

Snowmelt Timing Leads to Plasticity and
Alters Natural Selection on Leaf Traits in
Ipomopsis

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Abstract

Anthropogenic climate change is altering environments, which in turn has the potential to influence plant traits (through phenotypic plasticity) and natural selection. The effect of changing climate on the strength and direction of selection remains unresolved. In this study we quantified the plasticity and strength of selection on vegetative traits of the subalpine herbs *Ipomopsis aggregata*, *Ipomopsis tenuituba*, and hybrids. We used long-term data to quantify phenotypic plasticity in these traits and their effects on fitness over 12 years with varying dates of snowmelt in the Colorado Rocky Mountains. We hypothesized that the vegetative traits would be under selection that depended on snowmelt date, which affects early-season water availability and temperature. Specific leaf area (SLA) responded plastically to snowmelt timing, with lower SLA found in early snowmelt years and larger SLA found in late snowmelt years. Natural selection on water use efficiency (WUE) changed based on snowmelt date, with early snowmelt correlating with more positive selection on WUE and late snowmelt correlating with negative selection on WUE. Both plant traits and selection were affected by snowmelt timing and both responded in ways that theoretically should aid in acclimation and adaptation to drier conditions for *Ipomopsis*. Our results highlight how climate change can impact traits and alter natural selection by modifying abiotic factors such as snowmelt timing.

Introduction

Climate change is altering the distribution of organisms in diverse ecosystems ([Parmesan, 2006](#)). Yet, it remains largely unknown how the changing climate will impact trait values and impact evolution through a change in natural selection ([Hoffmann & Sgro, 2011](#)). Phenotypic plasticity refers to direct changes in expression of trait values with a change in environmental conditions ([Bradshaw, 1965](#)). This plasticity can be governed by environmentally-induced epigenetic changes such as histone modifications and DNA methylation ([Duncan et al., 2014](#)) and is hypothesized to help organisms combat environmental instability.

In addition to plastic responses, organisms can adapt to new environments through evolution. Evolutionary rescue is when adaptive evolution counters the decline of a population at risk of extinction when organisms are faced with new forms of natural selection in a changing environment ([Carlson et al., 2014](#)). The theoretical pattern of evolutionary rescue is characterized by population decline followed by recovery due to adaptive evolution to new conditions ([Gomulkiewicz & Shaw, 2013](#)). However, it is challenging to predict whether evolution can rescue populations facing novel environmental threats ([Gomulkiewicz & Shaw, 2013](#)), such as climate change. This research is part of a long-term study to understand how climate leads to adaptive evolution by influencing trait expression, selection and population growth.

Previous studies on plants have demonstrated potential ways in which climate change can impact evolution and plasticity. For example, *Boechera stricta* populations exposed to warmer and drier conditions have been shown to decline in genetic diversity, which can negatively impact the future survival of the population ([Bemmels & Anderson, 2019](#)). In addition, selection on floral traits in *Ipomopsis* is associated with the changing climate, potentially due to drier conditions in years of early snowmelt as well as changes in pollinator abundance and distribution ([Campbell &](#)

[Powers, 2015](#)). In *Ipomopsis*, vegetative traits can exhibit phenotypic plasticity based on changes in environmental conditions ([Navarro et al., 2022](#)). For example, summer precipitation and snowmelt timing impact vegetative traits such as stomatal conductance and water use efficiency (WUE) ([Navarro et al., 2022](#)).

Mountains in Colorado are experiencing shifts towards earlier snowmelt and snowmelt runoff ([Clow 2010](#)). Earlier snowmelt in Colorado lengthens the period of the foresummer drought, the period between snowmelt and onset of the summer monsoon rains, leading to drier conditions ([Sloat et al. 2015](#)). Water availability is expected to cause differences in selection on vegetative traits ([Dudley 1996](#)). The shift to earlier snowmelt in theory should favor selection for vegetative traits that maintain fitness in drier conditions.

Using a long-term data set on the subalpine plants *Ipomopsis aggregata*, *tenuituba*, and hybrids, I addressed the following hypotheses. Plants will respond plastically to the date of snowmelt in the spring, with lower specific leaf area, higher trichome density, higher water use efficiency and lower percent leaf water content in years of early snowmelt (Table 1). Selection will favor smaller specific leaf area in early snowmelt years and larger specific leaf area in late snowmelt years (Table 2), as thick leaves conserve water by reducing surface area and are often found in dry environments ([Poorter et al., 2009](#)). High trichome density will be selected for in years with earlier snowmelt (Table 2), as previous studies have indicated that trichome density increases during drought conditions ([Shahzad et al., 2021](#)). This may be because trichomes can reflect sunlight and may play a role in water retention ([Bickford, 2016](#)). Selection on water use efficiency will exhibit environmental sensitivity, with stronger positive selection in years with early snowmelt (Table 2), as drier conditions favor increased water retention.

Table 1. Hypotheses for the effect of snowmelt timing on plasticity on three vegetative traits.

<i>Trait</i>	<i>Plasticity with early snowmelt date</i>
Water use efficiency	Increase in water use efficiency
Trichome density	Increase in trichome density
Specific leaf area	Decrease in specific leaf area
Percent leaf water content	Decrease in percent leaf water content

Table 2. Hypotheses for the effect of snowmelt timing on selection for three vegetative traits.

<i>Trait</i>	<i>Selection with early snowmelt date</i>
Water use efficiency	Stronger for increase in water use efficiency
Trichome density	Stronger for higher trichome density
Specific leaf area	Stronger for lower specific leaf area

Methods

Study Site and Plants

We measured natural selection on vegetative traits of plants of the subalpine herbs *Ipomopsis aggregata*, *I. tenuituba* and their advanced generation hybrids of *I. aggregata* x *I. tenuituba* at three sites (Fig. 1) at Poverty Gulch, Gunnison County, Colorado, USA. The use of hybrids allowed us to examine a wider range of phenotypic traits and increased our ability to make generalizations to other plants and species. At Poverty Gulch, *I. aggregata* grows at elevations of 2900 m and below, *I. tenuituba* grows above 3100 m, and the hybrids grow in between (Fig. 1). Plants spend 2 to 10+ years as a vegetative rosette of leaves before flowering in a single season and then dying. The plant sites have been previously described in [\(Campbell et al. 1997\)](#). Traits and fitness were measured on two sets of plants. One set consists of plants started as seed in common gardens in 2007 and 2008 [\(Campbell & Powers, 2015\)](#) and was measured for vegetative traits in 2009 through 2014, with fitness measures continuing to 2018. The common gardens (planted at an *I. aggregata* site and at a hybrid site) included seeds of crosses between two *I. aggregata* plants, between two *I. tenuituba* plants, and F₁ and F₂ hybrids. The other set consists of in situ plants measured for vegetative traits in 2017 to 2022, with fitness measures from 2018 to 2022. Fitness for plants measured in 2022 will not be available until 2023. We also included a site in the East River Valley, “Vera Falls” (Fig. 2), at which natural plants of *I. aggregata* were measured from 2017 to 2022. We collected the same vegetative traits and used the same survival measures as we did at the Poverty Gulch sites.

Measurement of Vegetative Traits and Fitness

To add to the long-term data set, in 2022 we measured 62 previously and newly marked vegetative plants at the low site (*Ipomopsis aggregata*) and 30 at the hybrid site at Poverty Gulch for a total of 92 plants (Fig. 1). At each site four vegetative traits were measured: specific leaf area (SLA), trichome density, water use efficiency (WUE) and percent leaf water content. Early in the growing season (22/06/2022-29/06/2022) we collected one green leaf at random from all sampled plants. The leaves were stored in coin envelopes in a cooler and transported back to the lab. Before drying, the leaves were scanned (with the side of the leaf with trichomes facing down) in grayscale at 600 dots per inch (dpi). The wet weights of the leaves were measured on a balance to the nearest 0.00001 g. The leaves were then placed back in the coin envelope to bake in a drying oven at 70°C for 2 hours. The dried leaves were weighed using the same scale and precision as the wet weights. We calculated percent leaf water content by finding the difference between wet weight and weight and dividing that by the wet weight. We used ImageJ software to calculate the leaf area in cm². Trichomes were counted by hand using the scanned image at 200% magnification, and trichome density was calculated as trichomes/cm². To calculate the photosynthetic rate and WUE we used the LI-COR 6400 XT Portable Photosynthesis System. Each marked vegetative plant at both sites was measured between the hours 8:00 to 13:00 when plants are actively photosynthesizing. Measurements were taken using saturating light conditions (PAR = 1800 $\mu\text{mol}/\text{m}^2/\text{s}$), CO₂ concentration of 400 ppm, and leaf temperature of 27 °C following previous studies of *Ipomopsis* [\(Navarro et al., 2022\)](#). Two measurements per plant were logged and values averaged prior to analysis. To calculate photosynthetic rate per area and

conductance per area we obtained the area of the leaf inside the leaf chamber of the LI-COR 6400 XT. We did this by using a razor blade to cut the portion of the leaf that was in the leaf chamber and then scanned these portions and used ImageJ software to calculate the selected portion of the leaf area in cm^2 . To calculate the WUE we divided the light-saturated photosynthetic rate by the light-saturated stomatal conductance. To measure fitness we used three indices: survival to the next year, flowering in the next year and ever flowering (survival to flowering across the whole timespan)

We analyzed relationships of these plant traits to soil moisture in 2022. Soil moisture was measured as volumetric water content (vwc) using a Campbell Hydrosense 12 cm probe inserted into the soil near the base of the plant at the time a leaf was collected.

Statistical Analyses

We separately analyzed how four vegetative traits in 2022 (trichome density, specific leaf area, WUE and percent leaf water content) related to soil moisture using a model with the factor of site along with the continuous variable of vwc and the interaction between site and vwc. We included site as a factor to test whether the leaf trait really related to soil moisture rather than differences arising between the species of plant inhabiting locations with different soil moistures.

Phenotypic plasticity was assessed by using a linear mixed model to regress the plants' trait values in a given year against the date of snowmelt in the spring, using data from billy barr at the Rocky Mountain Biological Laboratory (RMBL, gothicwx.org). That site is 8 km distant from the nearest site at Poverty Gulch, but year to year variation in snowmelt date correlates well with year to year variation at Poverty Gulch ([Campbell & Powers, 2015](#)). As some plants were measured in multiple years, we did analyses with individual plants that were measured repeatedly, to test for developmental flexibility at the individual level. For that analysis, we used linear mixed models (function lmer in R) to model the trait value as a function of snowmelt date, the factor of site, the species, all interactions, and a random factor of the individual plant id. Species were categorized as *I. aggregata*, hybrid, or *I. tenuituba*.

Natural selection in a given year was quantified by regressing one of three binary fitness metrics on the trait value for an individual plant. The binary fitness metrics included survival (died = 0, lived = 1), flowering (did not flower = 0, flowered = 1), or ever flowering (never flowered = 0, flowered = 1). The intensity of selection was quantified by the standardized selection differential, which equals the slope of relative fitness (fitness divided by mean fitness) on the trait value in units of standard deviation ([Kingsolver et al. 2001](#)). To see how selection depends on snowmelt date, the standardized selection differential for a given year was then regressed against snowmelt date in the spring.

Results

Relationship of leaf traits to soil moisture in 2022

We found no statistically significant relationship between morphological leaf traits (trichome density, specific leaf area, and percent leaf water content) and soil moisture in 2022 ($P = 0.58$,

0.64 and 0.08 respectively; Table 3). Water-use efficiency, however, declined with increasing soil moisture ($P < 0.05$; Table 3), with the effect particularly evident at the hybrid site (Fig. 3). We found no statistically significant relationships of the other physiological traits (photosynthetic rate per area and conductance per area) to soil moisture in 2022 ($P = 0.37$ and 0.33 respectively). We also found that plants at the hybrid site had higher percent leaf water content than *I. aggregata* ($P = 0.02$; Fig. 4), but the sites did not differ significantly in SLA or trichome density (both $P > 0.05$).

Table 3. P values to test for relationship of leaf traits to soil moisture in 2022. The statistical model included site as a factor along with soil moisture as a continuous variable and their interaction.

<i>Trait</i>	<i>P Value for effect of soil moisture (vwc)</i>
Trichome density	0.58
Specific leaf area	0.64
Percent leaf water content	0.08
Photosynthetic rate per area avg	0.37
Conductance per area	0.33
WUE	0.0045*

* = $P < 0.05$.

Phenotypic plasticity of vegetative traits in response to snowmelt timing

In order to test for plasticity of vegetative traits we used a repeated measure analysis to examine how individual plants responded to snowmelt day across a span of years. Specific leaf area was shown to be plastic with meltdates ($p < 0.01$; Table 4; Fig. 5). However, the other tested vegetative traits (trichome density, photosynthetic rate, conductance, and WUE) did not show any significant relationship with snowmelt through the years (Table 4). In addition, we graphed SLA of individual plants across varying years to illustrate that individual plants show plasticity (Fig. 6).

Table 4. P values to test for plasticity of vegetative traits in response to meltdates.

<i>Trait</i>	<i>P Value</i>
Specific leaf area	$P < 0.01$ *
Trichome density	0.85
Photosynthetic rate	0.80

Stomatal conductance	0.08
WUE	0.26

* = $P < 0.05$.

Natural Selection

To analyze if natural selection changed with snowmelt timing we compared the standardized selection differential on different vegetative traits to the snowmelt date in the years between 2009-2022. We found a negative relationship between the standardized selection differential for WUE and snowmelt date where the fitness measure was whether the plant ever flowered ($P = 0.03$, slope = -0.0067 change of selection/day; Table 5, Fig. 7). WUE had positive selection in early snowmelt years and negative selection in late snowmelt years (Table 5). Selection on SLA was typically negative when the fitness measure was flowering (Range of $S = -0.67 - +0.11$; Effect of SLA on fitness (based on ever flowered) $P = 0.01$; Table 5, Fig. 7).

Table 5: Snowmelt date affect on selection differential on WUE and SLA

<i>Year</i>	<i>Snowmelt Date Jan 1 = 1</i>	<i>Selection differential on WUE (based on ever flowered)</i>	<i>Selection differential on SLA (based on flowering)</i>
2009	131	-0.026 +/- 0.002	-0.640 +/- 0.007
2010	132	-0.086 +/- 0.002	0.672 +/- 0.007
2011	158	No data	-0.513 +/- 0.007
2012	114	0.061 +/- 0.002	-0.475 +/- 0.007
2013	135	0.005 +/- 0.002	-0.419 +/- 0.007
2014	146	No data	0.095 +/- 0.007
2017	144	-0.124 +/- 0.002	-0.349 +/- 0.007
2018	125	-0.064 +/- 0.002	-0.050 +/- 0.007
2019	157	-0.208 +/- 0.002	-0.403 +/- 0.007
2020	129	No data	-0.116 +/- 0.007
2021	127	0.169 +/- 0.002	0.1070 +/- 0.007

Discussion

Relationship of leaf traits to soil moisture in 2022 at Poverty Gulch Sites

We found that water-use efficiency increases with decreasing soil moisture with this effect particularly evident at the hybrid site (Fig. 3). This data aligns with [\(Navarro et al., 2022\)](#) who showed that *Ipomopsis* leaves at the end of summer show an association between decreasing soil moisture and higher WUE. This may be a plastic response to retain water in drier conditions. Manipulated precipitation reduction increased WUE and precipitation addition decreased WUE [\(Navarro et al., 2022\)](#). WUE increases drought tolerance but also can lead to relatively slower rates of growth [\(Arntz & Delph., 2001\)](#). Therefore, an increase in WUE with decreasing soil moisture in 2022 is likely adaptive to drier conditions.

Phenotypic plasticity of vegetative traits in response to snowmelt timing

Specific leaf area showed plasticity with larger SLA in late snowmelt years and smaller SLA in early snowmelt years (Fig. 5). Earlier snowmelt leads to drier conditions [\(Sloat et al. 2015\)](#) so the production of thick leaves in early (drier) snowmelt years and thinner leaves in late (wetter) snowmelt years may be adaptive. For example, previous studies have demonstrated that leaf thickness is an adaptive feature of drought resistant plants [\(Nawazish 2006\)](#). In addition to plasticity of overall SLA, it would be interesting to investigate if certain cell types, ratio and organization of cell types and gene expression showed plasticity in *Ipomopsis* exposed to differing melt dates. Future studies can utilize transcriptomics/genomics/epigenomics and flow cytometry to characterize change in gene expression and cell types respectively.

We found that individual plants show plasticity (Fig. 6). We know leaves can last multiple years because green leaves have been found under the snow (N. Waser, pers. obs.). The proportion of leaves that last a single year or multiple years is somewhat elusive. If most leaves only last a single growing season, environmentally induced changes to leaf meristem cells may give rise to new leaves with varying SLA. If a substantial amount of leaves remain over multiple seasons then there could be potential for epigenetic regulation of SLA. In addition, it is possible that both these pathways are in play if some leaves last multiple seasons while new leaf out also occurs.

Future experiments can test if individual leaves are plastic. In the field leaves can be marked with special paint or tape that is resistant to all weather conditions. Long term data collected on the individual leaves with varying snowmelt date can reveal if leaf traits are plastic in individual leaves over years with varying snowmelt date. In addition, it is interesting to consider if the plasticity of SLA is more plastic in one direction than the other. Is it easier to go from thick leaves to thin or vice versa? Future experiments can test for the directionality of plasticity in SLA and in other traits.

It is also interesting to consider if the plants are responding directly to the snowmelt or to other correlated environmental factors. It has been previously suggested that soil moisture may act as a mechanism for plasticity to changes in snowmelt and precipitation [\(Navarro et al., 2022\)](#). Although, other mechanisms may be at play such as temperature buffering of snow cover [\(Navarro et al., 2022\)](#). It would be interesting to test if plants responded directly to soil moisture, snow pack, snowmelt timing or other environmental stimuli.

Natural Selection

Selection on water use efficiency changed based on snowmelt timing with positive selection in early snowmelt years, negative selection in late snowmelt years and little to no selection in years with intermediate snowmelt dates (Fig. 7). Snowmelt timing can impact vegetative traits such as WUE ([Navarro et al., 2022](#)), and there is a positive relationship between WUE and drought ([Yang, 2021](#)). In drier conditions *Ipomopsis* has more pressure to efficiently use its sparse water resources so positive selection on WUE in early snowmelt years is adaptive. In wetter conditions where selection is negative it is possible that decreasing WUE benefits *Ipomopsis* through other physiological pathways. Or perhaps a lower WUE requires less energy and the tradeoff between energy and water reserves changes in wetter years.

In addition we found that selection was typically negative on SLA (Fig. 7). The Colorado Rocky Mountains are shifting towards earlier snowmelt and snowmelt runoff dates ([Clow 2010](#)). Perhaps selection for thicker leaves is a response to drier conditions in the region caused by earlier snowmelt on average.

Relationship Between Plasticity and Natural Selection

This study evaluated plasticity and evolutionary rescue in separate contexts. However, plasticity itself can influence evolution by allowing phenotypes that might not be expressed under other environmental conditions to be selected for under novel conditions ([Schlichting & Wund, 2014](#)). In addition, natural selection itself can affect the selection of the molecular machinery that regulates plastic responses.

Theoretically, evolutionary rescue to unstable conditions may be able to lead to the evolution of more complex epigenetic regulatory machinery and in turn more plastic organisms. In turn more plastic organisms may be able to survive longer in unstable conditions allowing more time for selection to rescue a declining population. Understanding how these two factors impact each other is critical to understanding the climate crisis.

Conclusion

This study demonstrated that *Ipomopsis plants* experience phenotypic plasticity and changes in natural selection on certain traits based on snowmelt timing. Specifically, SLA was plastic with meltdate with a decrease in SLA in early snowmelt years, and selection on WUE changed with meltdate with positive selection in early snowmelt years and negative selection in late snowmelt years. This study reveals that an abiotic factor associated with climate change (snowmelt timing) can impact both plasticity and evolution. This study can serve as a model to see how diverse groups of organisms may respond to climate change and if species will be able to adapt through plasticity or evolutionary rescue. Studies like these are necessary to inform well thought out environmental policies that will allow society to positively react to anthropogenic climate change.

Literature Cited

- Bemmels, J. B., and J. T. Anderson. 2019. Climate change shifts natural selection and the adaptive potential of the perennial forb *Boechera stricta* in the Rocky Mountains. *Evolution* 73: 2247-2262.
- Bickford, C. P. (2016). Ecophysiology of leaf trichomes. *Functional Plant Biology*, 43(9), 807-814.
- Bradshaw, A. D. (1965). Evolutionary significance of phenotypic plasticity in plants. *Advances in genetics*, 13, 115-155.
- Campbell, D. R., & Powers, J. M. (2015). Natural selection on floral morphology can be influenced by climate. *Proceedings of the Royal Society B: Biological Sciences*, 282(1808), 20150178.
- Campbell, D.R., N.M. Waser, and E.J. Meléndez-Ackerman. 1997. Analyzing pollinator-mediated selection in a plant hybrid zone: hummingbird visitation patterns on three spatial scales. *American Naturalist* 149: 295-315.
- Carlson, S. M., Cunningham, C. J., & Westley, P. A. (2014). Evolutionary rescue in a changing world. *Trends in Ecology & Evolution*, 29(9), 521-530.
- Clow, D. W. (2010). Changes in the timing of snowmelt and streamflow in Colorado: a response to recent warming. *Journal of Climate*, 23(9), 2293-2306.
- Dudley, S. A. (1996). Differing selection on plant physiological traits in response to environmental water availability: a test of adaptive hypotheses. *Evolution*, 50(1), 92-102.
- Duncan, E. J., Gluckman, P. D., & Dearden, P. K. (2014). Epigenetics, plasticity, and evolution: How do we link epigenetic change to phenotype?. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, 322(4), 208-220.
- Gomulkiewicz, R., & Shaw, R. G. (2013). Evolutionary rescue beyond the models. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368(1610), 20120093.
- Hoffmann, A. A., and C. M. Sgro. 2011. Climate change and evolutionary adaptation. *Nature* 470: 479-485.
- Kingsolver, J. G., H. E. Hoekstra, J. M. Hoekstra, D. Berrigan, S. N. Vignieri, C. E. Hill, A. Hoang, et al. 2001. The strength of phenotypic selection in natural populations. *American Naturalist* 157: 245-261.
- Navarro, J., Powers, J. M., Paul, A., & Campbell, D. R. (2022). Phenotypic plasticity and selection on leaf traits in response to snowmelt timing and summer precipitation. *New Phytologist*, 234(4), 1477-1490.

Nawazish, S., Hameed, M., & Naurin, S. (2006). Leaf anatomical adaptations of *Cenchrus ciliaris* L. from the Salt Range, Pakistan against drought stress. *Pak. J. Bot*, 38(5), 1723-1730.

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annual review of ecology, evolution, and systematics*, 637-669.

Pociecha, E., Kościelniak, J., & Filek, W. (2008). Effects of root flooding and stage of development on the growth and photosynthesis of field bean (*Vicia faba* L. minor). *Acta Physiologiae Plantarum*, 30(4), 529-535.

Poorter, H., Niinemets, Ü., Poorter, L., Wright, I. J., & Villar, R. (2009). Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New phytologist*, 182(3), 565-588.

Schlichting, C. D., & Wund, M. A. (2014). Phenotypic plasticity and epigenetic marking: an assessment of evidence for genetic accommodation. *Evolution*, 68(3), 656-672.

Shahzad, M., Khan, Z., Nazeer, W., Arshad, S. F., Ahmad, F., Farid, B., ... & Riaz, H. (2021). Effect of drought on trichome density and length in cotton (*Gossypium hirsutum*). *Journal of Bioresource Management*, 8(1), 15.

Sloat, L. L., Henderson, A. N., Lamanna, C., & Enquist, B. J. (2015). The effect of the foreshummer drought on carbon exchange in subalpine meadows. *Ecosystems*, 18(3), 533-545.

Walker, C., & Burggren, W. (2020). Remodeling the epigenome and (epi) cytoskeleton: a new paradigm for co-regulation by methylation. *Journal of Experimental Biology*, 223(13), jeb220632.

Xu, Z., & Zhou, G. (2011). Responses of photosynthetic capacity to soil moisture gradient in perennial rhizome grass and perennial bunchgrass. *BMC Plant Biology*, 11(1), 1-11.

Yang, S., Zhang, J., Han, J., Wang, J., Zhang, S., Bai, Y., ... & Rong, Y. (2021). Evaluating global ecosystem water use efficiency response to drought based on multi-model analysis. *Science of the Total Environment*, 778, 146356.

Tables and Figures

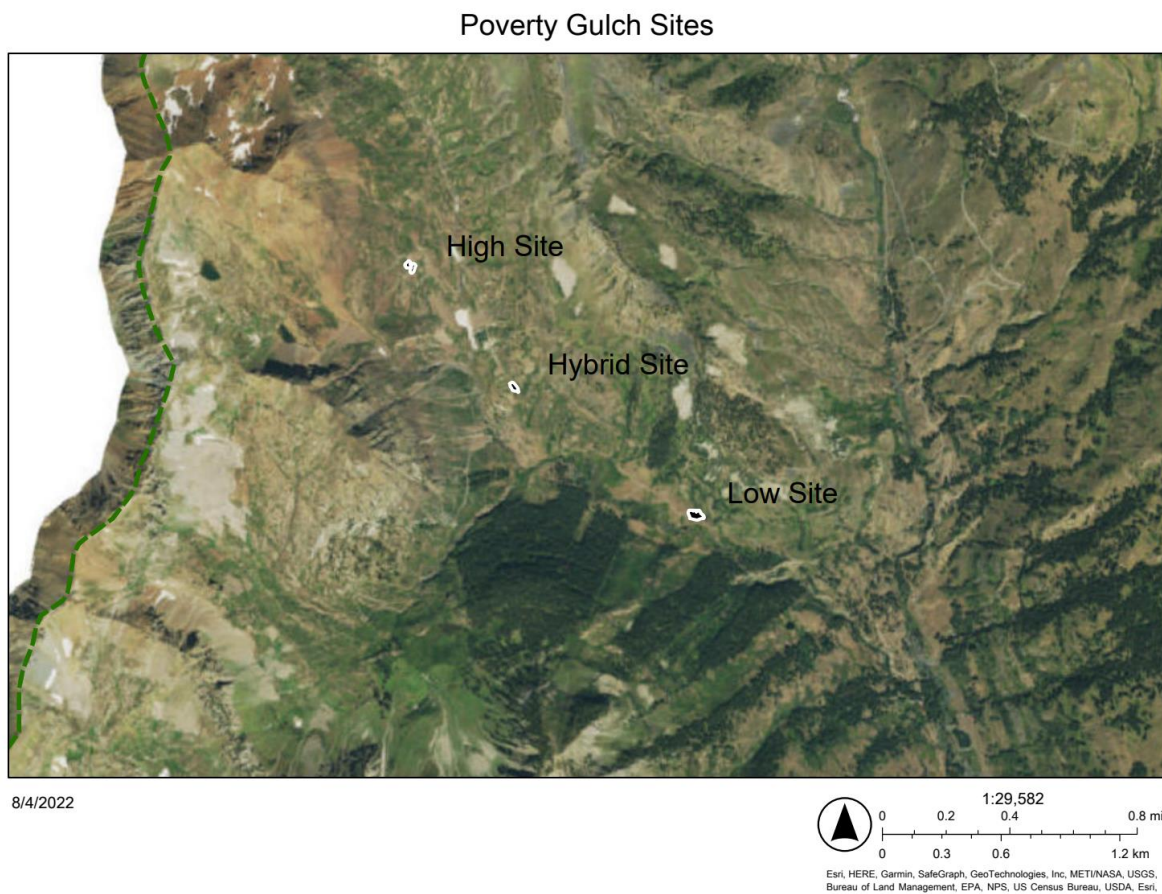
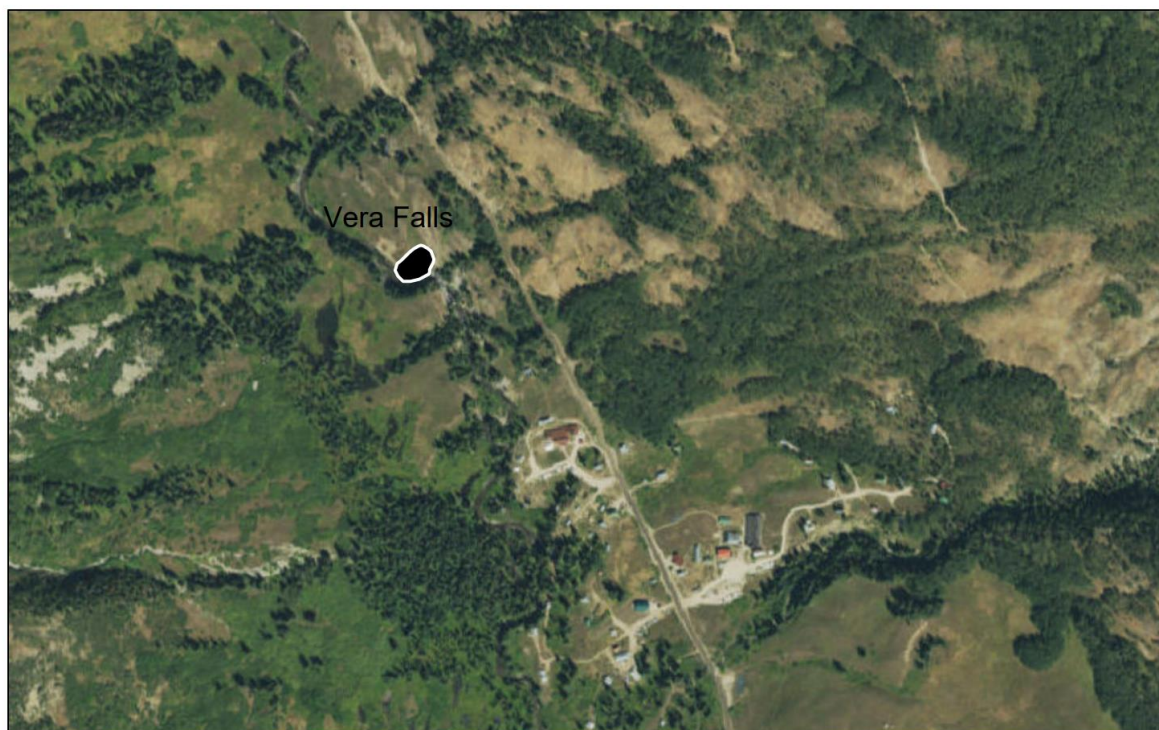


Figure 1: The three sites at Poverty Gulch Gunnison County CO, USA. Low site 2900m (*I. aggregata*), hybrid site 3050 m, and high site (*I. tenuituba*) 3200 m.

Vera Falls



8/4/2022

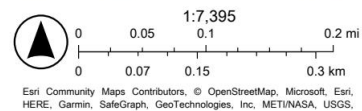


Figure 2: Vera Falls site (*I. aggregata*) ~2900m, Gunnison County CO, USA.

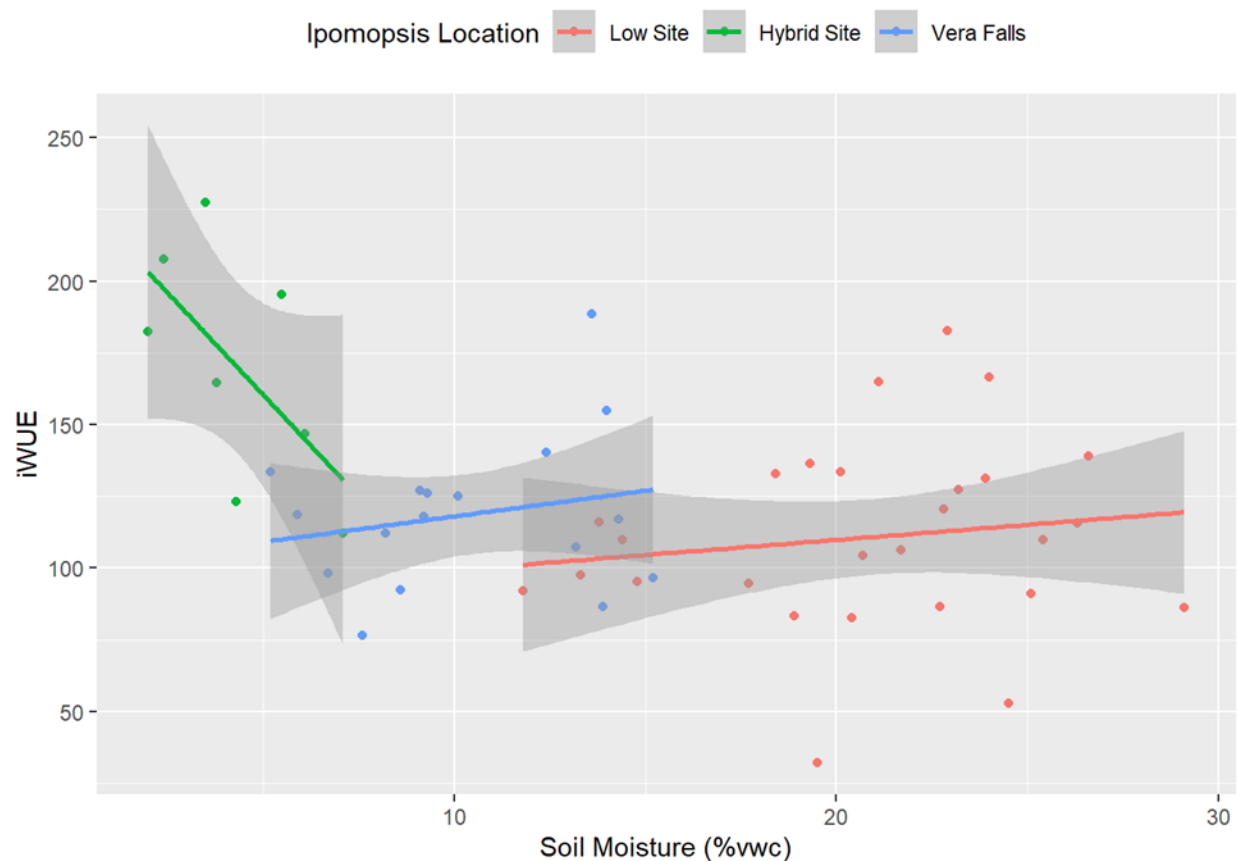


Figure 3. Soil moisture (%vwc) effect on iWUE at three sites in 2022.

Variable related to iWUE	Df	Sum Sq	Mean Sq	F Value	Pr (>F)
VWC	1	8917	8917	8.936	0.00448*
Site	2	13408	6704	6.718	0.00276*
Vwc:site	2	5223	2611	2.617	0.08388
Residuals	46	45903	998		

Table 6. ANCOVA table of iWUE relationship to variables.

Df = degrees of freedom

Sum Sq = Sum of squares

Mean Sq = Mean of squares

F Value = Mean squares treatment

Pr (>F) = P value

** = $P < 0.005$

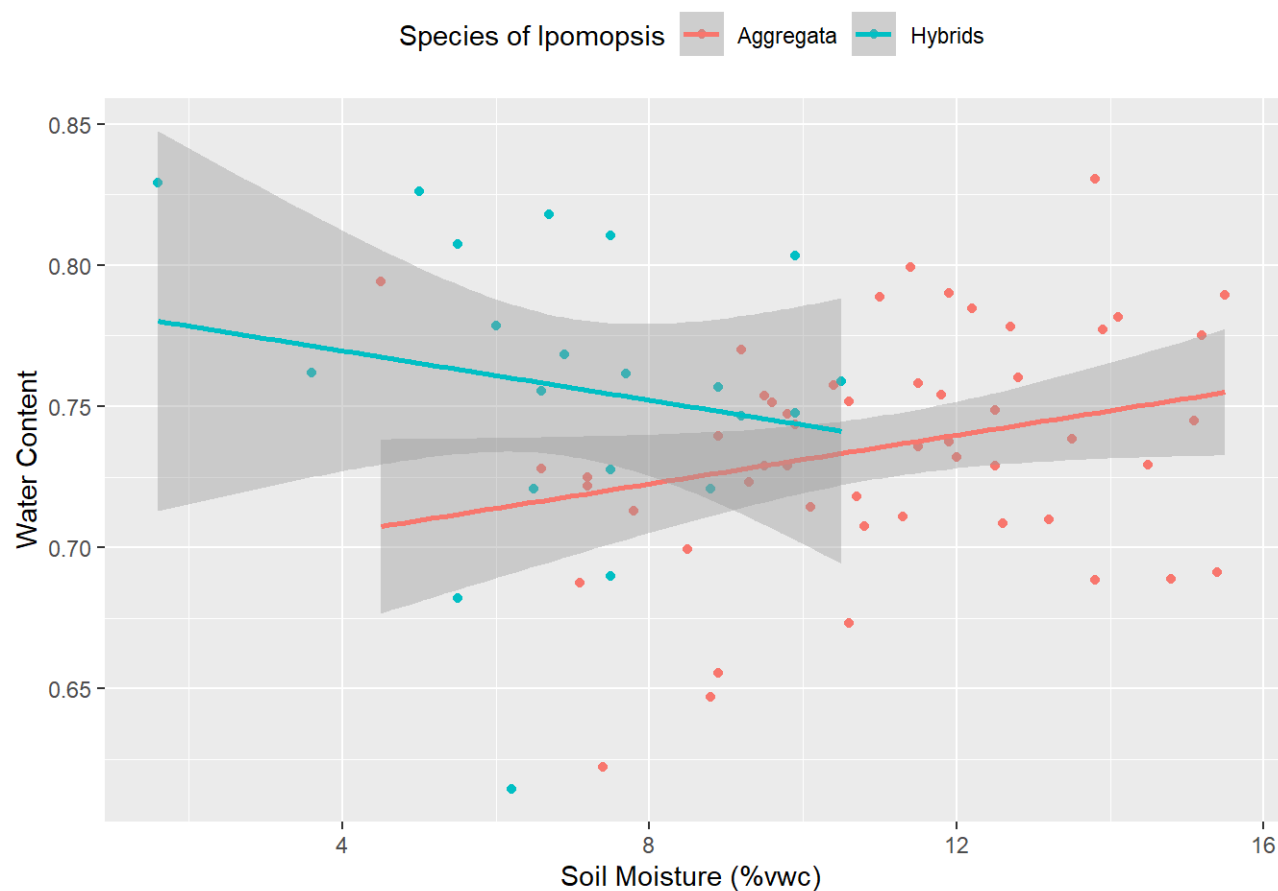


Figure 4. Soil moisture (%vwc) effect on percent leaf water content at Poverty Gulch in 2022.

Variable related to water content	Sum square	Df	F value	Pr (>F)
(Intercept)	1.21790	1	631.0894	$2 \times 10^{(-16)}$ ***
VWC	0.00619	1	3.2051	0.07780
Species	0.01020	1	5.2874	0.02451*
VWC:species	0.00551	1	2.8541	0.09565
Residuals	0.13316	69		

Table 7. ANCOVA table of water content relationship to variables

Sum Sq = Sum of squares

Df = degrees of freedom

F Value = Mean squares treatment

Pr (>F) = P value

*** = $P < 0.0005$

* = $P < 0.05$

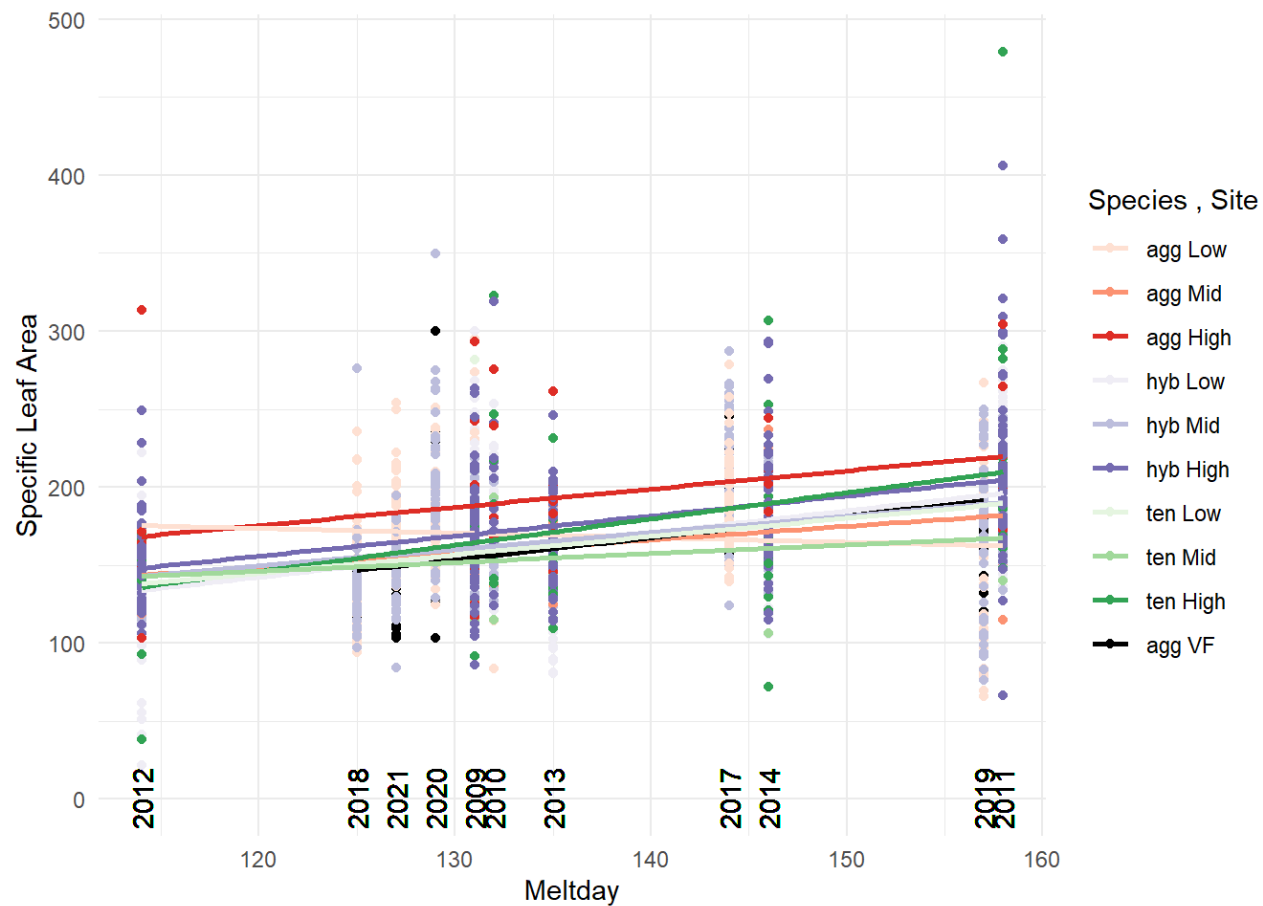


Figure 5. Plasticity of specific leaf area to snowmelt date.

Variable relationship to SLA	Chisq	Df	Pr (>Chisq)
(Intercept)	0.4463773	1	0.5040604
Meltday	8.9768593	1	0.0027342*
Site	12.4728173	2	0.0019569*
Species	2.0544726	2	0.3579950
Meltdate:site	14.2803761	2	0.0007926*
Meltday:species	1.6453113	2	0.4392636
Site:species	15.6440495	4	0.0035359*
Meltday:site:species	16.16733365	4	0.0022368*

Table 8. Chi-Sq table of SLA relationship to variables

Chisq = Chi-Square

Df = degrees of freedom

Pr (>Chisq) = P Value

* = $P < 0.05$

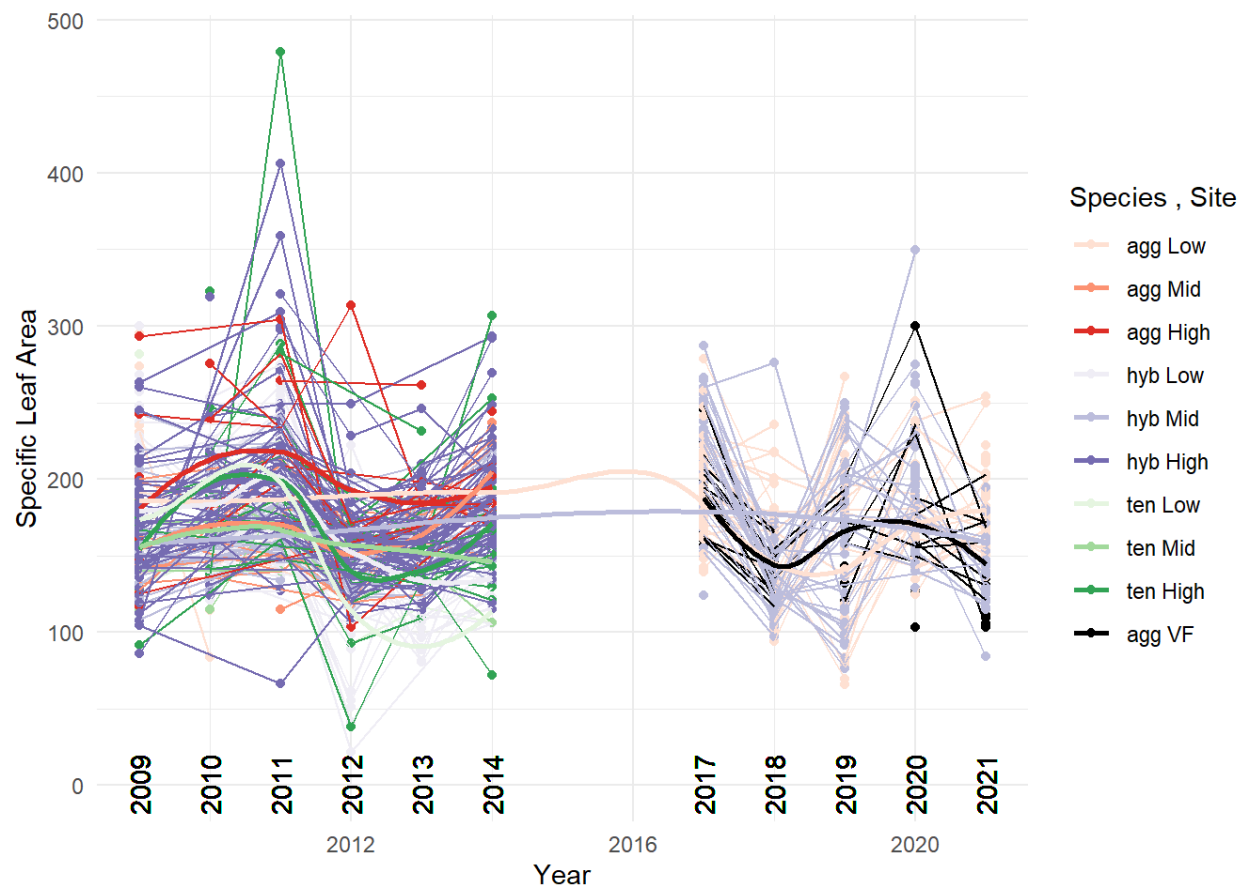


Figure 6. Plasticity of individual plants for specific leaf area. Each line follows an individual plant through the years. Curved lines show a LOESS fit for each species and site.

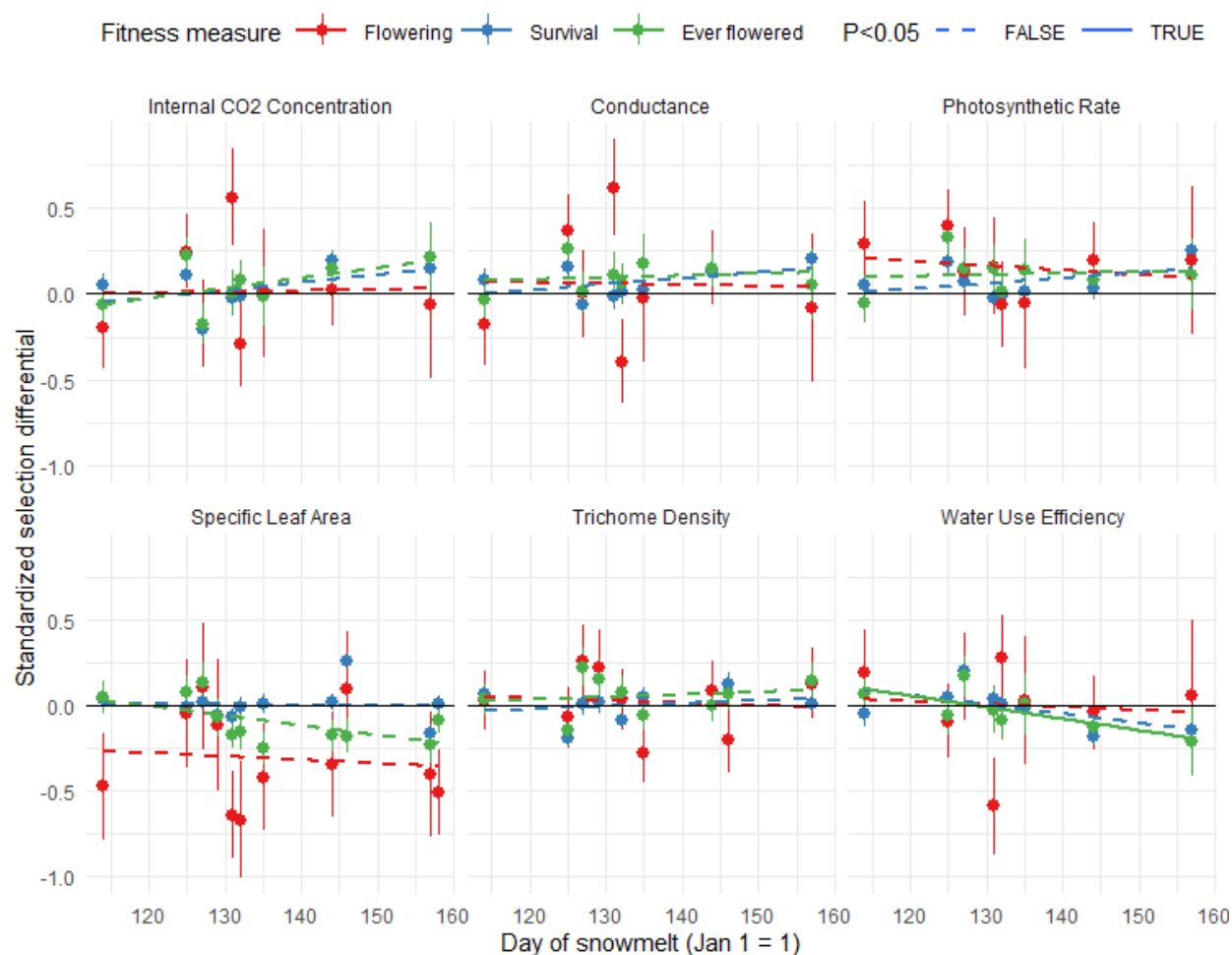


Figure 7. Standardized selection differential (S, with standard error) of six vegetative traits against snowmelt timing in 11 years.