

Investigating the relationship between plant morphology,  
density, and herbivory of *Thlaspi arvense*

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## Abstract:

The mustard family Brassicaceae includes many crop species, model plant systems, and invasive species. Many of these species produce a class of secondary defense to deter herbivory with a class of chemicals called glucosinolates (Carlsson et. al, 2009; Keeler & Chew, 2008). *Thlaspi arvense* is a non-native member of the Brassicaceae family that produces these chemicals, yet it remains a target for herbivory. Studying the interaction of both *T. arvense* and herbivores assists in understanding the factors of what influences a continued arms race of defense and attack (Rodman and Chew, 1980; Lee et. al, 2009). We will investigate how these two factors react to a change of density and an elevational gradient by measuring herbivory at similar phenological stages in a common garden in the Gothic townsite and in randomized field plots at sites of varying elevation. By manipulating density and seed source in the common garden, environmental factors are stabilized, and herbivory selection factors will become more prominent. We expect to find less herbivory per plant in denser populations and more herbivory in plants with seed sources from higher elevation will experience less herbivory.

## Intro:

A major question in ecology is how and why successful plant invasions occur and how those invaders interact with their new environments. Many characteristics contribute to the success of an invasive plant, including high fecundity, escape from natural predators in their original region, and a high rate of phenotypic plasticity (Cappucino & Carpenter, 2005). The addition of secondary defensive chemicals to this suite of traits can also improve the competitive ability of a non-native plant in a new territory (Callaway & Ridenour, 2004). Brassicaceae, or the mustard family, are an interesting group to investigate non-native spread and potential invasion as most of the plants in this family have a class of secondary defense chemicals called glucosinolates (Rask, 2000). These chemicals help deter damage from generalist herbivores and can act as a “novel weapon” in a new range, potentially increasing competitive advantage of the non-native plants (Callaway & Ridenour 2004; Harvey et. al, 2010). *Thlaspi arvense* is a non-native mustard plant that was introduced in the late 1800s to the Gunnison Basin. The townsite of Gothic is the edge of its non-native elevational range, and while *T. arvense* flourishes on roadsides and in disturbed areas, it is also present in subalpine meadows. There are many native mustard plants in the subalpine area where specialist herbivores have evolved alongside these mustard plants to seek out and digest the tissues containing glucosinolates (Lake & Leishman, 2004; Nakajima, 2013). The unique ecology of subalpine meadows in this area may be significantly impacted by the introduction of non-native Brassicaceae. We have observed significant levels of herbivory on *T. arvense* and both specialist and generalist herbivores have been found on these plants at varying levels, though ability to successfully digest plant tissue also varies (Boggs and Steward, pers. comm). The selection pressure that this herbivory poses on *T. arvense* across subalpine meadows remains an open question, and we aim to quantify this relationship in a common garden with manipulated seed source and density to control for

environmental variation across the range. We will also investigate how herbivory pressures on *T. arvense* vary across sites along the edge of its elevational range in the field.

My research questions are (1) do herbivores preferentially select in a common garden and (2) does herbivory vary by site in the field. I predict that as density of *T. arvense* increases, percent of herbivory will decrease in both the common garden experiment and in the field. I anticipate that denser populations will permit the herbivores to have a more abundant food source available and increase the ability to travel among plants, resulting in a lower percent of herbivory for each individual plant. However, I expect this relationship to vary by seed source. I predict that sites with earlier snowmelt timing will have larger plants as their life cycles will be longer with less stress than those in late snowmelt sites. I predict that the percent of herbivory will decrease as seed source elevation increases. Because the common garden takes place in the townsite of Gothic and the seed source of highest elevation is at Gothic, I predict that the seeds from higher elevation plants have experienced similar herbivory pressures and are more novel to this area where *T. arvense* is scarce. I also predict that sites with earlier snow melt will have greater herbivory, as the larger plants will attract more herbivores. Comparing the common garden data with data collected at our field sites will allow to see if observed relationships hold true outside of a common garden.

## Methods:

### **Common Garden Methods**

Our common garden represented four field sites (Gothic, Snodgrass Trailhead, Rainbow Park, Brush Creek Trail) with seeds from ten maternal plants at each site, for a total of 40 seed source plants. The sites were located across an elevational range from 2700-3000 feet, classified for this experiment as high, medium, and low.

We planted 2,400 seeds of *T. arvense* in 40 flat seed trays filled with water saturated standard potting soil. We placed the trays into a Weatherport to provide a stable environment for germination and later transferred them into two small portable greenhouses. Two weeks after 50% of plants have germinated, we transplanted 269 seedlings into a randomized block array. All four field sites were equally represented in each treatment with 171 plants planted in high density and 98 plants planted in low density. The high-density treatment consisted of 50 plants per tray and the low-density treatment is 25 plants per tray. We completed an initial herbivory survey one week after transplant. Every two days we scanned the common garden for flowering time of each individual plant. Once a plant has flowered, we recorded height, rosette leaf number, cauline leaf number, branch number, rosette diameter, and herbivory. The herbivory survey consisted of the number of leaf that the herbivory is on, type of herbivory (i.e. munching, surface damage), and estimated percent of herbivory per leaf. We minimized observation variation by using a flatbed leaf scanner to measure a subset of leaf areas and calculate exact tissue loss. We compared exact loss to estimated loss to confirm there is no statistical difference in measurements before

continuing the common garden herbivory estimates. For field estimates, two observers randomly confirmed estimates to maintain consistency. Monitoring herbivory at flowering time allowed us to have a phenological basis to compare measurements of herbivory across the common garden and the field site plots. Because of late growing season, this aspect of the experiment will not be included in results and discussion but will be added at a further date when full data is collected.

### **Field site methods**

We established ten field sites across an elevational gradient of 2700m-3000m (Fig. 1 & 2, Gothic, Deer Creek trail/Kettle Ponds, Snodgrass Trailhead, Lower Meander pumphouse, Hunter Hill, Mount Crested Butte Rec Path, Brush Creek Trail, West Brush Creek, Brush Creek/Pearl Pass, and Brush Creek/Deer Creek trail). We classified snowmelt times by presence of early season ephemeral flowering species, with snow melt times classified as early, medium and late.

We assigned each of the ten field sites classifications in the categories of snow melt timing, elevation, site disturbance, and density depending on their site characteristics. snow melt timing was categorized as early, medium, and late by timing of presence of early spring ephemerals (*claytonia* and *mertensia*). Elevation taken from GPS coordinates were separated into categories of low (2700-2800m), medium (2800-2900m), and high(2900-3000m) (Fig. X). Lastly the categories of site disturbance were separated into low, medium, and high based on observed levels of biotic – disturbance from mole hills and cow grazing - and abiotic – proximity to path or road - interferences.

In each field site we placed a 15 meter transect running through a population of *T. arvense* and select 0.5 x 0.5m plots with a quadrat randomly placed along the transect over patches of *T. arvense*. In each plot we counted the number and phenological stage of each *T. arvense*. There are five phenological stages that we categorized *T. arvense* into rosette, bolting (main stem  $\geq$ cm), flowering, seed setting, and senescence (Fig. 3). Once half the plants in the quadrat flowered, we conducted an herbivory survey as outlined in the common garden procedure. By recording phenological stage to compare herbivory in the common garden and in the field, we can understand if any trends observed in the common garden corresponded with those observed in the field.

### **Results:**

Density varied greatly across sites, with the greatest number of plants at West Brush Creek (low elevation class, early snowmelt, average plot density = 164 plants) and the lowest number of plants at Snodgrass (high elevation class, medium snowmelt, average plot density = 13 plants). We classified each plot into a density category based on total number of plants in the plot, low (1-50), medium (50-150) and high (150-300). We compared the height of individual *T. arvense* plants at flowering stage with plot density class. Using height as a size for proxy, we used an ANOVA to test for significance in relating height to snowmelt, elevation, disturbance level, and density. Plant height varied significantly by snowmelt ( $F_{2,255}=6.86$ ,  $p<0.01$ ) and density

was marginally significant ( $F_{2,225}=2.492$ ,  $p=0.084$ ). The height of individual plants varied by density class of plots, with higher density plots having taller plants (FIG A, high density  $\bar{x}=23.5\text{cm}$ , medium density  $\bar{x}=17.9\text{cm}$ ; low density  $\bar{x}=16.8\text{cm}$ ). In areas of early snow melt, individual plants had the greatest height in high density plots ( $\bar{x}=31.27\text{cm}$ ), with medium and low density plants around the same size (Figure B; early snowmelt, medium density  $\bar{x}=18.71\text{cm}$ ; early snowmelt, low density  $\bar{x}=21.00\text{cm}$ ). For sites of medium snow melt timing, the variation was less visible than the category of early snow melt (Figure B; medium snow melt, low density  $\bar{x}=16.06$ , medium density  $\bar{x}=19.18\text{cm}$ , high density  $\bar{x}=15.77\text{cm}$ ). The plots in the late snow melt category were not significantly different by density, however the late snow melt high-density class was not represented in this data set.

Using an ANOVA we tested the significance of density of plants, snow melt, elevation class, disturbance category, and date of measurement in relation to the amount of plants per quadrat with *any* herbivory damage. We found that the number of plants with herbivory damage varied significantly by the density of plants without herbivory damage ( $F_{1,37}$ ,  $p<0.01$ ) and snowmeltclass ( $F_{2,37}$ ,  $p<0.05$ ). Elevation class, disturbance category, and date of measurements were not significant. Plots found in areas of early snowmelt and high density had more individual plants with herbivory damage ( $\bar{x}=12$ ) than those of medium and late snowmelt (which figure?).

Running a beta regression model from R stats package betareg to test the variation in percent of herbivory per plant by snow melt, elevation class, density, and date of measurement, we found that snow melt, elevation, and days are significant (FIG X, late snow melt z value = 2.793,  $p<0.005$ ; medium snow melt z value = 2.577,  $p<0.05$ ; low elevation class z value = -2.216,  $p<0.05$ ; medium elevation class z value = -3.001,  $p<0.005$ ; following beta regression for modeling proportions; Ferrari and Cribari-Neto 2004). Density, however, was not insignificant. The highest average percent herbivory occurred at high elevation class sites with medium snow melt, 9.05% estimated whole plant herbivory. The lowest herbivory occurred at medium elevation class and medium snow melt, 2.9% herbivory.

Analyses were run in R Studio version 1.2.1335 and R version 3.5.2.

### Discussion:

At early snowmelt sites, higher density populations had larger plants, contrary to what we predicted. While we anticipated that higher density populations would have smaller plants, likely due to competition for resources, potentially the trend of higher density and larger plants occurs at sites with greater overall resources. This may be driven by water availability, as the soil types are consistent across sites. We found significant variation in plant size across snow melt timing (Fig. A), with earlier snow melt populations having significantly larger plants – particularly at high densities. This is consistent with earlier snowmelt populations having longer growing seasons, facilitating increased plant growth.. Later snowmelt populations have shortened seasons and less time to devote resources to growth over reproduction (Gray & Siobhan, 2016).

Density did not significantly impact herbivory as we predicted, as there was a significant positive relationship between number of plants with herbivory in a plot and number of plants without (Fig. A). However, the ratio of plants with herbivory and plants without varied by snowmelt timing. We found significant variation in total number of damaged plants and proportion of damaged plants across snow melt timing and elevation category. We'd expect that this is due to variation in insect communities across elevation classes, as phenological variation in herbivores is likely to trend with snowmelt timing as a major phenological cue (Best & McIntyre, I. G., 1972). Plants in both later snow melt and higher elevation had significantly higher proportions of herbivory per plant – these populations likely experiencing more herbivory stress (Fig D.). Because the sites at the edge of *T. arvense*'s elevational ranges are the ones most affected by herbivory, acting as a selection pressure for evolution (Callaway & Ridenour, 2004; Franks et. al, 2014). This suggests that the edge populations are under higher selection pressure from herbivore (Franks et. al, 2014).

As the introduced plant of this range spans North America, it seems limited by elevation. These edge populations are under higher pressure to evolve in the face of shorter growing seasons and higher herbivory. Further study on the defensive chemicals produced by these plants is needed to understand if glucosinolate quantity plays a role in herbivore choice and amount of herbivory experienced by a plant. While this study does not address secondary chemistry directly, it suggests that both environmental biotic and abiotic factors play a role in the amount of herbivory experienced by these plants.

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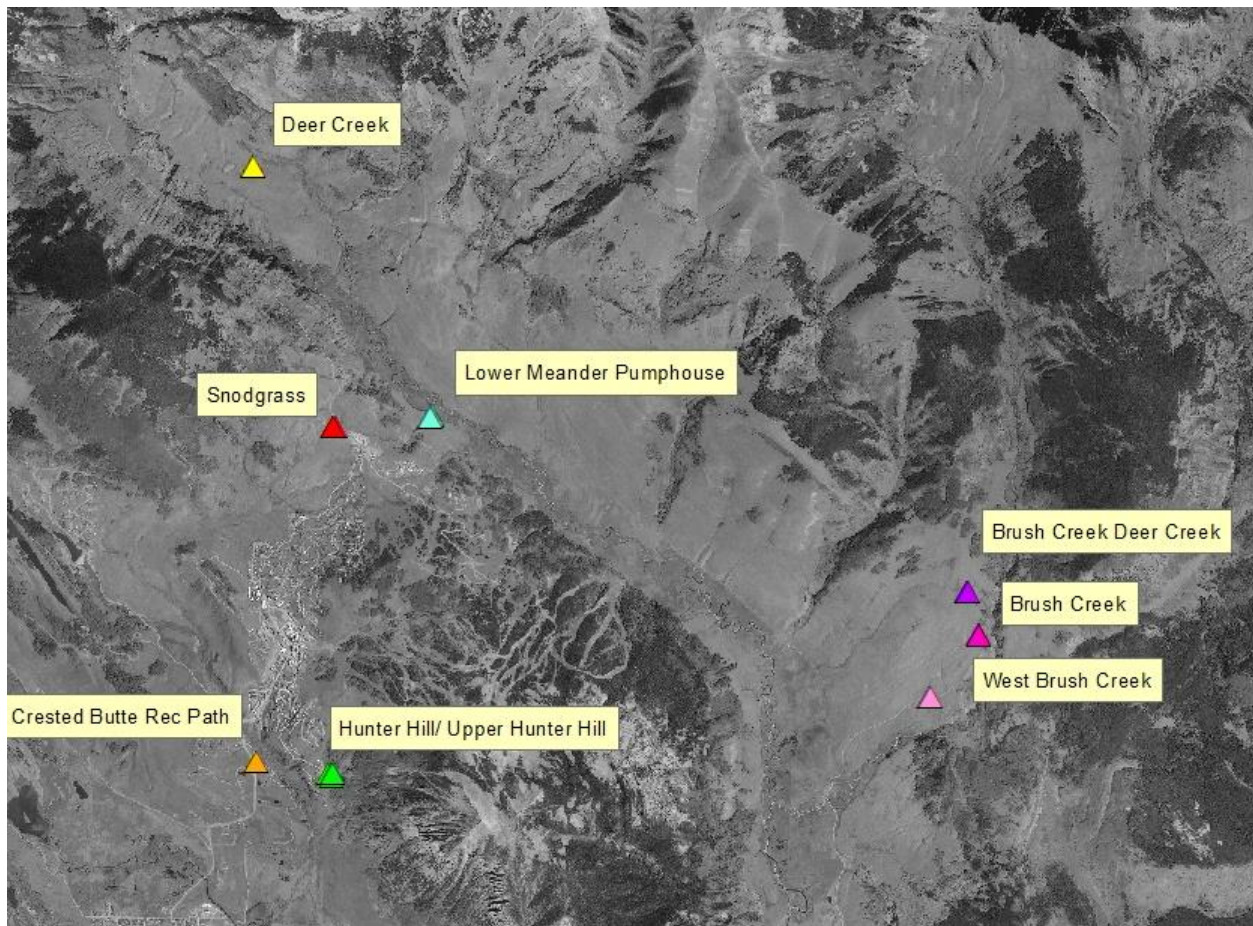


Figure 1. A map of the field sites generated in GIS based on GPS data. Two additional sites (Brush Creek Trail, Gothic Townsite) are located outside of the range of this map.

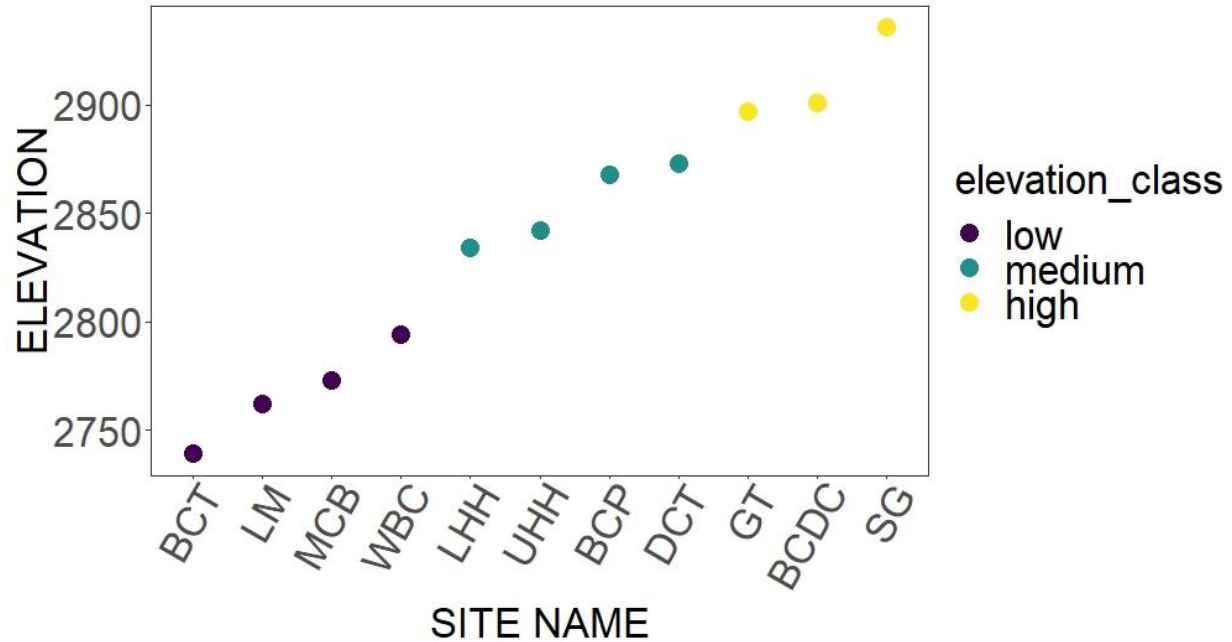


Figure 2. A representation of site elevation and elevation class. BCT = Brush Creek Trail, LM = Lower Meander, MCB = Mount Crested Butte, WBC = West Brush Creek, LHH and UHH = lower and upper Hunter Hill, BCP= Brush Creek Pearl Pass, DCT= Deer Creek Trail, GT = Gothic Townsite, BCDC = Brush Creek Deer Creek, SG= Snodgrass. Elevation class was separated into low (2700-2800m), medium (2800-2900m), and high (2900-3000m).

| Stage | Morphology |
|-------|------------|
| 1     | Rosette    |
| 2     | Bolting    |
| 3     | Flowering  |
| 4     | Seed Set   |
| 5     | Senescence |

Table 1. Phenological stages recorded during field surveys.

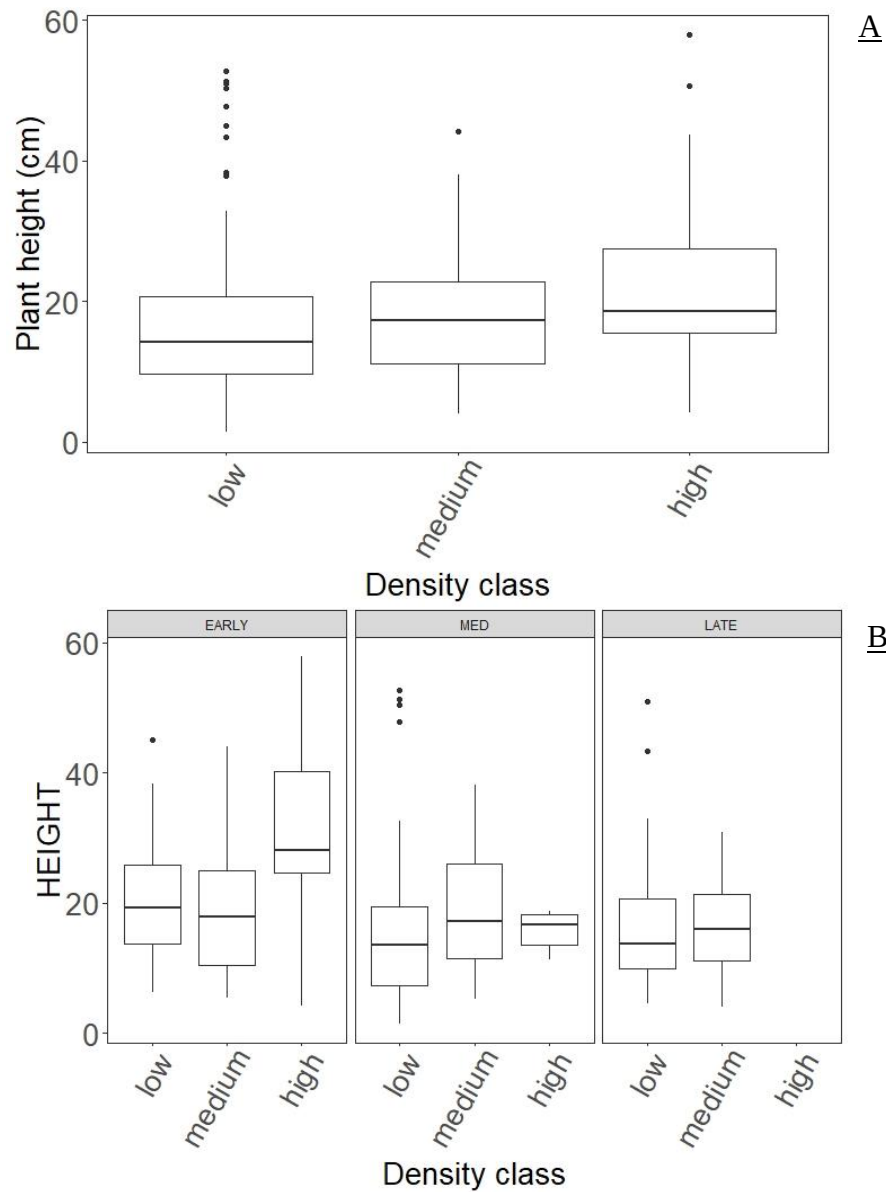


Figure 3A. A boxplot of plant height (cm) separated by density class and 3B. plant height by density class and snow melt timing (early, medium and late). Boxplot summary statistics line indicates median value, boxes indicate interquartile range. Filled circles indicate outliers and whiskers indicate max values within 1.5 times interquartile range

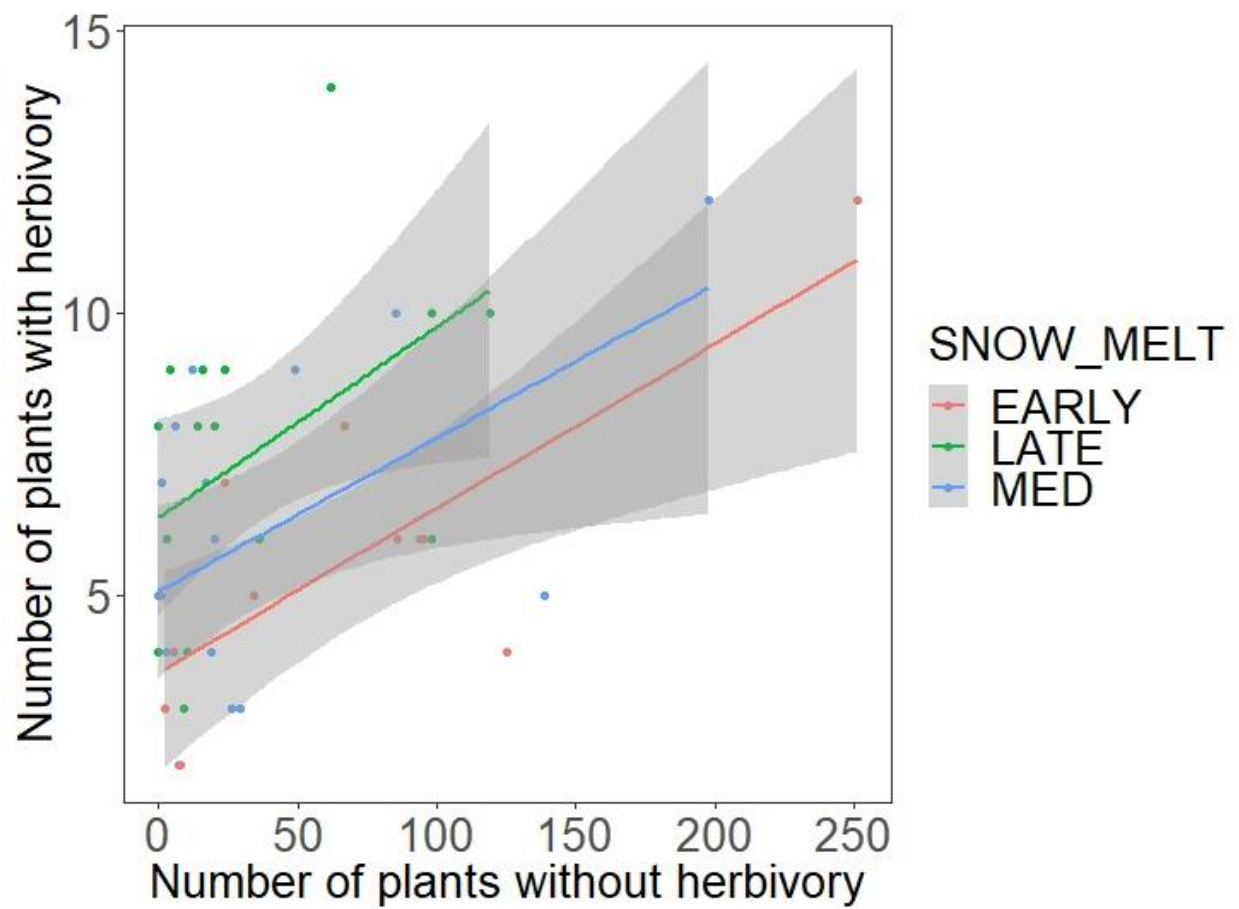


Figure 1 A () A scatter plot comparing the number of plants per quadrat without herbivory with the number of individual plants with herbivory. Regression lines estimated by snow melt classes.

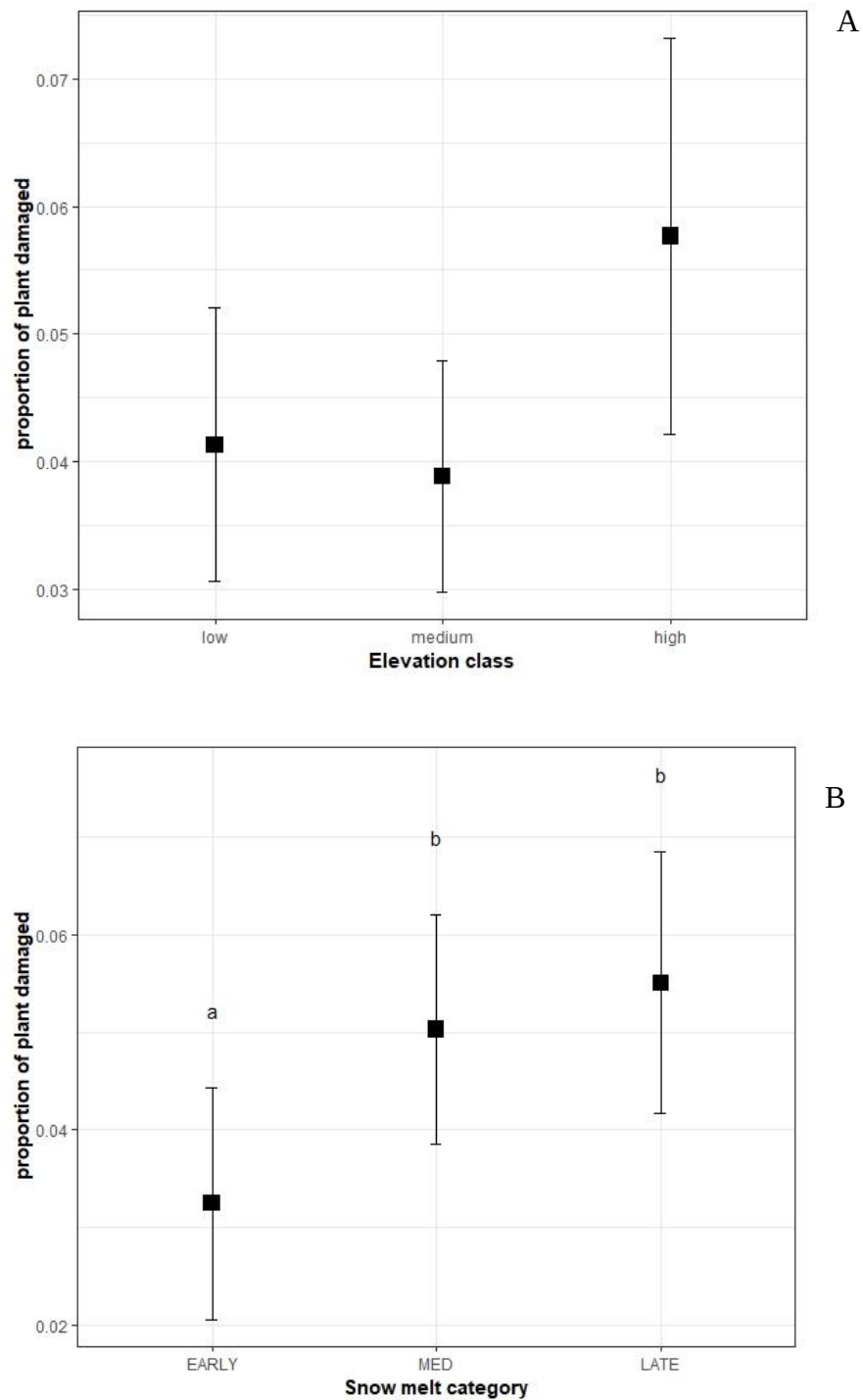


Fig X. A. Estimated proportion of herbivory on a plant by elevation class, high elevation  $\bar{x}$ =0.066, medium elevation  $\bar{x}$ =0.037, low elevation  $\bar{x}$ =0.045 and B. Estimated proportion of herbivory on a plant by snowmelt category, early  $\bar{x}$  = 0.033, med  $\bar{x}$  = 0.046, late  $\bar{x}$  = 0.057