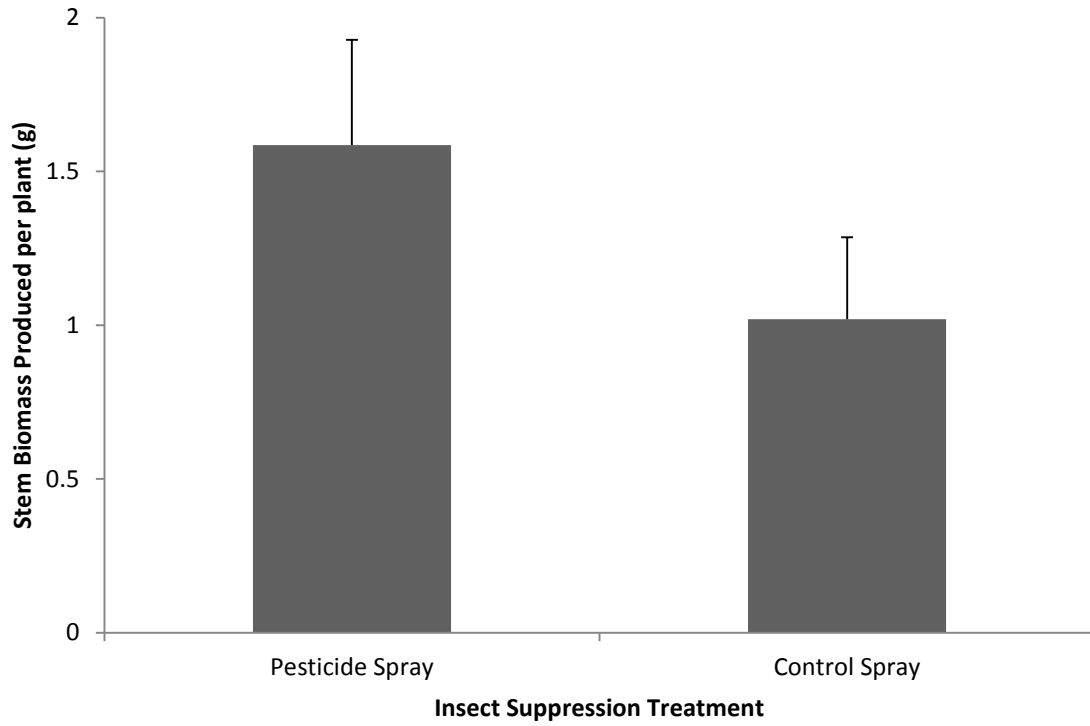
Figure 2.6: Effect of insect suppression treatment on mean stem biomass produced per plant. Values are backtransformed least square mean ± SE, n=143.
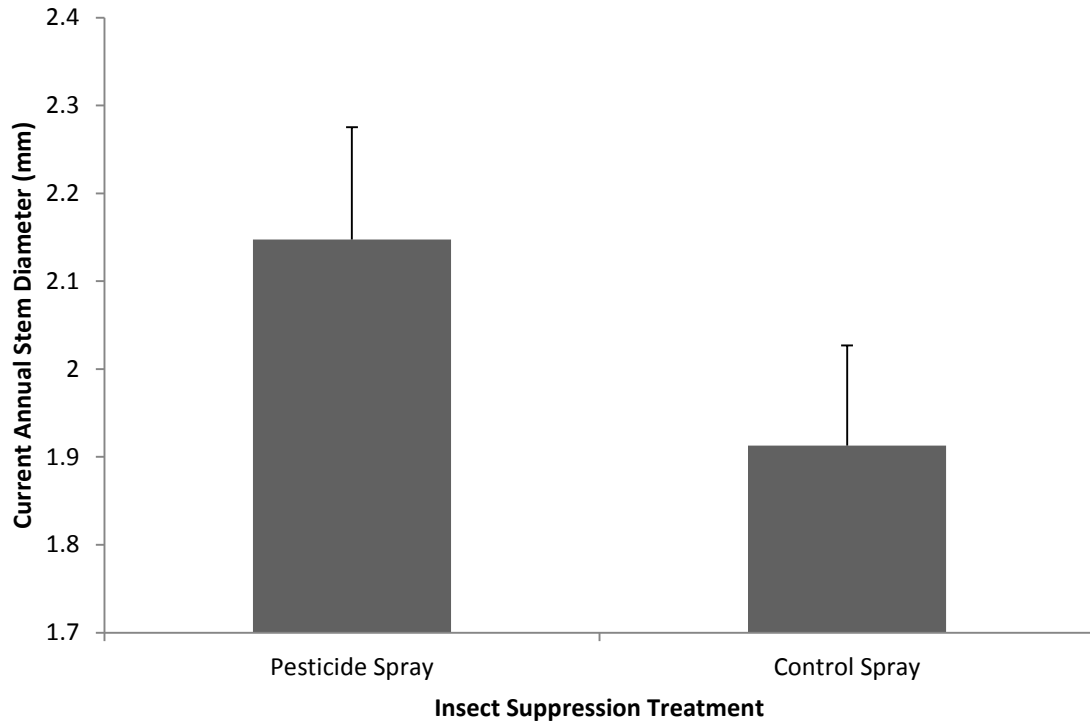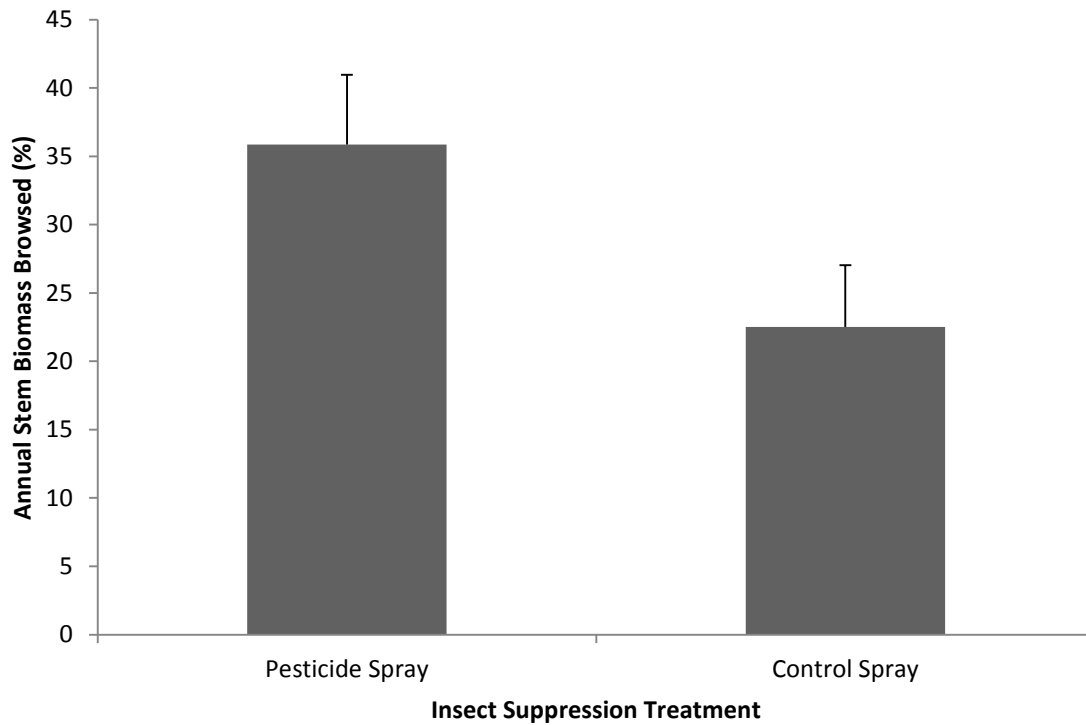
Figure 2.7: Effect of insect suppression on mean current annual stem diameter. Data are backtransformed least square means ± SE, n=140.

Figure 2.8: Effect of insect suppression on mean overwinter stem biomass browsed per plant. Data are backtransformed least square means ± SE, n=143.

Figure 2.9: Effect of insect suppression on proportion of current annual stems browsed by moose over winter. Data are backtransformed least square means ± SE, n=135.

Conclusion

In my studies, I found evidence that folivory may have indirect effects on mammal herbivores feeding overwinter in interior Alaska. Firstly, relatively low levels of defoliation reduce *S. interior* stem production, and secondly, heavy defoliation alters stem chemical composition. Similarly, previous work has found that temporally isolated herbivores can have indirect effects upon each other through herbivory-induced changes in plant characteristics (Ohgushi 2005, Schwenk and Strong 2011, Werner and Peacor 2003).

The defoliation experiment presented in chapter 1 provides evidence that defoliation of *S. interior* can alter the chemical composition of *S. interior* stems months later in winter. Manual defoliation of *S interior* significantly increased N concentration in stems (Fig 1.2A), and reduced stem C:N ratio (Fig 1.2E). In quaking aspen (*Populus tremuloides*), a mix of artificial defoliation and insect folivory also significantly increased winter stem nitrogen concentration (Lindroth et al. 2007). Conversely, in Chapter 2 I found no significant effect of defoliation on stem N concentration or C:N ratio (Table 2.2). However, leaf damage on plants in this study was much lower (by an order-of-magnitude) than the manual defoliation treatments described in Chapter 1. The levels of defoliation employed in the Chapter 1 experiment were typical of damage caused by insect outbreaks (FS-R10-FHP 2013, FS-R10-TP-123 2004). It is possible that higher levels of leaf damage resulted in a greater reduction in plant carbon fixation, which might explain the relatively higher N concentrations in current annual stems of more heavily defoliated plants in Chapter 1. These differing findings suggest that the severity of leaf damage can affect plant response to defoliation. In Chapter 2, insect suppression significantly increased stem production in terms of stem diameter (Fig 2.7) and consequently biomass (Fig 2.6). These results indicate

that even small amounts of insect folivory (<10% of leaf area damaged) can reduce *S. interior* stem production. These findings corroborate earlier work on the effect of willow leaf blotch miners on willow growth as measured by stem elongation (Wagner and Doak, unpublished). Previous research has similarly found that insect herbivory can reduce plant production in other species. In striped maple (*Acer pensylvanicum*), insect herbivory reduced both shoot growth and leaf number, even though insect herbivory removed only an average of 7% leaf area (Schwenk and Strong 2011). In white birch (*Betula pubescens*), defoliation significantly reduced plant growth (Hjalten et al. 1994). My findings indicate that *S. interior* stem production is significantly reduced by modest levels of insect herbivory.

Although I predicted lower PPC in damaged plants, my findings indicated a lack of tannin induction in response to either manual or insect defoliation. In both experiments, defoliation had no significant effect on stem PPC. By comparison, in quaking aspen defoliation decreased concentrations of both condensed tannins and phenolic glycosides in woody stems (Lindroth et al. 2007). However, in another study measuring the effects of leaf damage on wood chemical composition, simulated herbivory of white birch had no significant effect on total phenolic concentrations, which were used as a measure of chemical defense concentrations (Hjalten et al. 1994). Tannins are not universally inducible by insect herbivory, and vary by plant species or in response to environmental variables (Barbehenn and Constabel 2011).

Because defoliation both increases stem N concentration and decreases stem production, defoliation appears to have both positive and negative effects on the mammal winter forage. However, while defoliating 75% of the leaf area on *S. interior* in chapter 1

resulted in a 1.15-fold increase in stem nitrogen content, a 3-fold increase in insect defoliation in chapter 2 resulted in a 1.6-fold decrease in stem production. Thus, the negative effect of defoliation on stem production was relatively greater than the increase in stem nutritional quality, even in response to a lower average level of defoliation. This indicates that while insect folivory may improve the nutritional quality of forage, the overall effect on winter moose forage is likely negative.

In order to better understand the dynamics of the relationship between insect and mammal herbivores in interior Alaska, it may be necessary to study further indirect interactions, such as how moose feeding might also indirectly affect insect feeding. Previous research has shown that mammal browsing of stems during winter tissues can also affect insect feeding or success on the same plants the following year (Danell and Huss-Danell 1985, Ohgushi 2005, Schwenk and Strong 2011, Werner and Peacor 2003). Consequently, there may be a feedback effect of insect herbivory on mammal herbivory, where changes in mammal herbivory due to insect herbivory may then have secondary indirect effects on insect herbivores in subsequent seasons or years. Future work should incorporate possible responses and interactions between herbivores not only in the same year, but over multiple years, in order to better understand the interactive effects of herbivores feeding on the same plants.

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