**Projecting the Ecological Effects of Climate Change is Harder at High Elevations: A Case of Complex Dynamics Driving Insect Herbivory in a Perennial Subalpine Wildflower**

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**Abstract**

Because short growing seasons severely constrain plant growth and biomass accumulation in high elevation habitats, herbivory can have profound impacts on both individual fitness and community dynamics in these settings. Climate change is generally expected to increase the activity of insect herbivores, since higher temperatures are strongly correlated with the metabolic rates of exotherms. However, montane communities may not respond in the same ways as lowland ecosystems. To better understand the drivers of insect herbivory in an open, high-elevation habitat, from 2016-2018 we measured grasshopper herbivory on subalpine lupines along an elevational gradient in Mt. Rainier National Park. We found a significant increase in herbivory with elevation, despite decreasing temperatures. This pattern was related to both abiotic (snowmelt timing) and biotic (plant and insect phenotypes) drivers. Our findings highlight the complexity of predicting how species interactions will change with warming in alpine and subalpine ecosystems, where environmental plasticity or local adaptation lend tremendous complexity to ecological dynamics.

**Introduction**

Of the estimated 2.1 million extant species taxonomists have described to date, over 400,000 are land plants (IUCN Red List, 2020) and more than 1 million are insects or other invertebrates that make a living feeding on plant tissues (Fürstenberg-Hägg et al., 2013). Since virtually every plant species we know of hosts at least one insect herbivore (Strauss and Zangerl, 2002), the antagonistic relationship between a plant and an insect that feeds on it is arguably the most common type of pairwise species interaction on earth. It has been clear for decades that these relationships have a potential role to play in structuring plant dispersal and population dynamics across terrestrial ecosystems (Schultz et al., 2017; Agrawal et al., 2012; Maron and Crone, 2006; Fagan and Bishop, 1999; Crawley, 1989), and that they can strongly influence nutrient cycling (Metcalfe et al., 2013; Chapman et al., 2003; Belovsky and Slade, 2000).

The direct positive effects of temperature on insect metabolism, development, reproductive potential, and voltinism are relatively larger than its effect on primary production (deLucia et al., 2012; O’Connor et al., 2011; Bale et al., 2002), so global climate change is likely to transform plant-insect herbivore interactions. Forecasting these outcomes has been an important focus of ecological research in recent years (Pincebourde et al., 2016; deLucia at al., 2012; Cornelissen, 2011), and in general, theoretical predictions of increased consumption by insect herbivores in response to warming are supported by the results of experimental manipulations (Hamann et al., 2020; Birkemoe et al., 2016; Jamieson et al., 2014; Lemoine et al., 2014; Roy et al., 2004).

However, while temperature may be the dominant driver of herbivory under controlled or semi-controlled settings, in natural environments relationships between plants and the insects they host are influenced by myriad interacting biotic and abiotic factors. The variety of these potential drivers is especially marked in mountain habitats, where characteristics including but not limited to local topography, precipitation, wind speeds, snow regimes, solar radiation, edaphic constraints, community composition, vegetation cover, plant phenology, insect thermal requirements, and predation pressure operate at different scales and vary along spatial gradients, not necessarily monotonically (Tito et al., 2020; Moreira et al., 2018; Hodkinson, 2005). Such bedeviling conditions complicate the assumption that plants growing at higher elevations (and therefore cooler air temperatures) will experience lower rates of insect herbivory. Indeed, as described by Rassman et al., 2014, variable and even opposing elevational patterns have been observed in mountain ecosystems (e.g. Kristensen et al., 2020 vs. Pellissier et al., 2014).

The presence of multiple potentially relevant ecological variables also makes it more difficult to predict the future of montane plant-insect herbivore interactions under climate change, which itself could have both direct and indirect effects on each of these factors (Moreira et al., 2018). While many studies have used mountains as natural laboratories in which to investigate the effects of temperature on insect herbivory, relatively fewer have taken a more holistic approach that attempts to test the importance of multiple drivers of herbivory along an elevational gradient—particularly when it comes to the net outcome of interactions between biotic and abiotic factors, which may manifest as elevational trait differences driven by phenotypic plasticity or local adaptation (but see for example Barrio et al., 2016, Haider et al., 2015, and Pellissier et al., 2012). We are particularly interested in understanding differences in both plant and insect herbivore phenotypes that may influence levels of consumption, as well as how these differences are influenced by a population’s history within its environment. For example, cold-adapted plants may produce tougher leaves (Hagen et al., 2019), while the feeding rates of cold-adapted insects may show greater dependence on temperature (Buckley and Nufio, 2014).  Work that connects these differences to observed patterns of herbivory has the potential to provide deeper insights into the near- and far-term effects of climate change on montane plant-insect interactions.

Here we present the results of a three-year study in which we used a combination of observations and experiments to characterize the relationship between subalpine lupines, *Lupinus latifolius* var. *subalpinus* (Fabaceae, Piper & B.L. Robinson (C.P. Smith, 1907)), and Cascade timberline grasshoppers, *Prumnacris rainierensis* (Acrididae, Caudell, 1907), along an elevational gradient in a mountain range in western North America. Specifically, we asked the following questions:

1) How does grasshopper herbivory on lupines vary across sites at different elevations?

2) If elevational patterns exist, how do they relate to: a) abiotic drivers such as temperature and snowmelt timing? b) biotic drivers such as plant and/or insect phenotypes?

**Methods and Materials**

*Study area*

We conducted our research in Mt. Rainier National Park, WA (46.8529° N, 121.7604° W). Standing at 4,392 m, Mt. Rainier (traditionally known as Mt. Tahoma) is the tallest volcanic peak in the rugged Cascade Mountain Range that runs parallel to the Pacific Coast, from California to Canada. Our two species of interest both occur in the subalpine, a narrow band of elevation between conifer forests below and rocky alpine communities above. This unique meadow ecosystem hosts a remarkable diversity of plants and wildlife that make their living under challenging environmental conditions, including relatively cool average temperatures, low summer rainfall, and up to 40 weeks a year of deep snow cover.

We collected data on two transects, one each on the South and East sides of the mountain, spanning the total range of subalpine meadow habitat: approximately 1,490—1900 m on the South and 1,6660—2,020 m on the East side. (The elevational distribution of this plant community depends on snowfall, which is greater on the South side and keeps tree line lower.) On each transect we had five sites, roughly evenly spaced along the elevational gradient, with nine permanently marked 1 m2 plots at each site spaced at least 10 m apart. The plots on the South transect were previously established for a different study (Theobald et. al, 2017) such that at each elevation, three plots were located on ridge tops, three along slopes, and three in depressions. While we did not necessarily consider such local topographic variation to be a direct driver of insect herbivory, it is strongly correlated with the timing of snowmelt (Ford et al., 2013), which could influence herbivory through phenology; therefore, we situated plots on the East transect to match the design of the South transect. We measured canopy cover at each plot with a spherical densiometer, and collected soil moisture readings at each visit using a Decagon Procheck meter and GS3 sensor (Pullman, WA). Finally, to estimate snow disappearance dates, we buried HOBO Pendant temperature sensors (Onset Computer Corporation) just below the soil surface at each plot (as in Sethi et. al, 2020).

*Study species*

*L. lat. var. subalpinus* (subalpine lupine) is a common herbaceous perennial of the northwestern United States, with a distribution range stretching from northern Oregon to Alaska. In the mountains of western Washington, most populations are found at 1200 m or higher in elevation, growing amid the forest understory as it approaches tree line, and in large patches on open ridges and meadows. The dense foliage, charismatic blue-violet inflorescences, and bean-like seeds of this species support a rich array of invertebrate, mammal, and bird species, including bumble bee and hummingbird pollinators and foragers like hoary marmots and golden-mantled ground squirrels. As nitrogen-fixers, these plants also contribute to soil nutrient availability for the high-elevation communities to which they belong, and are often among the earliest colonizers of recently exposed soils left by deglaciation or permafrost disturbance (Cray and Pollard, 2015; Henderson, 1973).

*P. ranierensis* (Cascade timberline grasshopper) is a stout, short-winged (flightless), high-elevation grasshopper with yellow-green to coppery brown coloration, black lateral stripes, and legs marked with chevron-shaped ridges. It is endemic to the Cascade Mountains of Oregon and Washington and extremely locally abundant within our study area. Eggs laid the previous season that have overwintered beneath a heavy layer of snow hatch at snowmelt, and mating adults are thick—in the thousands—in subalpine meadows on Mt. Rainier by the end of the summer. Although they are generalist forb-eaters that feed on many subalpine wildflower species, these grasshoppers are known to be particularly closely associated with *L. lat.* var *subalpinus* (Helfer, 1972; pers. obs.). Our identification of this species as *P. rainierensis* was confirmed by Andrew Carmichael at the Systematic Entomology Laboratory (SEL), part of the Agriculture Research Service in the US Department of Agriculture, after we sent three collected specimens for inspection.

*Herbivory*  
We monitored grasshopper herbivory on *L. lat.* var. *subalpinus* between 2016 and 2018. Since lupines reproduce both by seed and clonally, through short rhizomes (Celis et al., 2017), we could not identify genetically unique plants without destructive sampling. Our analysis therefore treats whole plots, which were far enough apart (>10 m) to be unlikely to contain the same individual, as replicates. There was clear variation in herbivory among “clumps”—clusters of smaller stems surrounding a main stem—so we monitored all lupine clumps in all study plots, except in a few plots with particularly high lupine populations where we monitored only the first 8 plants that leafed out (mean = 4.3 clumps per plot, sd = 1.5). Every two weeks during the growing season each year (approximately 12 weeks, from late June-September), we visited each plot and recorded % leaf area lost to chewing insect herbivory on 10-30 haphazardly selected, fully expanded, non-senescent lupine leaves per clump. We did this using simple visual estimation, classifying each leaf into one of eight damage classes (0%, 0-1%, 1-5%, 5-10%, 10-25%, 25-50%, 50-75%, and 75-100%) and calculated apparent leaf damage (ALD) per clump as ∑(# leaves in each class \* median % damage in that class) / total leaves sampled). Since there were differences in lupine density between plots, we used the median ALD observed across lupine clumps within each plot at each visit as the outcome variable in all our analyses.

We note that visual estimation is a commonly used method of quantifying invertebrate herbivory damage in the field (e.g. Scheidel and Bruelheide, 2001; Dostálek et al., 2018; Kristensen et al., 2020), and human observers have been shown to be capable of precise and accurate estimates of herbivory comparable to those produced by digital methods (Johnson et al., 2016). This is especially true with chewing at the margins of leaves as opposed to leaf interiors, which is the type of damage typically left by grasshoppers (Gangwere, 1966; Avanesyan and Culley, 2015). The patterns of damage associated with chewing insects are unambiguous and easily diagnosable to functional group (Green and Maloy, 1990), and while other chewing insect herbivores that feed on lupines are present at our sites (including other orthopterans and several butterfly larvae), sweep searches suggest their numbers relative to *P. rainierensis* are extremely small (Sethi, unpublished data). We are confident that the vast majority of the insect herbivore damage recorded in our dataset was produced by *P. rainierensis.*

In addition to foliar herbivory observations, we recorded pre-dispersal seed predation in 2018 and 2019 by collecting 10 ripening seed pods from each of 5 haphazardly selected plants at each elevation where they were available). Seed pods were transported back to the lab in moist ziplock bags, and examined under a microscope for signs of invasion and seed predation. Invertebrates found within seed pods were counted and identified to family.

*Experimental Feeding Trials*

Because our first year of data suggested greater grasshopper herbivory on lupines at higher sites vs. lower ones, despite lower average ambient air temperatures at high elevations, we conducted experiments to disentangle the effects of feeding temperature, lupine population, and grasshopper population on grasshopper feeding rates. In late August/early September 2017 and 2018, when mature grasshoppers were present at all elevations, we visited our highest and lowest sites on each transect and collected adult *P. rainierensis* individuals, as well as fully unfurled, non-senescent, undamaged lupine leaves (stems attached). Field-collected specimens were transported back to the lab the same day, held at room temperature, and used for feeding trials within 24 hours of collection. In the meantime, leaves were placed in individual floral tubes filled with water to prevent evapotranspiration, and grasshoppers in individual 16 oz. polyurethane cups along with a damp paper towel and access to additional leaves.

We used a fully factorial design with eight treatment groups (average n=38 per treatment) comprising all combinations of temperature (25 vs. 32 °C), grasshopper source (low vs. high elevation), and lupine source (low vs. high elevation). After being fasted for 12 hours to ensure their stomachs and guts were empty at the time of the experiments, grasshoppers were placed in incubators and acclimated to their assigned test temperature for 1 hour before being provided with 1-2 lupine leaves from their assigned population, depending on the size of the leaf. Trials lasted a total of 8 hours, to provide sufficient time for all grasshoppers to ingest, fill their crops, and clear their guts (after Buckley and Nufio, 2014, and Harrison and Fewell, 1995). Leaves were weighed before and after each trial to determine feeding rates. We also placed control leaves from each population source inside empty polypropylene cups in each incubator for the duration of the experiments. As these leaves lost negligible mass (an average of <0.5% in 8 hours), we were able to rule out potential changes in mass due to evapotranspiration rather than grasshopper consumption.

*Leaf traits and photosynthetic performance:*

To assess the role of lupine leaf traits in explaining variation in herbivory rates at different sites, we measured three traits that were likely to affect food quality for arthropod herbivores: Leaf Mass per Area (LMA) as a proxy for leaf toughness (He and Sun, 2016), the ratio of carbon to nitrogen (C:N, Barbour et al., 2015), and constitutive defenses (in the form of quinolizidine alkaloid compounds, which are the main chemical deterrence used by lupines: see Wink, 1992, and Mithöfer and Boland, 2012).

To calculate LMA and C:N ratio, we collected leaves with stems attached from at least 10 replicate plants at each elevation and stored them in moist sealed plastic bags in a cool box until processing. Leaves were rehydrated overnight before being gently rubbed dry and scanned. We calculated LMA using leaf area estimated from the scans with ImageJ (Schneider et al., 2012) and dry mass measurements taken after the leaves were dried in an oven at 45°C for one week. Finally, we ground and homogenized the leaves in a ball mill, encapsulated 2mg replicate samples in tin foil, and measured C:N concentrations using an elemental analyser (CHN Analyzer 2400 Model, PERKIN ELMER Co). For chemical defense analysis, we collected leaves from 5 replicate plants at each elevation, flash froze them immediately in liquid nitrogen in the field, and transferred them to an -80°C freezer until they were processed and analyzed at the Murdock Metabolomics Laboratory at Washington State University (Pullman, WA).

To explore the potential effects of herbivory on plant photosynthetic performance, we used a handheld device to measure a suite of photosynthetically relevant parameters (MultispeQ, PhotosynQ Inc; Kuhlgert et al., 2016) in light-adapted undamaged leaves (n=469) and leaves with at least 5% of area lost to herbivory (n=283). These measurements were collected across a range of temperature and light conditions at each elevation to account for environmental variation. More details about data collection and analyses of plant traits and performance can be found in the supplementary material.

*Statistical Analysis*

All analyses were carried out in R 4.0.3 (R Core Team, 2020). To understand potential drivers of the patterns of leaf area damage we observed, we created a linear mixed effects model (LMM) to test for the effects of elevation, snowmelt date, time since snowmelt, and canopy cover (continuous predictors) as well as of transect and year (categorical predictors) on herbivory. We included plot as a random effect, since each plot was sampled multiple times. We used the top-down variable selection process outlined in Zuur et al., 2009, preferring Bayesian Information Criterion (BIC) over Akaike Information Criterion (AIC) scores if the two differed because our analytical goal was hypothesis testing, rather than predictive performance (Aho et al., 2014). In addition, we chose to implement our LMM with R package “nlme” (Pinheiro et al., 2013), because it allowed us to account for heteroscedasticity in the residual errors by modifying the variance structure we used in our final model (Supplementary Material XX).

We used a three-factor ANOVA to explore the effects of temperature, grasshopper source, and lupine source on feeding rates in our experimental trials, linear regressions followed by ANOVA to test for effects of herbivory on photosynthetic performance, and linear regressions to analyze elevational relationships with LMA, C:N ratio, and pre-dispersal seed predation. Finally, we used both linear regressions and principal components analysis (PCA with the “prcomp” function in stats) to test for differences in alkaloid concentration and composition across elevation.

**Results**

*Herbivory*

There was a high incidence of grasshopper herbivory on lupines in the subalpine meadows we monitored, with 76.9% of our observations recording visible tissue loss. The magnitude of this damage was generally low; we estimate that the average lupine clump in our study plots lost only 3.2% of the total leaf area it produced to grasshopper herbivory each season. There was, however, a long tail of higher losses; in one out of four of all plot-year combinations, at least one lupine clump lost over 10% of its total leaf area to herbivory, and some clumps experienced ten times that much tissue loss (maximum estimated mean ALD in a season: 36%). Our best-fitting mixed-effects model showed that grasshopper herbivory on lupines was significantly affected by the main effects of elevation, year, snowmelt date, and time since snowmelt, as well as by the pairwise interactions between elevation and transect, elevation and year, and elevation and time since snowmelt. (Table 1).

Because we centered and scaled each of our numeric predictors, we were able to directly compare their model-estimated coefficients to identify their relative importance (Table 1). Elevation had the largest effect, with plots at higher elevations experiencing greater herbivory. The slope of the elevation-herbivory relationship was steeper on the East transect than the South transect in all years, and on both transects in 2018 (Fig. 1, Table 1). Snowmelt date and time since snowmelt both had positive relationships with herbivory (plots that melted out later had higher herbivory, and herbivory increased with time since snowmelt). In addition, plots at higher elevation experienced greater increased in herbivory with time since snowmelt.

Besides folivory, we also observed a remarkably high level of pre-dispersal seed predation. 46% of lupine pods (n=601) collected from the East transect and 39% of pods from the South transect (n=678) showed clear signs of invasion by specialized invertebrate herbivores, mainly fly, leaf beetle, and sawfly larvae. While most invaded pods contained some intact seeds at the time of collection, an average of 34% of seeds within invaded pods had been predated to the point where we would assume they were no longer viable (>25% tissue loss). A linear regression showed a significant positive relationship between elevation and seed predation (p<0.0001), but this explained a vanishingly small amount of the variation in seed predation (R2=0.02), and is based on incomplete data because no lupine plants successfully set seed in some elevations in some years. (In 2018, lupines at the two highest elevations on both transects appeared to have failed to set seed, and in 2019 this was true of lupines at the second-highest elevation on the East transect.)

*Experimental Feeding Trials*

We found significant treatment effects on grasshopper feeding rates in our experimental trials (Fig. 2A). Consumption was higher at 32°C than 25°C (F value = 21.78, p<0.0001). Low-elevation lupine leaves were consumed more rapidly than high-elevation leaves (F value=10.72, p<0.001). Finally, high-elevation grasshoppers fed faster than low-elevation grasshoppers (F value =6.53, p<0.01). The interaction between temperature and grasshopper source was not significant (F value=2.78, p=0.09), but visualizing the interaction suggests high-elevation grasshoppers may be more sensitive to temperature than low-elevation counterparts (Fig. 2B).

*Plant Traits and Photosynthetic Performance*We found that LMA increased with elevation (Fig. 3a), as did C:N ratio (Fig. 3b). The concentration of sparteine, a major lupine alkaloid, also increased with elevation (Fig. 3c); however, this relationship was very noisy, and lupanine, another major defensive alkaloid in lupines, did not differ significantly with elevation. PCA of all detected alkaloids collectively showed no distinction between sites or elevations in terms of the overall composition of chemical defense compounds (Fig. S1).

MultispeQ measurements revealed no differences between damaged and undamaged leaves in terms of the efficiency of photosynthesis, defined as the relationship between PAR (Photosynthetically Active Radiation) and Phi2 (the quantum yield of photosystem 2). But leaves that had experienced herbivory showed significantly lower levels of non-photochemical quenching (PhiNPQ, p=<0.001) and significantly higher levels of non-regulated energy dissipation that can lead to photoinhibition (PhiNO, p=0.008) at a given light intensity (Fig. S2). In addition, damaged leaves had a lower mean level of relative chlorophyll than undamaged leaves, though this difference was not statistically significant (p=0.07).

**Discussion**

*Overall patterns of insect herbivory on subalpine lupines*

 Resource limitation theory predicts that environmental filtering should result in higher resistance to herbivory among slow growing plants in climatically stressful habitats such as the montane meadows we studied (Grime, 2006). In agreement with most other empirical data on insect herbivory in similar open, high-elevation systems (e.g. Haider et al., 2015; Pellesier et al., 2014), we found that on average, the magnitude of grasshopper damage experienced by subalpine lupines was relatively low. In our study, it was associated with an estimated 3.2% loss of annual primary production—minor when compared, for example, to the damage from chewing insects reported in temperate woodlands (12% annually, Visakorpi et al., 2021) or pastures (13% annually, Coupe and Cahill, 2003). However, foliar herbivory on lupines was widespread, affecting 88% of all individual plants in our dataset, and for some individuals, high—about 20% of the population experienced the loss of 10% or more of the total leaf area they produced in a season. In addition, pre-dispersal seed predation by insects was extremely common, affecting 42% of collected pods.

We would argue that this level of chronic background herbivory presents a meaningful challenge to the overall health of subalpine lupines on Mt. Rainier. There is convincing evidence that even low intensities of insect damage can have important effects on individual plant performance and community dynamics (Visakorpi et al., 2021; Dostálek et al., 2018; Zvereva et al., 2012), including via depressed photosynthetic rates that can extend well beyond the area of removed leaf tissue and persist for several days after the damage occurs (Zangerl et al., 2002). Here, we did not find a significant relationship between herbivory and photosynthetic efficiency, but because we do not know how much time passed between the time leaves were attacked and our measurements, we cannot rule out the possibility that photosynthetic yields were temporarily reduced by chewing damage. We did find that lupine leaves with at least 5% area loss to insect herbivory appeared to have a reduced ability to harmlessly dissipate high levels of incoming radiation, and to direct a greater proportion of excess energy towards pathways that have the potential to cause photo-oxidative damage to chloroplasts.

In other words, grasshopper herbivory may contribute to increased stress and ultimately lower productivity for subalpine lupines, especially among plants growing at the very highest elevations that are already facing considerable levels of abiotic stress. Addressing the potential fitness effects of herbivory on lupines is beyond the scope of this work, but given the high rates of pre-dispersal seed predation by invertebrates, which suggest that achieving reproductive success for lupines requires a great deal of energetic investment, it would be surprising if these were nonexistent. Interestingly, though our analyses of lupine chemical defenses largely failed to show clear differences among sites, we did find an elevational increase in one alkaloid, sparteine, that acts as a deterrent. We can only speculate about the biological significance of this pattern, but it may be a marker of previous selection for higher defenses driven by the pressure of insect herbivory.

*Potential abiotic and biotic drivers of herbivory*

Mean and minimum daily air temperatures in subalpine meadows on Mt. Rainier decrease predictably with elevation (Ford et al., 2013). Despite this, leaf area loss at our sites was significantly higher, rather than lower, at higher elevations. This result was consistent over both transects and all three years of data collection, and is in line with the sizable minority—22%—of the studies of elevational gradients and plant-insect herbivore interactions reviewed by Moreira et al., 2018 which reported a positive relationship between elevation and damage sustained by plants. A characteristic feature of high-elevation life that goes some way toward explaining this pattern is the extremely constrained growing season for both plants and insects. In our study system, there may be as little as 12 weeks between snowmelt and the return of snow cover. This sets tight boundaries on the development time available to grasshoppers, especially for populations living at higher elevations where seasons are shorter and available heat sums lower.

Previous work has shown that alpine grasshoppers may deal with this limitation by speeding up development times (Berner et al., 2004), which could take place via greater metabolic sensitivity to temperature. For example, high-elevation Colorado populations of *Melanoplus boulderensis*, a cold-adapted grasshopper in the same subfamily as *P. rainierensis*, exhibited higher feeding rates than their low-elevation conspecifics at all temperatures; they also showed increased thermal sensitivity of feeding rates (Buckley and Nufio, 2014). Similarly, in our experiments, we saw that *P. rainierensis* individuals from high elevations consumed at higher rates than those collected from low elevations at all temperatures, and there was a suggestion that they might also exhibit greater thermal dependence, though this trend was not significant. It is worth noting that in the field, behavioral thermoregulation is likely to have a marked effect on the effective body temperature grasshoppers are able to achieve, and high-elevation grasshoppers may be under especially strong selection for taking advantage of warm temperatures by basking in sunny areas (Buckley and Nufio, 2014). Not only does the intensity of solar radiation increase with elevation (Germino, 2014), but canopy cover decreases as well, so higher-elevation sites are less shaded and provide greater opportunities for thermal basking. All of this together suggests that lupines growing at higher elevations may experience greater herbivory because they host more rapacious populations of grasshoppers racing against time to complete their development so that they can have a chance of achieving reproductive success.

Consistent with this idea, snowmelt timing had a positive relationship with herbivory; later snowmelt (and therefore shorter growing seasons) was associated with greater leaf area losses. An experimental snow manipulation in an arctic habitat found a similar pattern on dwarf birch (Torp et al., 2010). However, Torp et al. speculate that because of the insulation provided by snow, winter soil microbial activity and therefore nitrogen availability was higher in plots with higher snow cover, thereby increasing the palatability of leaves to nitrogen-limited insect herbivores. In contrast, we hypothesize that herbivory levels in our system may be partly driven by compensatory feeding, in which herbivores consume larger amounts of low-quality food to balance their nutritional needs (Berner et al., 2005). A meta-analysis of leaf functional traits along elevational gradients suggests that despite mixed results from individual studies, in general C:N ratios should increase with elevation (Read et al., 2013), leading to lower nutritional quality for invertebrates. This is in fact what we found (Fig. 3B). Since lupines are a nitrogen-fixing species, they are already among the most nitrogen-rich host plants available at our study sites, making it unlikely that grasshoppers can resolve their need for nitrogen by switching to a different food source. However, they may increase the total amount of lupine tissue they consume if the nutrition they gain from a given leaf mass decreases. If this is the case, it could be an important explanation for the high levels of herbivory we observed at higher elevations.

*P. rainierensis* is a generalist herbivore, so even if nitrogen limitation is an important driver of its food choices, it is possible that some of the variation in leaf damage on lupines could be explained by shifting host-plant preferences among grasshoppers—perhaps based on elevational differences in plant community composition, defense, and/or other nutrients (Barrio et al., 2016; Lemoine et al., 2013). However, we believe that this is unlikely to be the case. During the first year of our observations, we additionally monitored folivory in the eight most abundant subalpine wildflower species at our study sites after lupine, and found no evidence that lupine herbivory was correlated with herbivory on other species consumed by grasshoppers (Fig. S3, TK). Another potentially relevant factor we did not measure was the strength of top-down controls, including predation, pathogens, and parasitism. There is some evidence that biotic controls of herbivorous insects are generally stronger in warmer environments (Zhang and Adams, 2011). If this were true in our system, it would be consistent with the lower levels of leaf damage we found at lower elevations.

*Conclusion*

Montane plant species like subalpine lupines face challenging climatic conditions, including low temperatures, nutrient-deficient soils, and short growing seasons. These constraints drive up the construction costs of producing new leaf tissues, and therefore also the potential fitness costs of herbivory when it does occur (Germino, 2014; Dostálek et al., 2018). Here we find that high-elevation lupine populations currently experience higher levels of herbivory despite lower air temperatures, and that this pattern is associated with multiple interacting abiotic and biotic factors. In the short term, given that grasshopper feeding rates appear to be an important driver, high-elevation lupines are likely to be even harder hit as summer temperatures warm and the number of heat waves in the region increase (Mauger et al., 2015). However, our results highlight the difficulty of predicting how montane plant-insect herbivore dynamics will respond to climate change in the long run, especially as we were unsuccessful in rearing our study species under laboratory conditions, and were therefore unable to disentangle the relative proportion of either lupine or grasshopper phenotypes that could be attributed to local adaptation vs. environmental plasticity. We recommend that future work take on the challenge of conducting common garden experiments with multiple generations of field-collected plants and insects in order to better understand how climate change will shape these relationships in natural settings.

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**Figures and Tables**

Table 1: Estimated coefficients, confidence intervals, and significance values from our best-fitting model

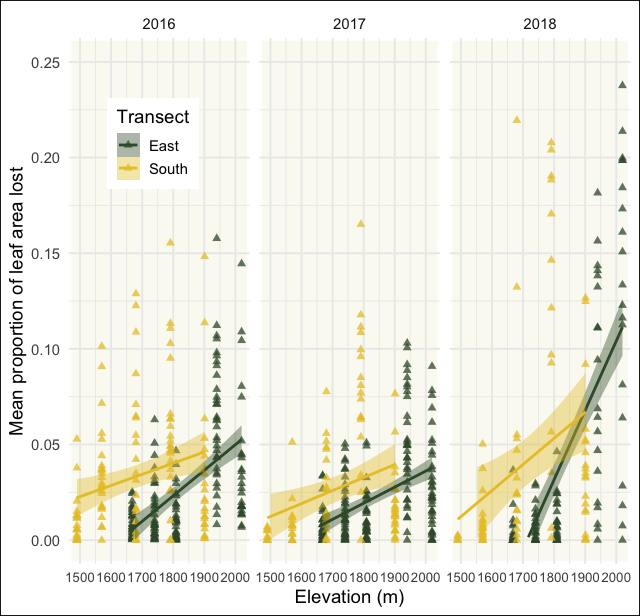
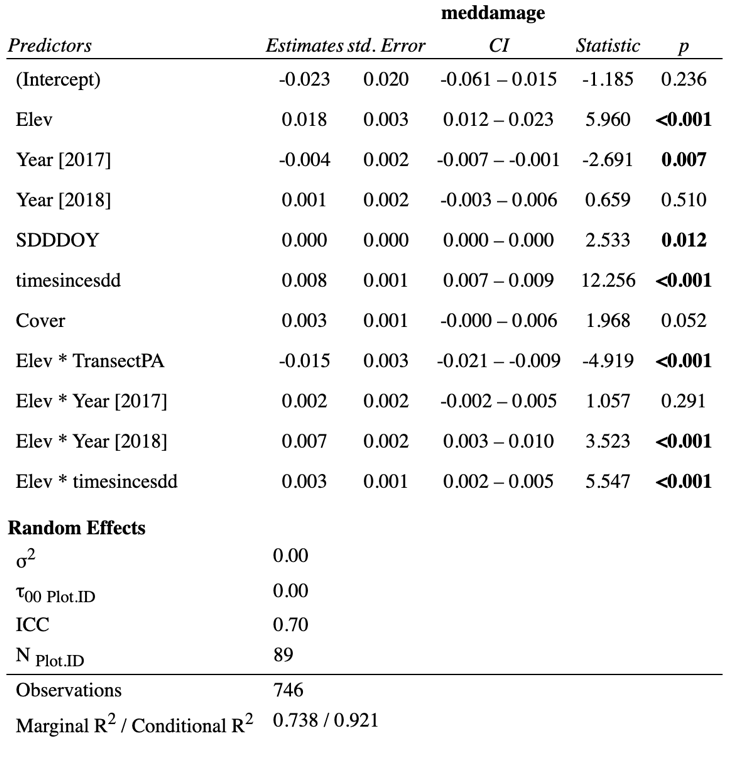


Figure 1: Estimated mean leaf area lost per plot across all years of the study.

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Figure 2A: Results of experimental feeding trials; boxplots show means and quartiles. Figure 2B: Interaction plot showing mean consumption of high vs. low elevation grasshopper populations by temperature.

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Figure 3: Elevational patterns in leaf traits: A (LMA), B (C:N ratio), C (Sparteine concentration).

**Supplementary Figures**

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Figure S1: Principal Component Analysis (PCA) plot of alkaloid composition by elevation and transect, showing the first two dimensions of the analysis.

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Figure S2: Effect of herbivory damage on the amount of excess photosynthetically available radiation (PAR) that is safely dissipated by through nonphotochemical quenching (A) vs. that is passed to nonregulated photoinhibitive processes.