

The spatial variation of leaf traits in *Ipomopsis* according to soil moisture and snow depth

Student: Rina Talaba

Mentor: Diane Campbell

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Abstract

This study analyzed how soil moisture and snow depth correlates with leaf traits of *Ipomopsis aggregata* across and within populations in the Crested Butte and Gothic, Colorado area. Earlier snowmelt due to global warming is lessening soil moisture in early summer in the Rocky Mountains and potentially affecting leaf traits that aid in photosynthesis. We expected that *I. aggregata* plants with low water availability to have leaf traits that more efficiently photosynthesize and conserve water. Variation in soil moisture within sites had weak or no effects on the leaf traits, except that stomatal density increased with soil moisture in sites that also had low snowpack. Sites with lower snow depth had lower leaf water content, lower specific leaf area (SLA), and trichome density but showed no average change in stomatal density and diameter. The variation across sites in leaf water content, SLA, and trichome density could be attributed to aiding in efficient photosynthesis and less transpiration in areas where there is drought-stress.

Introduction

The impacts of climate change on temperature and precipitation often affect soil moisture and, where precipitation declines, can potentially cause droughts that stress plants (Walter 2018). In the Rocky Mountain ecosystem, earlier snowmelt due to warming causes drier soil moisture earlier in the summer (Sherwood 2013). As increasing temperatures cause earlier snowmelt, subalpine plant populations may decrease as shown for *Ipomopsis aggregata*, which experiences reductions in seed production and seedling establishment as well as reduced survival to the year following early snowmelt (Campbell 2018). Apart from whole population effects, these changes in soil moisture with climate change may also influence the expression of traits on individual plants, including leaf traits (Ordoñez 2009).

Only a few studies have examined how timing of snowmelt influences leaf traits. For example, a study of the subalpine plant *Boechera stricta* showed changes in leaf traits in response to earlier snowmelt that aids in the prevention of water loss (Anderson & Gezon 2015). Specific leaf area (SLA), the area of a leaf divided by its dry biomass, was lower for plants in a common garden taken from lower elevation where snowmelt was earlier, as predicted because that trait value conserves water (Ludwig et al. 2004). A previous study of *I. aggregata* also demonstrated higher photosynthetic water-use efficiency when summer precipitation was reduced (Campbell & Wendlandt 2013), although that study did not detect changes in SLA.

Neither of these previous studies, however, directly tied changes in leaf traits to soil moisture, either at the scale of individual plants or among sites that differ in soil moisture, for example because of changes in snowpack. We hypothesize that in areas where soil moisture is low, SLA will be smaller because this trait value conserves water more efficiently. Similarly, trichomes, or leaf hairs, can help prevent water loss by increasing reflection of solar radiation and keeping the leaf cool (Abdulrahman & Oladele 2011). We hypothesize then, that in areas where soil moisture is low, trichome density will be high to reflect solar radiation and to conserve water.

This study focuses on how leaf traits in the subalpine plant *Ipomopsis aggregata* change within and among populations with soil moisture and snowpack. Overall, we expect *I. aggregata* leaf traits to be more efficient in water conservation as water availability is lower.

Research Questions

1. Do *I. aggregata* plants with lower soil moisture show lower leaf water content, lower SLA, higher trichome density, lower stomatal density, and lower stomata diameter compared to other plants in the population? Measuring soil moisture along with these traits will help elucidate mechanisms for any changes in trait expression.
2. Do plants in sites with low snow depth in late spring have lower soil moisture and also lower water content, lower SLA, higher trichome density, lower stomatal density, and lower stomata diameter than plants in other sites? Answering this question will allow us to see if results from smaller scale studies (such as Campbell & Wendlandt 2013) extend to large spatial scales, and whether snowmelt data can be used as a proxy for measurements of soil moisture.

Methods

Study System

Ipomopsis aggregata is a common subalpine plant in the family Polemoniaceae and is native to the Rocky Mountains. *I. aggregata* can stay in a vegetative state from 2 to 10 years before flowering (Campbell 1997). As a vegetative plant, it stays close to the ground and can grow multiple rosettes. Once flowered, it usually dies within the year. For the purposes of this study, we analyzed the leaf traits of vegetative *I. aggregata* plants.

We used three long-term study sites and four new sites. The three long-term sites include: Vera Falls on the Rocky Mountain Biological Laboratory (RMBL) property, Poverty Gulch off of the Slate River, and Maxfield Meadow, 1 km south of RMBL. In each long term site, vegetative rosettes of *I. aggregata* in unmanipulated areas were identified and tagged in 2017-2020. In 2021, we tagged additional *I. aggregata* plants in each location to make a total of 184 plants and measured water content, specific leaf area, trichome density, and stomatal density and diameter. The four new sites were chosen to generate a wider range of date of snowmelt in the spring, estimated from spatial maps of snowpack prepared by Ian Breckheimer from satellite data (Painter 2018). These new site locations include Dry Meadows, Lupine, Avalanche, and Ender's. The seven locations span a range of snowpacks, according to the area's snow depth recorded on

March 31, 2018 (See Table 1). Snowpack on March 31, 2018 correlates highly with the date of snowmelt.

Table 1. Research location sites' approximate location and snow depth (m) from lowest to highest recorded on March 31, 2018.

Location	Approximate Coordinates	Snow Depth (m)
Lupine	N/A	0.00
Dry Meadows	38.9607°N, 106.9842°W	0.39
Ender's	38.9584°N, 106.9872°W	0.46
Maxfield	38.9495°N, 106.9908°W	0.76
Vera Falls	38.5743°N, 106.5933°W	0.78
Avalanche	38.9639°N, 106.9937°W	0.87
Poverty Gulch	38.5721°N, 107.0432°W	0.91

Soil Moisture

Soil Moisture was recorded as a percentage of volumetric water content (VWC%) at 12 cm snow depth. We used a Campbell Scientific Hydrosense II, a probe instrument that records the percentage of water in the soil. Soil moisture was recorded at least twice during the summer for each tagged plant. One of the measurements was taken on a single day across all the sites on July 15, 2021 to account for potential changes in weather. In order to measure VWC for each plant we standardized VWC through the following procedure:

1. Relative VWC = individual plant VWC - overall site mean VWC
2. Averaged relative VWC for each plant across all dates
3. Standardized VWC = mean relative VWC + site mean on July 15, 2021

The standardized VWC of each individual plant was then used for analysis.

Specific Leaf Area (SLA)

The SLA of *I. aggregata*, area of the leaf divided by its dry mass, was obtained through leaf samples acquired from each tagged vegetative plant. Each leaf is then packed into a manila envelope and returned to the laboratory on ice. There we scanned the leaf, and used the program ImageJ to measure the area from the scan. In order to get the dry mass, the scanned leaves were placed into an oven at 65 degrees Celsius for two hours. Once they were dried, they were weighed individually.

Water Content

In order to find the water content of the *I. aggregata* leaves, the collected samples were individually weighed before and after being dried as described above. Collected samples were weighed to ± 0.0001 g before drying in a convection oven. The oven was preheated to 65°C and the leaves were inserted to dry for two hours. After drying, the leaves were reweighed and the difference of weight before and after drying was recorded as the water content percentage of the leaf.

Trichome Density

Trichome density is described as the ratio of number of leaf hairs divided by the area of the leaf. Similarly, trichome density was measured according to a scanned cumulative sheet of all the leaves. All the individual trichomes on each of the leaves were recorded. Total trichomes was divided by the SLA to acquire the trichome density.

Stomatal Density

Stomatal density is the ratio of stomata to the area of the leaf. In order to study *I. aggregata* stomata, acrylic imprints of the leaves were copied onto glass slides. Using simple acrylic, the backside of *I. aggregata* leaves were painted and removed using clear tape. The first three base leaflets of a single leaf were chosen to imprint. The acrylic imprint was then added onto a slide and analyzed under a compound microscope using a 10x/0.25. Stomata were counted and divided by the squared counted in a 10x10 grid in the microscope eyepiece to acquire the stomatal density. The length of the 10x10 grid was measured to be 1 cm making a 1 cm² area.

Stomata Diameter

The stomata diameter was measured with the same acrylic leaf imprints used for stomatal density. Using a 100x/1.25 eyepiece, stomata were measured in micrometers using a 10x10 microscope eyepiece grid. The length of the 10x10 grid was measured to be 10 μ m and thus each 1 μ m square was used to measure the diameter. Three stomata from each leaf were measured and averaged to get the final stomata diameter.

Data Analysis

We used analysis of covariance (ANCOVA) for each trait measured at the seven long-term sites. The model had fixed effects of the factor site, the continuous variable of soil moisture, and their interaction. In this ANCOVA, a positive slope of SLA on soil moisture, and negative slopes of trichome density and WUE, would support the hypotheses under question 1. To answer question 2, we examined differences among sites in the ANCOVA and also separately ran a regression of each leaf trait on snow pack at its site.

Results

Soil Moisture and Snow Depth

There was no significant relationship found between the environmental variables of soil moisture in mid-summer and snow depth in late spring ($P < 0.05$; See Figure 1).

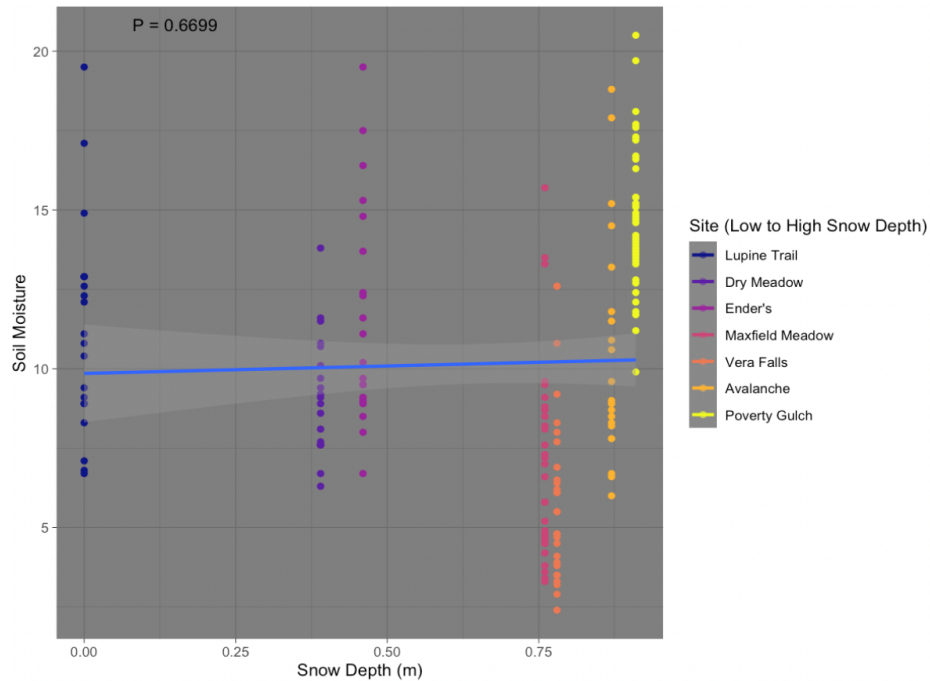


Figure 1. Linear regression between snow depth (m) on March 31, 2018 and soil moisture on July 15, 2021.

Leaf traits of I. aggregata according to soil moisture

In the ANCOVA, there was no main effect of soil moisture on any of the leaf traits (all $P > 0.05$; See Table 1 & Figure 2). However, the relationship of stomatal density to soil moisture varied according to site ($P = 0.0312$), with positive slopes in the low snowpack sites (e.g. Lupine, Enders, Dry Meadow) and negative slopes in the other sites (Figure 2). Since there was no detectable interaction between site and soil moisture for the other leaf traits, we removed the interaction term from the model and reran the ANCOVAs with just main effects to test for average effects of site. Using those simplified models, all other leaf traits differed among sites (all $P < 0.0001$; Table 2).

Table 2. P-values for the relationship between leaf traits and soil moisture, site, and the interaction of soil moisture and site. The interaction was dropped from the model if it was not statistically significant ($P > 0.05$).

Trait	Soil Moisture	Site	Soil Moisture x Site
Stomata Density	0.1205	0.1159	0.0312
Trichome Density	0.8048	<0.0001	-
Specific Leaf Area	0.9770	<0.0001	-
Water Content	0.3366	<0.0001	-
Stomata Diameter	0.6339	<0.0001	-

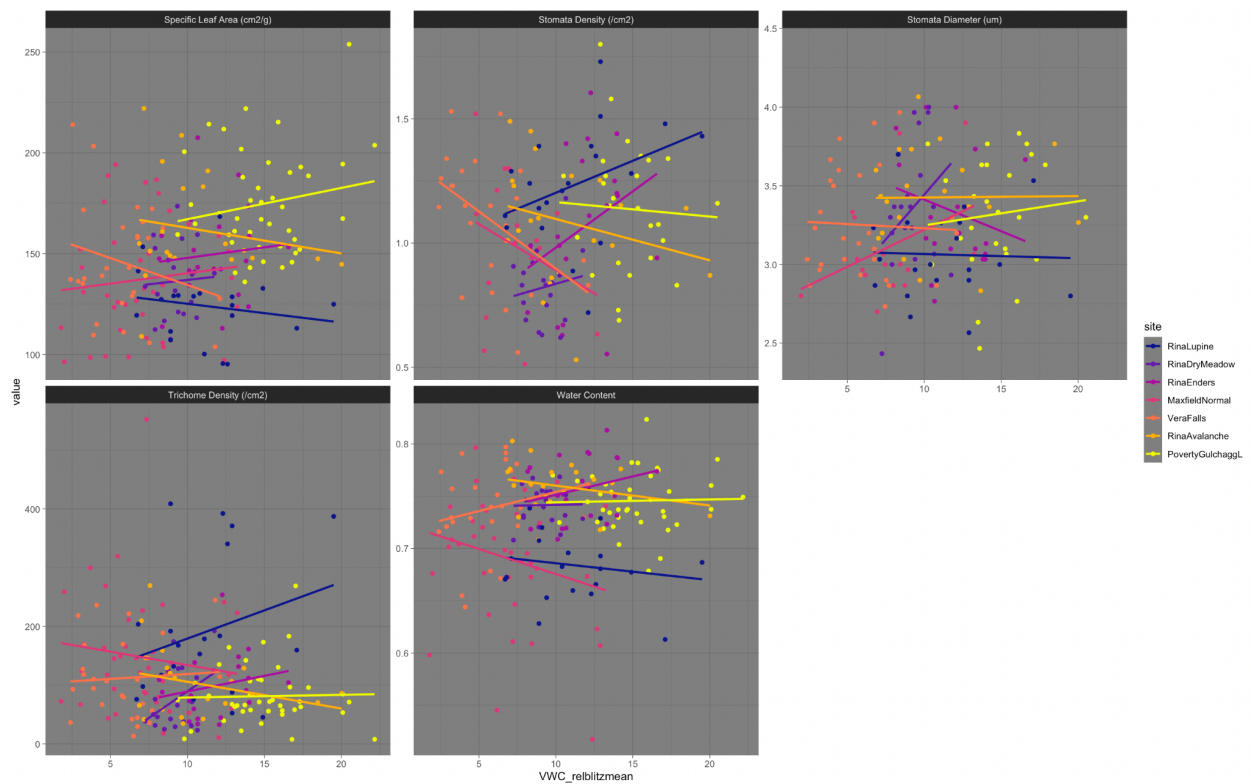


Figure 2. Linear regression of the relationship between leaf traits and soil moisture in each of the sites

Leaf traits of I. aggregata according to varied snow depth

The specific leaf area (SLA) of *I. aggregata* is larger as the snow depth is higher, thus a positive relationship exists between SLA and snow depth ($P < 0.0001$; Figure 3). Stomata density ($P = 0.8014$; Figure 3) and stomata diameter ($P = 0.1727$; Figure 3) did not have significant relationships with snow depth. Trichome density was found to have a negative relationship with snow depth ($P = 0.0008$; Figure 3). Leaf water content had a positive relationship with snow depth ($P = 0.0008$; Figure 3).

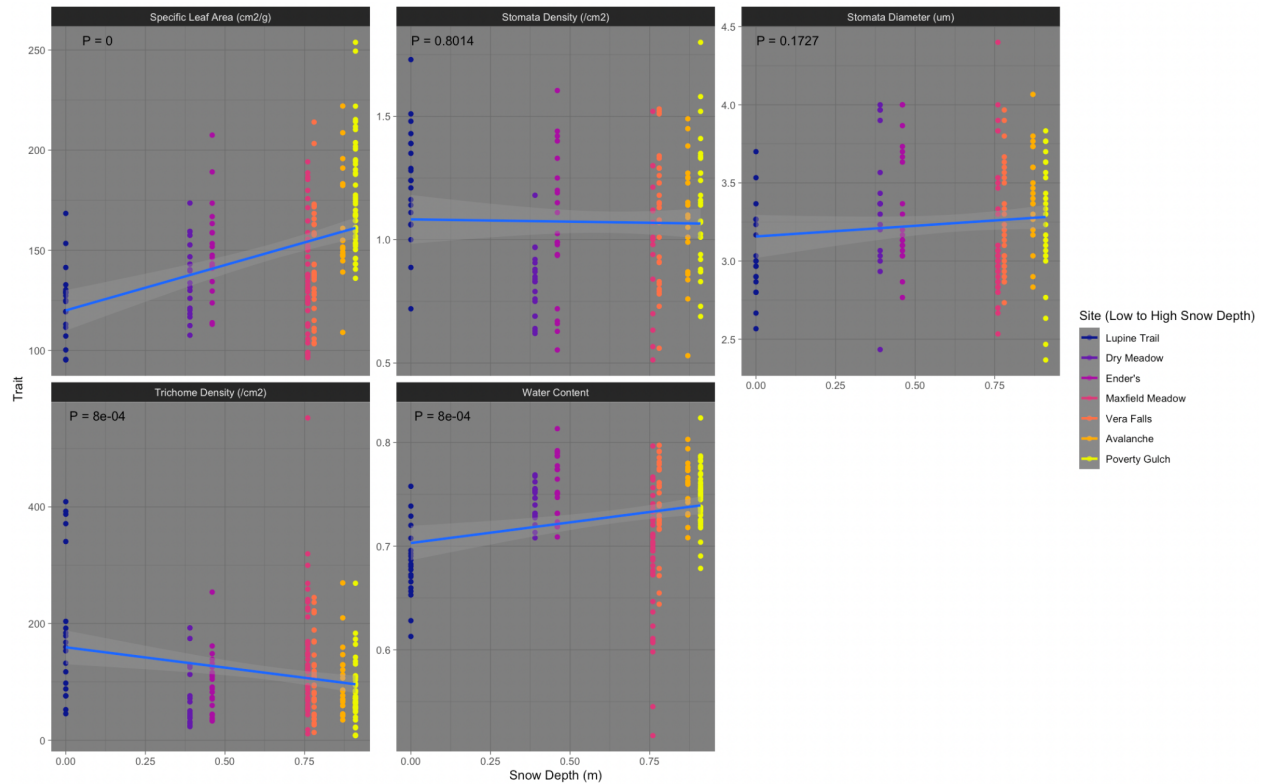


Figure 3. Specific leaf area, stomata density, stomata diameter, trichome density, and water content according to snow depth.

Correlations Between Different Leaf Traits

Between all the leaf traits measured there was no significant correlation ($P > 0.05$) aside from the relationship between SLA and water content and between SLA and trichome density. SLA and water content were positively correlated ($P < 0.05$) in a non-linear way ($P < 0.05$; Figure 4). SLA and trichome density were negatively correlated ($P < 0.05$) and also in a nonlinear way ($P < 0.05$; Figure 5).

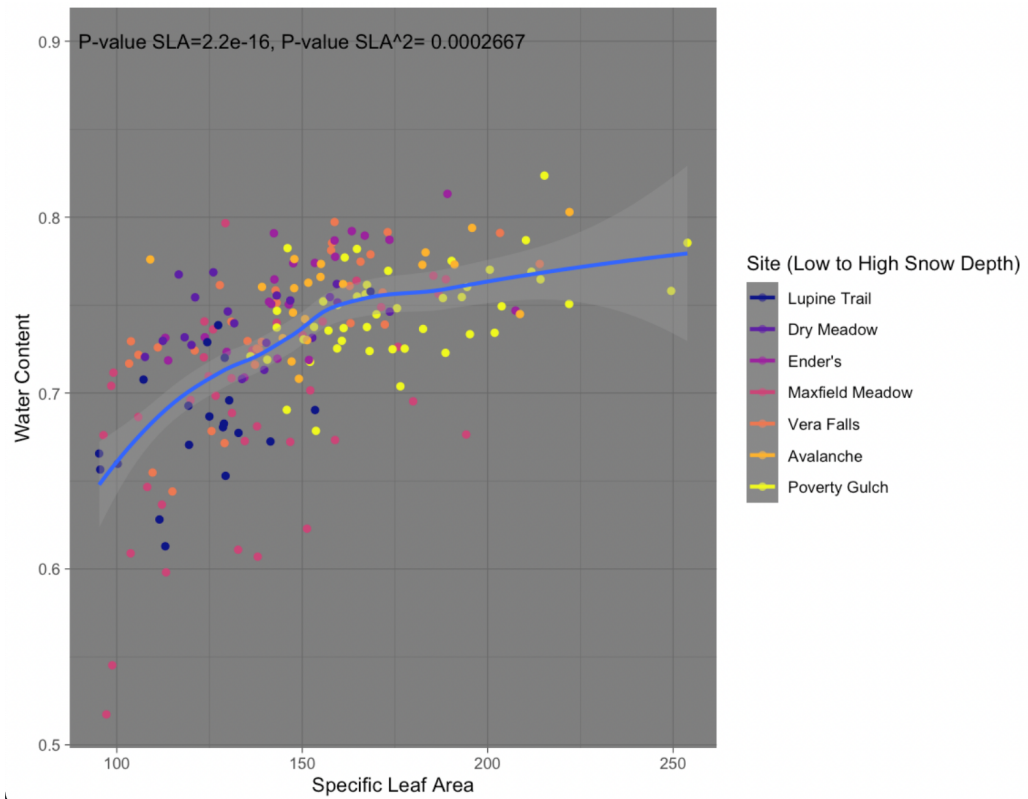


Figure 4. Quadratic loess regression between specific leaf area and water content. Individual plants are differentiated according to research sites.

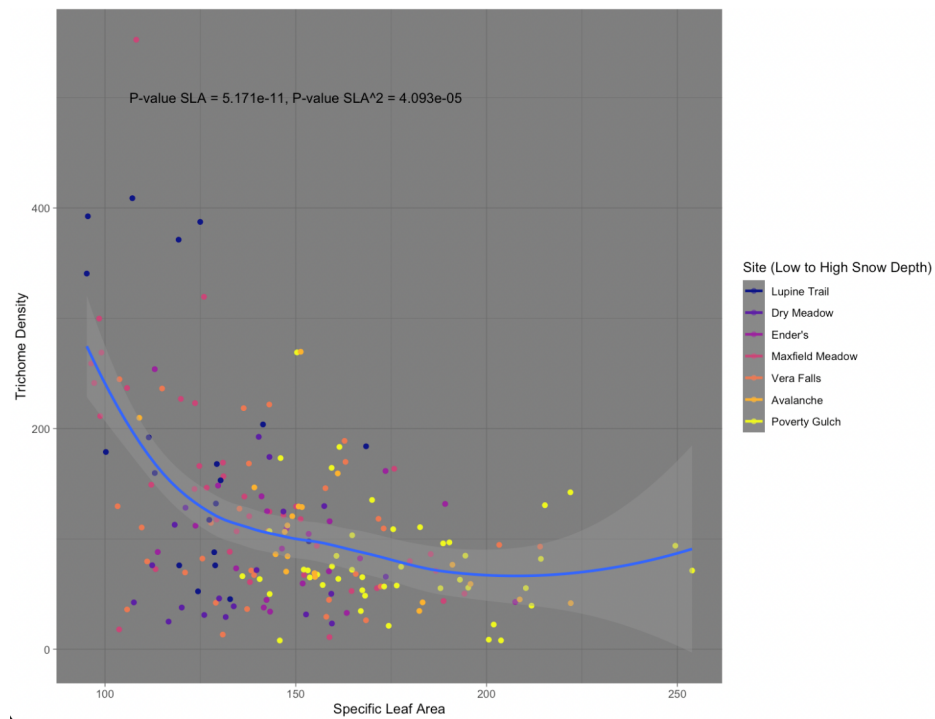


Figure 5. Quadratic loess regression between trichome density and specific leaf area. Individual plants are differentiated according to research sites.

Discussion

By analyzing soil moisture and snow depth we were able to observe spatial effects on certain leaf traits in *Ipomopsis aggregata*. Snow depth was not determined to be a viable proxy for soil moisture in mid-summer as there was no detectable relationship between the two environmental variables. This outcome went against our hypothesis that suggested that varying snow depth can predict soil moisture. Our measurements of soil moisture were taken, however, in mid-summer (July 15) after the monsoon rains had begun in 2021 (June 24, gothicwx.org) and only one day after a rain. It would be very useful to retest this relationship throughout June when the impacts of snowmelt timing are most likely to occur. The strongest effects on leaf traits we found were due to snow depth in late spring, rather than soil moisture in mid-July, suggesting that environmental conditions earlier in the year might be more important. Snowpack, however, can remain on the Rocky Mountains throughout the summer, suggesting that snow depth could have once been a stronger determining factor of summer soil moisture but no longer is due to the earlier snowmelt recorded (Sherwood 2013).

Within sites, plants with lower soil moisture did not have lower leaf water content, lower SLA, higher trichome density, lower stomatal density, or lower stomata diameter as originally hypothesized. However, we found that low snow depth sites had plants with lower leaf water content, lower SLA, and higher trichome density than plants in higher snowpack sites despite there being no changes in stomatal density or diameter. Such relationships potentially suggest these traits could aid in efficient gas exchange and minimal transpiration under low water availability early in the growing season. Some traits (e.g. SLA, leaf water content, trichome density) were intercorrelated, suggesting that they may act together to influence gas exchange.

Soil Moisture and Leaf Traits

Variation in soil moisture within a site affected only the leaf trait of stomatal density, with a positive relationship specifically in the three lowest snow depth sites--Lupine Trail, Dry Meadows, and Enders. A potential reason for this trend is that non drought-stressed plants can have higher stomatal density to increase leaf gas exchange without the concern for water loss. Though this trait may seem beneficial to reduce water loss, a study done by Hughes et al. (2017) suggests that reduced transpiration results in less nutrition uptake in comparison to other plants with high transpiration. When it comes to the variation of *I. aggregata* leaf traits, we should be concerned whether drought-stressed plants are negatively affected.

Snow Depth and Leaf Traits

Snow depth had a positive relationship with water content and SLA but also a negative relationship with trichome density, however, stomatal density and diameter remained the same. In low snow depth sites, these traits can be interpreted as efficient ways to avoid water loss. High snow depth sites may have higher water availability earlier in the year, making those trait values less adaptive.

Leaf water content was lower in sites where there was low snow depth. These low snow depth *I. aggregata* plants also have more compact and thicker leaves which may help to retain water where there was little available. In a similar vein, trichome density which is said to reflect solar radiation to prevent transpiration, is a trait that low snow depth *I. aggregata* have. All of these traits suggest plasticity or genetic changes that help to retain water. When it comes to stomatal density and diameter, we found no correlation with snow depth.

These trait variations should be particularly of concern when thinking about how they can affect plants long-term. We can similarly see variations in plant traits according to varying site dryness in a study done by Anderson and Gezon (2015). Manipulating snowmelt to occur earlier, and thus induce drier conditions, found *Boechera stricta* flowering times to also occur earlier (Anderson & Gezon 2015). These earlier flower times lower the fitness of *B. stricta* when combined with traits of high-elevation plants such as smaller flowers and shorter flowering durations (Anderson & Gezon 2015). Future considerations should thus include how *I. aggregata* leaf trait variation may affect individual fitness.

Leaf Trait Correlations

Across all sites, we found there to be a positive relationship between SLA and water content and a negative relationship between SLA and trichome density. As leaves are thicker and more compact there is less water contained than in leaves that are flatter. These thicker leaves are more evident within sites with low snow depth.

Leaves with low SLA were found to have higher trichome density. Varying SLA suggests varying availability of water which may indirectly affect trichome count.

Potential causes of leaf trait variation

Several leaf traits, including SLA and trichome density, but not stomatal characteristics, varied across sites that differed in snowpack depth in early spring. If snow depth determines the soil moisture earlier in the year, then its effects may have influenced leaf traits through adaptive plasticity or genetic variation. Adaptive plasticity would occur if leaf water content, SLA, and trichome density respond directly to snow depth and thus early soil moisture, and those plastic changes increase the fitness of the plant. An example of plasticity can be seen in a study done by Caruso (2016), who by manipulating soil moisture conditions, found plasticity in inflorescence. Genetic variation, on the other hand, would occur if differences in leaf trait alleles are due to differences in natural selection in different environmental conditions that influenced leaf traits through natural selection. For example, we can see genetic variation across different oceanic islands of the same endemic plant species who have stayed on separate islands for generations (Stuessy et al. 2014). Though we saw variation in leaf traits across sites of differing water availability, it is unclear whether the leaf traits aid in fitness at low snow depth sites. Despite the trait of thicker, compact leaves, water content is lower in those leaves that are at low snow depth sites. This relationship suggests that *I. aggregata* is conserving water through reducing transpiration rather than maximizing photosynthesis at those sites.

To see if the spatial variation of leaf traits is aiding in gas exchange, future research should analyze the effects on water use efficiency (WUE), the ratio of photosynthetic rate to stomatal conductance. Recording WUE across sites of varying snow depth may give us insight into whether photosynthesis is maximized where there is high water availability. If photosynthetic rate remains the same at all sites, then these varied leaf traits could be helping potentially drought-stressed plants to conserve water through higher water-use efficiency.

Cited Sources

- Abdulrahaman, A. A., & Oladele, F. A. (2011). Response of trichomes to water stress in two species of *Jatropha*. *Insight Botany*, 1(2), 12.
- Anderson, J. T., & Gezon, Z. J. (2015). Plasticity in functional traits in the context of climate change: a case study of the subalpine forb *Boechera stricta* (Brassicaceae). *Global change biology*, 21(4), 1689-1703.
- Campbell, D. R. (2019). Early snowmelt projected to cause population decline in a subalpine plant. *Proceedings of the National Academy of Sciences*, 116(26), 12901-12906.
- Campbell, D. R., & Wendlandt, C. (2013). Altered precipitation affects plant hybrids differently than their parental species. *American Journal of Botany*, 100(7), 1322-1331.
- Campbell, D. R. (1997). Genetic and environmental variation in life-history traits of a monocarpic perennial: a decade-long field experiment. *Evolution*, 51(2), 373-382.
- Caruso, C. M. (2006). Plasticity of inflorescence traits in *Lobelia siphilitica* (Lobeliaceae) in response to soil water availability. *American Journal of Botany*, 93(4), 531-538.
- Hughes, J., Hepworth, C., Dutton, C., Dunn, J. A., Hunt, L., Stephens, J., ... & Gray, J. E. (2017). Reducing stomatal density in barley improves drought tolerance without impacting on yield. *Plant physiology*, 174(2), 776-787.
- Jaleel, C. A., Gopi, R., Sankar, B., Gomathinayagam, M., & Panneerselvam, R. (2008). Differential responses in water use efficiency in two varieties of *Catharanthus roseus* under drought stress. *Comptes Rendus Biologies*, 331(1), 42-47.
- Ludwig, F., Rosenthal, D. M., Johnston, J. A., Kane, N., Gross, B. L., Lexer, C., ... & Donovan, L. A. (2004). Selection on leaf ecophysiological traits in a desert hybrid *Helianthus* species and early-generation hybrids. *Evolution*, 58(12), 2682-2692.
- Ordoñez, J. C., Van Bodegom, P. M., Witte, J. P. M., Wright, I. J., Reich, P. B., & Aerts, R. (2009). A global study of relationships between leaf traits, climate and soil
- Painter, T. 2018. ASO L4 Lidar Snow Depth 3m UTM Grid, Version 1. Boulder, Colorado USA. NASA National Snow and Ice Data Center Distributed Active Archive Center. doi: <https://doi.org/10.5067/KIE9QNVG7HP0>.
- Sherwood, J. (2013). Experimentally simulating environmental change in a montane meadow system via reduced snowpack and passive warming: soil and plant responses.
- Stuessy, T. F., Takayama, K., López-Sepúlveda, P., & Crawford, D. J. (2014). Interpretation of patterns of genetic variation in endemic plant species of oceanic islands. *Botanical Journal of the Linnean Society*, 174(3), 276-288.
- Walter, J. (2018). Effects of changes in soil moisture and precipitation patterns on plant-mediated biotic interactions in terrestrial ecosystems. *Plant Ecology*, 219(12), 1449-1462.