The effect of an outbreak by the leaf miner *Micrurapteryx salicifoliella* on the performance of multiple *Salix* species in interior Alaska

Journal:	<i>Botany</i>
Manuscript ID	cjb-2018-0050.R1
Manuscript Type:	Article
Date Submitted by the Author:	20-Apr-2018
Complete List of Authors:	Wagner, Diane; University of Alaska Fairbanks, Institute of Arctic Biology and Department of Biology and Wildlife Doak, Patricia; University of Alaska Fairbanks, Institute of Arctic Biology and Department of Biology and Wildlife
Keyword:	<i>Salix arbusculoides</i> , <i>Salix bebbiana</i> , <i>Salix niphoclada</i> , <i>Salix pseudomyrsinites</i> , Herbivory
Is the invited manuscript for consideration in a Special Issue? :	N/A

SCHOLARONE™
Manuscripts

**The effect of outbreak by the leaf miner *Micrurapteryx salicifoliella* on the performance of multiple
Salix species in interior Alaska**

Diane Wagner (corresponding author)

Institute of Arctic Biology and Department of Biology and Wildlife, University of Alaska, Fairbanks, AK

99775; diane.wagner@alaska.edu, 907-474-5227

Patricia Doak

Institute of Arctic Biology and Department of Biology and Wildlife, University of Alaska, Fairbanks, AK

99775; pdoak@alaska.edu

Abstract

The genus *Salix* tends to be tolerant of herbivory, but severe outbreaks of herbivorous insects may compromise growth. First documented in Alaska in 1991, the willow leaf blotch miner, *Micrurapteryx salicifoliella*, is now responsible for frequent and widespread foliar damage to *Salix* in interior Alaska. We experimentally tested the effect of leaf mining on the performance of four susceptible *Salix* species across two years of outbreak, and placed the results in the context of a broader survey of leaf mining damage. Across the four species, reduction of leaf mining damage increased average stem elongation, numbers of leaves per shoot, and leaf area. Leaf mining damage was negatively related to leaf water content, a consequence of the leaf miner's habit of breaching the cuticle on the underside of leaves. Growth deficits due to leaf mining are likely caused by both the loss of leaf tissue to leaf miner feeding and subsequent leaf desiccation. The results are notable in the context of a general decline in productivity in interior Alaska over the past few decades, and because the leaf miner impacts *Salix* species also used by vertebrate browsers of cultural and economic importance, such as moose.

Key words: herbivory, *Salix arbusculoides*, *Salix bebbiana*, *Salix niphoclada*, *Salix pseudomyrsinites*

Introduction

Insect outbreaks have profound effects on North American forests (Volney and Fleming 2000; Logan et al. 2003), and the frequency and severity of outbreaks are predicted to increase as the climate warms (Gray 2008; Dukes et al. 2009; Bentz et al. 2010). Over the past three decades, temperatures at high latitude have increased more rapidly than the global average (IPCC 2013), and boreal forest of interior Alaska has undergone a decades-long decline in productivity as indicated by satellite observations of photosynthetic activity (Beck and Goetz 2011; Verbyla 2015). While the Alaskan browning trend coincides with a warmer, drier growing season climate, insect damage is a possible contributor (Goetz et al. 2005; Verbyla 2008; Verbyla and Parent 2010).

The genus *Salix* is common and ecologically important in interior Alaska. Common on floodplains and disturbed sites, *Salix* is the major source of forage for browsers in Alaska, including moose, snowshoe hare, and ptarmigan. During autumn and winter, willow stems are a critically important and preferred food source for mammalian browsers (Peek 1974; Bryant and Kuropat 1980). In recent decades, many species of *Salix* have been subject to periods of severe leaf damage by *Micrurapteryx salicifoliella* (Chambers 1872), a gracilliarid leaf miner native to North America. Though previously recorded as far north as the Northwest Territories of Canada, *M. salicifoliella* was only first documented in Alaska in 1991 (USFS 1992). Since that time, estimates of damage based on aerial fly-overs have recorded the spread of *M. salicifoliella* throughout the Alaskan interior, with particularly high areas of infestation reported approximately every 5 - 6 years (USDA Forest Service 1992; 1999; 2004; 2010; 2016). At its peak in 2010, total area infested by *M. salicifoliella* exceeded 225,000 ha (USFS 2010). The leaf miner attacks at least ten of the 29 species of *Salix* that are broadly sympatric within interior Alaska (Furniss et al. 2001; Collet 2004). Though insect herbivory is rarely considered for its effect on vertebrate forage, it could impact the quantity of tissue available to browsers through negative effects on survivorship, photosynthesis, and stem production (Osier and Lindroth 2004; der Herder et al. 2009;

Schwenk and Strong 2011; Wagner and Doak 2013). However, some *Salix* species can compensate for even high levels of tissue loss (Shen and Bach 1997; Guillet and Bergstrom 2006) by drawing on carbohydrate reserves and increasing carbon fixation by undamaged tissues (Bryant et al. 1983; Houle and Simard 1996; Amichev et al. 2014).

The motivation for this study was the need to better understand the impact of *M. salicifoliella* outbreaks on *Salix* growth in interior Alaska. We surveyed leaf damage across nine *Salix* species and experimentally tested the effect of leaf mining by *M. salicifoliella* on the growth and leaf characteristics of four susceptible *Salix* species.

Methods

Natural history of the herbivore

M. salicifoliella is univoltine. In June, females deposit eggs singly on the abaxial (bottom) side of the leaves of susceptible species. The first and second larval instars mine the epidermis, and the third through fifth instars feed on the mesophyll, hollowing the internal spaces between leaf veins and leaving a discolored blotch on the adaxial (top) surface. The later-instar larvae also exit mines, move along shoots and branches, and initiate new mines within progressively younger leaves that they enter by cutting slits in the abaxial surface. As a result of this larval mobility, many more leaves receive mining damage than receive eggs. In addition to damage caused directly by feeding, exit and entry holes breach the integrity of the epidermis and thereby could increase foliar water loss. Larvae pupate under silken pads spun on the outer surface of leaves.

Survey of leaf damage

We quantified natural levels of leaf mining damage on nine *Salix* species during two consecutive outbreak years at sites in the Fairbanks area (1 – 4 sites per willow species; Tables S1 and S2). In mid-July of 2010 and 2011, when most leaf miners had completed feeding, we collected one shoot from each of six ramets per species and site (274 shoots and ramets; 4231 leaves total). Shoots were sealed in plastic bags and refrigerated until processed. The position of each leaf along the shoot was noted, with position 1 being proximal to the main stem. For each leaf, we quantified leaf mining damage by visually estimating the percentage of the top leaf surface area that was discolored by a blotch mine. Observers were tested prior to collecting data by comparing their visual estimates of leaf mining damage (3 *Salix* species, 20-30 leaves per species) to measurements of the same leaves made using image analysis software (Image J, National Institutes of Health, Bethesda, Maryland). Visual estimates of damage were strongly related to measurements made using image analysis ($R^2 \geq 0.95$ for all observers), and there were no systematic differences in damage estimates among observers ($P > 0.9$).

Experimental reduction of leaf mining damage

In order to measure the effects of leaf miners and other insect herbivores on plant performance, we experimentally reduced insect feeding damage on four *Salix* species across two growing seasons. We chose focal species that, based on our observations in previous years, had a range of susceptibility to *M. salicifoliella*. In May 2010, we tagged 40 - 80 ramets per species (*S. arbusculoides*, *S. bebbiana*, *S. niphoclada*, and *S. pseudomyrsinites*) across 1 -2 study sites per species (total $n = 250$ ramets; Table S1). Ramets ranged in height from 0.5 to 3.0 m. Focal ramets were randomly assigned to treatment and control for the duration of the experiment. On one occasion in early June of both 2010 and 2011, we applied the insecticide spinosad (Conserve SC, EPA registration number 62719-291; Dow AgroSciences, Indianapolis, Ind.; 1.56 ml l^{-1} , plants sprayed to runoff) to the treatment ramets and an equivalent

amount of water to the controls. Insecticide application occurred when *M. salicifoliella* oviposition had slowed but larvae were still in early instars.

In late July of each year, after *M. salicifoliella* larvae had ceased to feed, we assessed leaf damage and leaf area on focal plants in a nondestructive manner. For every leaf on 2-3, haphazardly-chosen shoots per ramet, we noted leaf position and visually estimated the percentage of leaf area that was mined and missing. Observers were tested to ensure accuracy of their estimates, as described previously. Leaf length and width (at the widest point) were measured to the nearest mm. Leaf area was calculated from species-specific predictive equations generated from a separate set of leaves. To generate the predictive equation for each species, we digitally scanned 20-30 leaves, measured leaf area, width, and length using image processing software (Image J, National Institutes of Health), and regressed leaf area against leaf width, length, and the product of width and length ($R^2 \geq 0.98$ in all cases). The estimate of leaf area produced from our field measurements represents the potential area available to capture light energy, prior to any tissue removal by herbivores. In August of each year, when growth had largely ceased, we measured the average length of the three longest shoots per ramet.

In order to assess the effect of leaf mining by *M. salicifoliella* on leaf moisture, we measured the moisture content of leaf samples from experimental and control ramets of the species *S. arbusculoides* and *S. bebbiana* from a single study site ($n = 80$ ramets total). A single shoot was collected from each ramet during early September, 2010. Leaves were assessed for mining damage as described above. All leaves within a shoot were pooled and weighed to the nearest 0.01 g, dried to constant weight at 60° C, then reweighed; leaf moisture content was calculated as the difference between fresh and dry mass divided by fresh mass ($\times 100$).

Mortality and dieback were monitored during the course of making other measurements but were not an intended focus of this study, which was too short in duration and too small in scope for a robust test of herbivore-induced mortality.

109 **Data analysis**

110 For both the survey and experimental data sets, data were analyzed using general linear mixed
111 models. The goal of the survey data was to provide context for the experimental work by quantifying the
112 amount of leaf mining damage received by a wide range of *Salix* species during the two years of the
113 study. To this end, the percentage of leaf area mined was modeled with the fixed effects of *Salix* species,
114 year, and their interaction, the covariate leaf position, and the random effects of site and individual
115 ramet nested within species and year. The goal of the experimental study was to assess the cost of leaf
116 damage at naturally-occurring, relative to artificially-reduced, levels across the two-year span of the
117 study. Three dependent variables were analyzed separately: leaf area, leaves per shoot, and the average
118 length of the three longest shoots. The statistical model for leaf area included the fixed effects of
119 treatment, *Salix* species, and their interaction, the covariate leaf position, and the random effects of
120 year, site, shoot nested within ramet, and ramet nested within treatment and species. Models used to
121 analyze leaves per shoot and average length of the three longest shoots were similar but lacked leaf
122 position and shoot-within-ramet effects. To assess the impact of the experimental treatment on leaf
123 mining and feeding by non-target herbivores, we analyzed the dependent variables percentage leaf area
124 mined and percentage of leaf area removed by externally-feeding herbivores, respectively. These
125 variables were analyzed using the same model structure as leaf area. When relevant, means were
126 compared using Tukey HSD tests.

127 To assess whether leaf moisture was negatively related to leaf mining damage, we modeled the
128 average percentage leaf water as a function of average percentage leaf mining, *Salix* species, and their
129 interaction. Unlike the models described previously, the effect of treatment was not included in this
130 analysis.

Results

Survey of leaf damage

All leaf mining damage observed in the survey was characteristic of *M. salicifoliella*, and no other internal feeders were detected. *Salix* species differed strongly in their susceptibility to mining by *M. salicifoliella* during two years of population outbreak ($F_{[8,243]} = 20.07$, $P < 0.0001$), with *S. alaxensis* sustaining very little damage and *S. pulchra* and *S. pseudomonticola* sustaining >50% leaf mining damage on average across years (Fig. 1A). All four species on which we focused our experimental manipulation were susceptible to attack by the leaf miner and these species did not differ significantly from one another in average mining damage during the survey (Tukey HSD, all $P > 0.05$). On average, mining damage was higher in 2010 than 2011 ($F_{[1,265]} = 32.04$, $P < 0.0001$), but the magnitude by which the mining declined across the two years differed among species (Fig. 1), resulting in a significant year-by-species interaction ($F_{[8,267.7]} = 3.19$, $P = 0.0008$). Average leaf mining declined from proximal to distal positions along the shoot ($F_{[1,4266]} = 333.33$, $P < 0.0001$).

At the time of the survey in late July, the percentage of leaf area damaged by externally-feeding herbivores was an order of magnitude lower than that caused by leaf miners (Fig. 1). *Salix* species differed in the percentage of leaf area lost to external feeders, with *S. pseudomonticola* sustaining the highest average level of damage and *S. arbusculoides*, *S. bebbiana*, and *S. lasiandra* sustaining the least (Fig 1B). Averaging within species, the percentage leaf damage by miners and external chewers was weakly positively correlated ($r = 0.34$, $n = 9$, $P = 0.37$).

Experimental reduction of leaf mining damage

Experimental treatment successfully reduced herbivory, and was considerably more effective at reducing damage caused by leaf miners than external feeders. On average, leaf mining damage was 7.8 times lower on ramets treated with insecticide ($5.2\% \pm 0.4$ SE, $n = 124$ ramets) than controls ($24.1\% \pm$

1.2 SE, $n = 127$ ramets) (Fig. 2; $F_{[1,241]} = 204.3$, $P < 0.0001$). The four *Salix* species did not differ in average percentage of leaf area mined ($F_{[3,7]} = 3.48$, $P = 0.08$). Treatment significantly reduced leaf mining for all four species (Tukey's HSD, $P < 0.05$), though differences in the magnitude of the reduction among species led to a significant species-by-treatment interaction (Fig. 2; $F_{[3,236]} = 5.44$, $P < 0.001$). As a percentage of leaf area affected, externally-feeding insect herbivores caused less damage than leaf miners overall (Fig. 2). Insecticide treatment did not significantly reduce the average percentage of leaf area removed by external feeders ($F_{1,246} = 2.92$, $P = 0.09$) and did not interact with species ($F_{[3,243]} = 2.10$, $P = 0.10$). Species did not differ significantly in percentage leaf area missing ($F_{[3,9]} = 3.40$, $P = 0.07$).

Leaf water content declined as leaf mining damage increased (Fig. 4; $F_{[1,68]} = 95.45$, $P < 0.0001$). Relative to *S. arbusculoides*, the leaves of *S. bebbiana* contained less water on average (ARBU: 60.2 ± 1.1 SE, $n = 35$; BEBB: $55.1\% \pm 1.4$, $n = 38$) and the relationship between leaf water content and leaf mining was more strongly negative (ARBU: $\beta = -0.20 \pm 0.04$ SE; BEBB: $\beta = -0.32 \pm 0.04$; interaction $F_{[1,68]} = 5.00$, $P < 0.03$); however the negative relationship between mining and water content was statistically significant for both species ($P < 0.05$).

Plant performance responded positively to insecticide treatment. Insecticide treatment increased average leaf area by 11%, average number of leaves per shoot by 8%, and the average length of the three longest shoots by 22%, relative to controls (Fig. 3, Table 1). There were no statistically significant interactions between treatment and species for any of these variables (Table 1). Ramet mortality during the two years of the experiment was low. Across all 249 focal ramets, only 8 (3.2%) of ramets died (6 controls and 2 treated).

Discussion

In this study, we demonstrate that natural levels of damage sustained during an outbreak of the leaf miner *M. salicifoliella* reduced the performance of four susceptible species of *Salix*. Willows exposed to ambient levels of herbivory were not more likely to die during the two-year experiment, but on

average produced shorter shoots with fewer, smaller leaves relative to plants treated with insecticide. Although both internal and external feeding has the potential to impact plant performance, two lines of evidence suggest that, in this case, the growth differences between the insecticide-treated and control plants were more strongly influenced by leaf miners than by externally-feeding herbivores. First, on control plants subject to ambient levels of herbivory, the magnitude of damage caused by leaf miners greatly exceeded that caused by externally-feeding insects. A similar pattern was detected in the survey of natural leaf damage across *Salix* species. Second, the response to insecticide treatment was strong and statistically significant for leaf mining but weak and marginally non-significant for external leaf feeding.

Unlike some leaf mining species that confine feeding to non-photosynthetic tissues (Hering 1951), in their later instars *M. salicifoliella* consume the mesophyll, virtually hollowing the leaf. Moreover, slits that late-instar *M. salicifoliella* larvae make in the lower leaf surface when they initiate new mines likely increase evaporative water loss from leaves, further disrupting leaf physiology. Though *M. salicifoliella*'s effects on plant physiology have not been studied in detail, based on the insect's feeding behavior we expect strong negative impacts on the photosynthetic capacity of damaged leaves.

In this study, *Salix* leaf size responded positively to insecticide treatment and the associated decrease in mining damage. A similar effect was reported previously for *Populus tremuloides* treated to reduce leaf mining by *Phyllocnistis populiella* (Wagner and Doak 2013). Mining damage may result in a reduction in leaf expansion due to reduced photosynthesis, possibly exacerbated by the competing demands of mounting an induced defense (Dale 1988; Moore et al. 2003; Ruuhola and Julkunen-Tiitto 2003). Alternatively, smaller leaf area might be a response to water loss from mined leaves, reflecting a reduction in turgidity or, as has been documented in *Poplar* subject to water limitation, a reduction in cell wall extensibility (van Volkenburgh and Taylor 1996). In either case, reduction in leaf area decreases the potential for light capture.

The species we studied experimentally were of moderate-to-high susceptibility to *M. salicifoliella*, relative to the full 9 species surveyed. Across the nine species of *Salix* surveyed, variation in susceptibility to the *M. salicifoliella* was extreme, ranging from an average of <5% of leaf area mined for *S. alaxensis* to >60% for *S. pseudomonticola*. Species-specific differences in the susceptibility of Alaskan *Salix* to *M. salicifoliella* are related to differences in the expression of physical defense. Across species, the density of *M. salicifoliella* eggs on leaves is negatively related to foliar trichome density, likely because trichomes prevent the close contact between egg and leaf epidermis necessary for larvae to transition successfully from egg to leaf interior (Wagner and Doak 2017). In contrast, differences in egg and leaf damage among species are not well explained by chemical defense (Wagner and Doak 2017).

Several of the *Salix* species heavily impacted by *M. salicifoliella* are also important resources for mammalian browsers such as moose and snowshoe hare. Of the four focal *Salix* species in the experimental portion of this study, three (*arbusculoides*, *bebbiana*, and *pseudomyrsinites*) are important sources of browse for moose in interior Alaska (Wolff 1976; Oldemeyer et al. 1977; Bryant and Kuropat 1980; Rissenhover 1989; Seaton 2002). Our results suggest that repeated outbreaks of *M. salicifoliella* have the potential to impact the food supply of browsers highly valued within Alaska's subsistence culture and economy (Loring and Gerlack 2009; Titus et al. 2009).

The boreal forest of Alaska is undergoing rapid change due to climate warming and associated changes in species' ranges (Johnstone et al. 2010; Sanderson et al. 2012). *M. salicifoliella* was first recorded as an agent of disturbance to the Alaskan landscape less than three decades ago; since that time, outbreaks have become widespread and recursive. Our results indicate that leaf mining damage by *M. salicifoliella* causes a decline in productivity across multiple susceptible *Salix* species, which together extend across interior Alaska (Collett 2004). Outbreaks such as that of *M. salicifoliella* may contribute to a general decline in productivity observed within the Alaskan boreal forest (Trugman et al. 2018).

Acknowledgements

This study was funded by USDA Forest Service Alaska Region State and Private Forestry grant #2010-DG-11100100-206 to DW and PD. The authors thank Brian Allman, Tanis Bourke, Amy Breen, Melody Durrett, Brett Parks, and Keane Richards for assistance with data collection, and the Fairbanks International Airport for land access.

References

- Amichev, B.Y., Hangs, R.D., Konecsni, S.M., Stadnyk, C.N., Volk, T.A., Bélanger, N., Vujanovic, V., Schoenau, J.J., Moukoumi, J., and Van Rees, K.C.J. 2014. Willow Short-Rotation Production Systems in Canada and Northern United States: A Review. *Soil Sci. Soc. Am. J.* **78**(S1): S168-S182. doi: 10.2136/sssaj2013.08.0368nafsc.
- Beck, P.S.A., and Goetz, S.J. 2011. Satellite observations of high northern latitude vegetation productivity changes between 1982 and 2008: ecological variability and regional differences. *Environ. Res. Lett.* **6**(4): 029501. doi 10.1088/1748-9326/7/2/029501.
- Bentz, B.J., Régnière, J., Fettig, C.J., Hansen, E.M., Hayes, J.L., Hicke, J.A., Kelsey, R.G., Negrón, J.F., and Seybold, S.J. 2010. Climate change and bark beetles of the Western United States and Canada: direct and indirect effects. *BioScience*, **60**(8): 602-613. doi: 10.1525/bio.2010.60.8.6
- Bryant, J.P., Chapin III, F.S., and Klein, D.R. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos*, **40**(3): 357-368.
- Bryant, J.P., and Kuropat, P.J. 1980. Selection of winter forage by subarctic browsing vertebrates: the role of plant chemistry. *Annu. Rev. Ecol. Syst.* **11**: 261-285.
- Collett, D.M. 2004. Willows of interior Alaska, US Fish and Wildlife Service, Yukon Flats National Wildlife Refuge, Fairbanks, AK, USA.
- Dale, J.E. 1988. The control of leaf expansion *Annu. Rev. Plant Phys. Plant Mol. Biol.* **39**: 267-295. Doi

- 10.1146/annurev.pp.39.060188.001411.
- der Herder, M., Bergstrom, R., Niemela, P., Danell, K., and Lindgren, M. 2009. Effects of natural winter browsing and simulated summer browsing by moose on growth and shoot biomass of birch and its associated invertebrate fauna. *Ann. Zool. Fenn.* **46**(1): 63-74. doi 10.5735/086.046.0107.
- Dukes, J.S., Pontius, J., Orwig, D., Garnas, J.R., Rodgers, V.L., Brazee, N., Cooke, B., Theoharides, K.A., Stange, E.E., Harrington, R., Ehrenfeld, J., Gurevitch, J., Lerdau, M., Stinson, K., Wick, R., and Ayres, M. 2009. Responses of insect pests, pathogens, and invasive plant species to climate change in the forests of northeastern North America: what can we predict? *Can. J. For. Res.* **39**(2): 231-248. doi 10.1139/X08-171.
- Furniss, M.M., Holsten, E.J., Foote, M.J., and Bertram, M. 2001. Biology of a willow leafblotch miner, *Micrurapteryx salicifoliella*, (Lepidoptera: Gracillariidae) in Alaska. *Environ. Entomol.* **30**(4): 736-741. doi 10.1603/0046-225X-30.4.736.
- Gray, D.R. 2008. The relationship between climate and outbreak characteristics of the spruce budworm in eastern Canada. *Clim. Change*, **87**(3-4): 361-383. doi 10.1007/s10584-008-9478-x.
- Hering, M. 1951. Biology of the leaf miners Dr. W. Junk, The Hague, Netherlands.
- Houle, G., and Simard, G. 1996. Additive effects of genotype, nutrient availability and type of tissue damage on the compensatory response of *Salix planifolia* ssp. *planifolia* to simulated herbivory. *Oecologia*, **107**(3): 373-378. doi 10.1007/bf00328454.
- IPCC. 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. *Edited by* T. F. Stocker et al. Cambridge Univ. Press, Cambridge, UK.
- Johnstone, J.F., Hollingsworth, T.N., Chapin, F.S., and Mack, M.C. 2010. Changes in fire regime break the legacy lock on successional trajectories in Alaskan boreal forest. *Global Change Biol.* **16**(4): 1281-1295. doi 10.1111/j.1365-2486.2009.02051.x.

- 274 Logan, J.A., Regniere, J., and Powell, J.A. 2003. Assessing the impacts of global warming on forest pest dynamics.
275 Front. Ecol. Environ. **1**(3): 130-137. doi 10.1890/1540-9295(2003)001[0130:ATIOGW]2.0.CO;2.
- 276 Loring, P.A., and Gerlach, S.C. 2009. Food, culture, and human health in Alaska: an integrative health approach
277 to food security. Environ. Sci Policy, **12**(4): 466-478. doi 10.1016/j.envsci.2008.10.006.
- 278 Moore, J.P., Taylor, J.E., Paul, N.D., and Whittaker, J.B. 2003. Reduced leaf expansion as a cost of systemic
279 induced resistance to herbivory. Func. Ecol. **17**(1): 75-81. doi 10.1046/j.1365-2435.2003.00708.x.
- 280 Oldemeyer, J.L., Franzmann, A.W., Brundage, A.L., Arneson, P.D., and Flynn, A. 1977. Browse quality and the
281 Kenai moose population. J. Wildl. Manag. **41**(3): 533-542.
- 282 Osier, T.L., and Lindroth, R.L. 2004. Long-term effects of defoliation on quaking aspen in relation to genotype
283 and nutrient availability: plant growth, phytochemistry and insect performance. Oecologia, **139**(1): 55-
284 65.
- 285 Parent, M.B., and Verbyla, D. 2010. The browning of Alaska's boreal forest. Remote Sens. **2**(12): 2729-
286 2747. doi 10.3390/rs2122729.
- 287 Peek, J.M. 1974. A review of moose food habits studies in North America. Nat. Can. **101**: 195-215.
- 288 Risenhoover, K.L. (1989) Composition and Quality of Moose Winter Diets in Interior Alaska. J. Wildl. Manag.
289 **53**(3): 568-577.
- 290 Ruuhola, T., and Julkunen-Tiitto, R. 2003. Trade-off Between synthesis of salicylates and growth of
291 micropropagated *Salix pentandra*. J. Chem. Ecol. **29**(7): 1565-1588. doi:10.1023/a:1024266612585.
- 292 Sanderson, L.A., McLaughlin, J.A., and Antunes, P.M. 2012. The last great forest: a review of the status of
293 invasive species in the North American boreal forest. Forestry, **85**(3): 329-340. doi
294 10.1093/forestry/cps033.
- 295 Schwenk, W.S., and Strong, A.M. 2011. Contrasting patterns and combined effects of moose and insect
296 herbivory on striped maple (*Acer pensylvanicum*). Basic Appl. Ecol. **12**(1): 64-71. doi
297 10.1016/j.baae.2010.10.002.

- 298 Seaton, C.T. 2002. Winter foraging ecology of moose in the Tanana flats and Alaska range foothills. M.S. thesis,
299 University of Alaska, Fairbanks.
- 300 Shen, C., and Bach, C. 1997. Genetic variation in resistance and tolerance to insect herbivory in *Salix cordata*.
301 Ecol. Entomol. **22**(3): 335-342. doi 10.1046/j.1365-2311.1997.00078.x.
- 302 Titus, K., Haynes, T.L., and Paragi, T.F. 2009.. The importance of moose, caribou, deer, and small game in
303 the diets of Alaskans. *In* Ingestion of Lead from Spent Ammunition: Implications for Wildlife and
304 Humans. *Edited by* R. T. Watson, M. Fuller, M. Pokras, and G. Hunt. The Peregrine Fund, Boise,
305 Idaho. pp 1-7. doi 10.4080/ilsa.2009.0312.
- 306 USDA Forest Service, Alaska Region. 1992. Forest health conditions in Alaska - 1992. R10-TP-40. Anchorage,
307 Alaska.
- 308 USDA Forest Service, Alaska Region. 1999. Forest health conditions in Alaska - 1999. R10-TP-82. Anchorage,
309 Alaska.
- 310 USDA Forest Service, Alaska Region. 2005. Forest health conditions in Alaska - 2005. R10-PR-3. Anchorage,
311 Alaska.
- 312 USDA Forest Service, Alaska Region. 2010. Forest health conditions in Alaska - 2010. R10-PR-23. Anchorage,
313 Alaska.
- 314 USDA Forest Service, Alaska Region. 2016. Forest health conditions in Alaska - 2016. R10-PR-39. Anchorage,
315 Alaska.
- 316 Van Volkenburgh, E., and Taylor, G. 1996. Leaf growth physiology. *In* Biology of Populus and Its Implications for
317 Management and Conservation. *Edited by* R. F. Stettler, H. D. J. Bradshaw, P. E. Heilman, and T. M.
318 Hinckley. NRC-CNRC, Ottawa, Canada, pp. 283-299.
- 319 Verbyla, D. 2008. The greening and browning of Alaska based on 1982–2003 satellite data. Glob. Ecol. Biogeogr.
320 **17**(4): 547-555. doi 10.1111/j.1466-8238.2008.00396.x.
- 321 Wagner, D., and Doak, P. 2013. Long term impact of a leaf miner outbreak on the performance of quaking

- 322 aspen. Can. J. For. Res. **43**(6): 563-568. doi 10.1139/cjfr-2012-0486.
- 323 Wagner, D., and Doak, P. 2017. Oviposition, larval survival and leaf damage by the willow leaf blotch miner,
324 *Micrurapteryx salicifoliella*, in relation to leaf trichomes across 10 Salix species. Ecol. Entomol. **42**(5):
325 629-635. doi 10.1111/een.12431.
- 326 Wolff, J.O. 1976. Utilization of hardwood browse by moose on the Tanana floodplain of interior Alaska.
327 Research Note PNW-267. USDA Forest Service, PNW Forest and Range Experiment Stations, Portland,
328 OR.

Draft

Table 1. Linear mixed model analysis of the effects of insecticide treatment on average levels of (a) leaf area, (b) leaves per shoot, and (c) length of the three longest shoots. Statistically significant p-values appear in bold font.

Dependent variable	Source	d.f.	<i>F</i>	<i>P</i>
a. Leaf area	Treatment	1, 238.6	4.34	0.04
	Species	3, 10.95	16.44	0.0002
	Treatment*Species	3, 239.8	0.42	0.73
	Leaf position	1, 1177.8	265.42	<0.0001
b. Leaves per shoot	Treatment	1, 227.4	9.18	0.003
	Species	3, 11.3	1.30	0.32
	Treatment*Species	3, 226.9	0.97	0.40
c. Shoot elongation	Treatment	1, 236.2	9.57	0.002
	Species	3, 12.2	1.57	0.25
	Treatment*Species	3, 236.1	0.45	0.72

Figure legends

Fig. 1. Natural levels of leaf damage sustained by 9 species during 2010 and 2011. Panel (a) shows damage caused by the leaf miner *M. salicifoliella*. Panel (b) shows damage caused by externally-feeding chewers, including defoliation and skeletonization. Note the difference in scale on the y-axes. Bars show average and standard error with ramet as the unit of replication. Different lowercase letters above the bars indicate significant differences between species across years (Tukey HSD, $P < 0.05$). Abbreviations for *Salix* species: ALAX: *alaxensis*; ARBU: *arbusculoides*; INTE: *interior*; BEBB: *bebbiana*; LASI: *lasiandra*; NIPH: *niphoclada*; PSMY: *pseudomyrsinites*; PSMO: *pseudomonticola*; PULC: *pulchra*.

Fig. 2. Effect of experimental insecticide treatment on average levels of foliar damage across two years. Panel (a) shows the percentage of leaf area mined; panel (b) shows the percentage of leaf area removed by externally-feeding herbivores. Bars show the average and standard error across ramets. See Fig. 1 for a key to species abbreviations.

Fig. 3. Effect of experimental insecticide treatment on the performance of *Salix* ramets of four species. Panel (a) shows the average leaf area; panel (b) shows the average number of leaves per shoot; panel (c) shows the average of the three longest shoots. Error bars are standard errors. See Fig. 1 for a key to species abbreviations.

Fig. 4. Relationship between leaf mining damage and leaf water content for two species of *Salix*: (a) *S. arbusculoides*, and (b) *S. bebbiana*. Each symbol represents one ramet. Both insecticide-treated and control ramets are included.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.