

Effects of water availability on expression of vegetative traits in Ipomopsis across space and time

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ABSTRACT

In subalpine ecosystems, climate change has contributed to an earlier snowmelt as a result of warmer temperatures in the winter and spring. This shift may act to limit water availability for plant communities by prolonging the period between initial snowmelt and late-season monsoon rains, placing these systems at risk of more frequent and severe drought. The genus *Ipomopsis*—a perennial subalpine plant—has been the subject of a long-term study at RMBL examining the effects of climate change on phenotype. Previous research has identified several vegetative traits in the genus whose expression can vary based on environmental factors including snowmelt date and soil moisture. This project aims to examine how water availability, specifically soil moisture, affects the expression of vegetative traits in *Ipomopsis* using both spatial (2024 data) and long-term temporal comparisons. Four traits—trichome density, specific leaf area (SLA), leaf volumetric water content (VWC), and photosynthetic water use efficiency (WUE)—were measured, along with soil moisture, from plants in three populations. Additionally, historical data were analyzed to compare vegetative traits and snowmelt date over 15 years. Relationships between trait expression and water availability were found to be stronger in historical comparisons than in spatial comparisons, likely as a result of abnormally wet conditions during June of 2024. Higher soil moisture, however, predicted higher leaf VWC in this year's data, and years with later snowmelt date had higher SLA. Trichome density did not change significantly with snowmelt date. Finally, WUE was influenced by an interaction between site and snowmelt date.

INTRODUCTION

In subalpine ecosystems, including many of those within close proximity to the Rocky Mountain Biological Laboratory (RMBL) in Colorado, climate change has contributed to an earlier snowmelt timing as a result of warmer temperatures in the winter and spring (Wadgymar et al. 2018). Earlier snowmelt can affect plants in three primary ways: by increasing vulnerability to frost, shifting blooms to earlier in the season, and limiting water availability by prolonging the period between initial snowmelt and the summer monsoon rains which occur later in the season (Campbell et al. 2022). The last of these, combined with increasingly arid conditions, is predicted to place subalpine systems at risk of more frequent and severe drought (Bolinger et al. 2023).

The genus *Ipomopsis*—a monocarpic perennial—has been the focus of long-term study at RMBL. However, some local *Ipomopsis* populations are expected to drop below replacement within 20–40 years (Campbell 2019). Both floral and vegetative traits have been examined over time and in the context of changing environmental conditions—including water availability—in order to identify evolutionary mechanisms. Additional steps have been taken to assess the genus’ potential for evolutionary rescue: a phenomenon by which rapid evolutionary change allows a declining population to stabilize and eventually experience positive growth (Carlson et al 2014). Previous studies have identified several morphological traits in *Ipomopsis* whose expression differs based on both environment and year. Three vegetative traits—trichome density, specific leaf area (SLA), and photosynthetic water use efficiency (WUE)—were found to display variation in their expression over time and under different environmental conditions (Navarro et al. 2022). Each of these traits has the potential to be expressed adaptively under drought conditions. Trichomes—hair-like structures found on plant surfaces—are thought to reduce water loss in some species by reflecting solar radiation (Agrawal et al. 2008). Lower values of SLA reduce water loss during photosynthesis, as does higher WUE. Both affect survival to flowering in *Ipomopsis aggregata* (Navarro et al. 2022). The study also identified strong associations between these three traits and snowmelt date using data collected over three years (Navarro et al. 2022). However, while we know that these associations exist, we do not know whether changes in the expression of these traits act to increase fitness in an increasingly arid environment—and if so, whether the rate of change is sufficient to allow these populations to persist.

This project expands on the earlier three-year experiment to examine how water availability, specifically soil moisture, affects the expression of vegetative traits in natural populations of *Ipomopsis* using both spatial and temporal comparisons. First, vegetative traits were recorded from individuals in three different populations (one located at Vera Falls on RMBL property, and two at Poverty Gulch in the Slate River valley), and soil moisture was

measured for each plant. Second, historical data from the same three populations were analyzed to compare vegetative traits and snowmelt date over 15 years. In both cases, we hypothesized that the expression of vegetative traits will change with low water availability, with decreased SLA, and increased trichome density and WUE in areas with low soil moisture and in years with an earlier snowmelt date.

METHODS

Data for this study were collected from three sites, each containing a distinct population of *Ipomopsis*. The first of these was Vera Falls (located within the Gothic townsite), populated by the species *Ipomopsis aggregata*. The remaining two sites were located at Poverty Gulch, and consisted of one lower-elevation site (“L”) populated by *Ipomopsis aggregata* and one higher-elevation site (“I”) populated by *Ipomopsis aggregata*-*Ipomopsis tenuituba* hybrids. A total of 112 plants were measured: 28 from the Vera Falls population, 42 from the “L” population, and 42 from the hybrid “I” population.

Four vegetative traits—trichome density, SLA, WUE, and leaf volumetric water content (VWC)—were measured across the three sites. One leaf was collected from each plant included in the study, and soil moisture was recorded concurrently using a 12 centimeter probe. Soil moisture was measured at Vera Falls on June 18, and July 5 and 10 at the Poverty Gulch sites. Leaves were scanned, photographed, and examined under 200% power. Trichomes were counted in ImageJ software, and total counts were divided by leaf area in order to obtain the trichome density for each plant. SLA was calculated as leaf area (cm²) divided by leaf dry mass (g). The resulting area-to-biomass ratio indicates leaves’ potential for water loss, with a higher SLA correlating to a higher proportion of water lost to transpiration. Measurements for WUE were taken in the field using a LI-COR system (LI-6400). Photosynthetic rate, stomatal conductance, and internal CO₂ concentration were recorded. Two consecutive measurements were taken for each plant to be averaged for use in calculations, and additional measurements were taken for soil moisture. Measured leaves were returned to the laboratory, and leaf areas were calculated to represent the area contained within the LI-COR chamber. WUE was then measured as carbon assimilated (μmol) divided by water transpired (μmol) for the plant. In order to accurately compare WUE between individuals, values were adjusted based on leaf area. Lastly, leaf VWC was calculated by dividing the difference between leaves’ wet and dry weight (both measured in grams) by wet weight, with the resulting proportion indicating the percentage of water composing each leaf’s biomass.

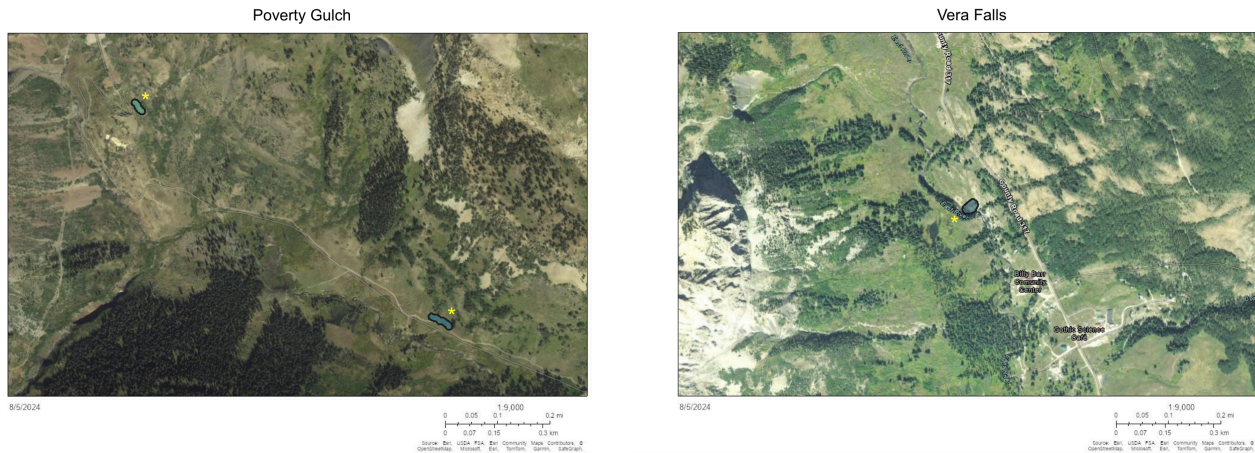


Figure 1. Maps of field sites. **(a)** Poverty Gulch site “I” (left) and Poverty Gulch site “L” (right). **(b)** Vera Falls.

Furthermore, this project utilized the data collected in previous years as part of a long-term study conducted by Dr. Diane Campbell. This included data on trait expression in the same three populations from 2009 to 2023, as well as the snowmelt dates for each year during which measurements were taken. Snowmelt dates were taken from the long-term records collected by billy barr (<https://www.gothicwx.org/long-term-snow.html>).

Statistical analyses for this project were conducted using RStudio and R software. The variables for vegetative traits—trichome density, SLA, leaf VWC and WUE—were compared with soil moisture (for data collected over the course of this summer) and snowmelt date (for historical data) to determine whether these environmental variables are significant predictors of trait expression. To conduct the spatial analysis, each trait for an individual plant was analyzed as a function of the fixed effects of population and soil moisture using function *lm*. Population was treated as a factor and soil moisture as a continuous variable in order to determine how traits changed with moisture. For each trait, we first tested if the effect of soil moisture varied across populations by examining the interaction between population and soil moisture. If the interaction was not significant, we removed it from the model to obtain an overall slope of the trait value on soil moisture (trait ~ population + soil VWC). To conduct the temporal analysis, each trait was analyzed with the function *lmer* using a model with fixed effects of population and snowmelt date and the random effect of plant ID (included to account for repeated measures on the same plant in multiple years).

RESULTS

Soil moisture at the time that leaves were collected varied significantly across the three populations (one-way ANOVA, $p < 0.001$). Poverty Gulch site “L” showed the highest average moisture content (12.50%), and Vera Falls the lowest (3.96%). Poverty Gulch site “I”—containing the hybrid population—had an intermediate average moisture content (5.94%).

Neither SLA nor trichome density in 2024 depended significantly on soil moisture. SLA differed significantly among sites ($p < 0.001$) after controlling for soil moisture, with Vera Falls, Poverty Gulch site “L”, and Poverty Gulch site “I” averaging 202.08, 213.09, and 176.47 g/cm^2 , respectively. There was no significant difference in average trichome densities across sites. However, leaf VWC differed across sites ($p < 0.001$) and also increased significantly with soil moisture, particularly in the two populations with lower soil moisture (figure 2; $p < 0.05$).

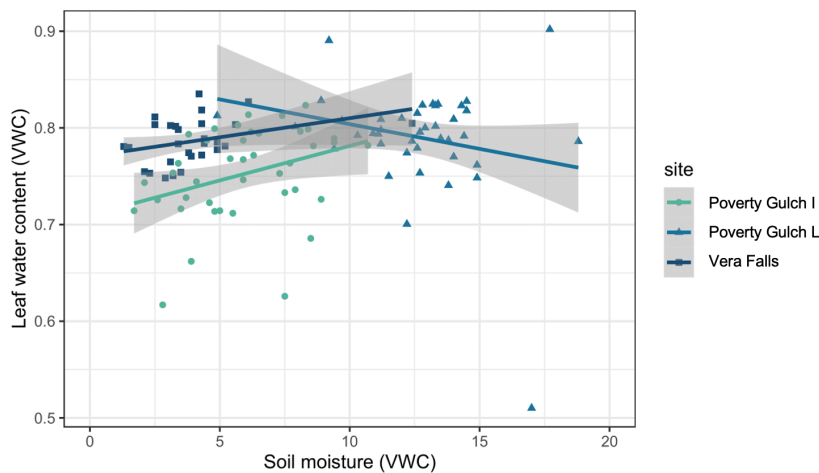


Figure 2. Leaf VWC changes with soil moisture, exhibiting a positive trend across all three sites ($p = 0.0458$).

So far we have analyzed WUE only at the Vera Falls site. In that population, WUE did not increase significantly with soil moisture ($p = 0.4360$ in linear regression), but the sample size was small.

Following spatial analyses, correlation tests were conducted to examine the relationships between leaf traits using the data collected in 2024. This analysis found a negative correlation between trichome density and SLA (figure 3a; $r = -0.42$, $p < 0.001$), a negative correlation between leaf VWC and trichome density (figure 3b; $r = -0.27$, $p < 0.01$), and a positive correlation between SLA and leaf VWC (figure 3c; $r = 0.63$, $p < 0.001$).

Temporal analysis of historical data for SLA and snowmelt timing found snowmelt date

to be a statistically significant predictor of SLA (figure 4a; $p < 0.001$). Furthermore, snowmelt timing had a larger effect on SLA than did the site. At each of the three sites plants had higher SLA values in years with later snowmelt dates, suggesting plasticity in this trait.

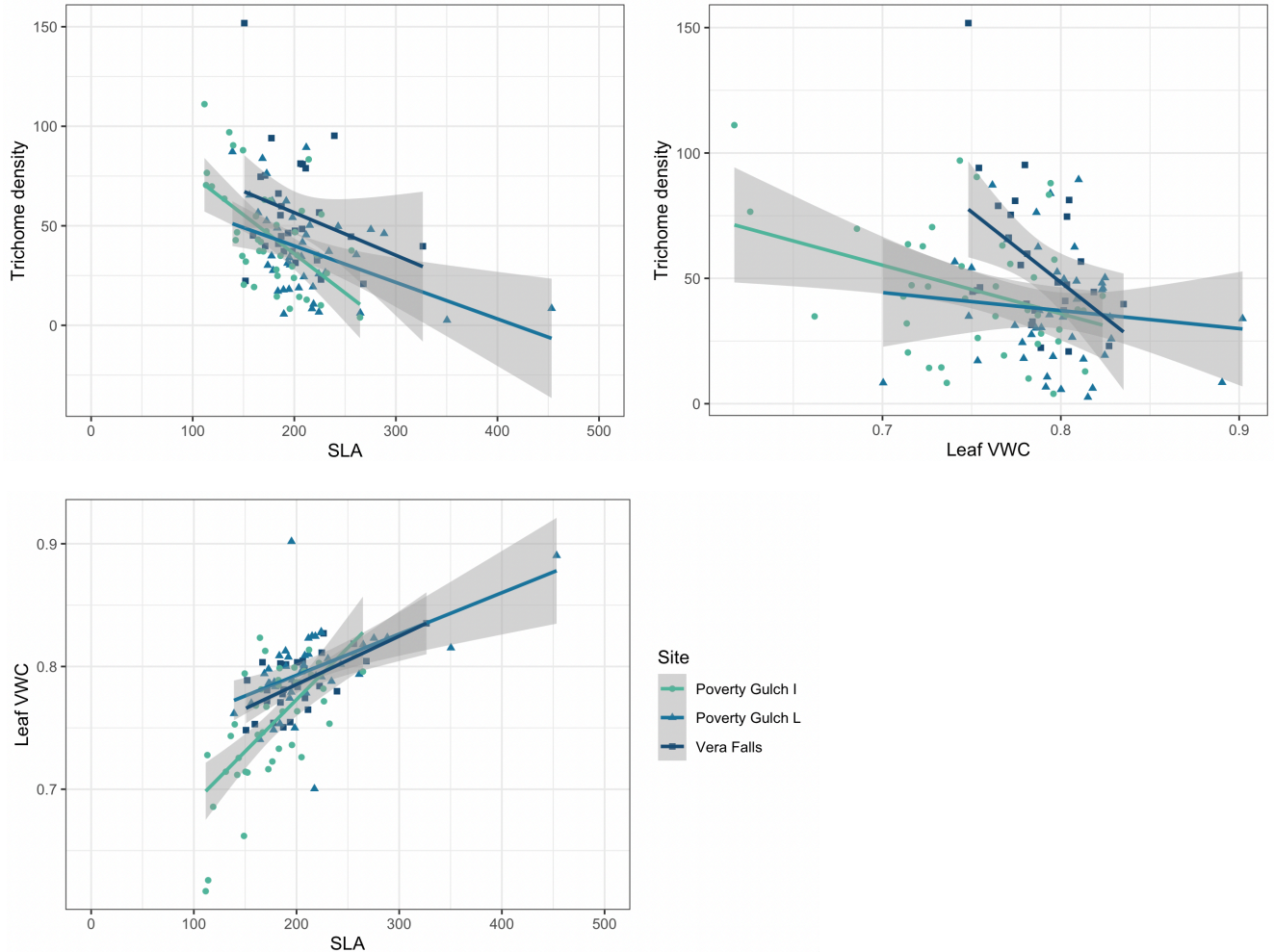


Figure 3. Trait correlations across sites, 2024 data. **(a)** Trichome density decreases with SLA ($r = -0.42$, $p = 5.33e-6$). **(b)** Trichome density decreases with leaf VWC ($r = -0.27$, $p = 3.55e-3$). **(c)** Leaf VWC increases with SLA ($r = 0.63$, $p = 1.49e-13$).

Analysis of historical data for trichome density found snowmelt date to be a less significant predictor of the trait (figure 4b; $p = 0.0664$), with differences in site contributing more significantly to variation in trichome density ($p < 0.001$). However, across all sites plants tended to have higher trichome densities in years with earlier snowmelt dates. Analysis of historical data for WUE found an interaction between the effect of snowmelt date and site (figure 4c; $p < 0.01$), showing that sites differed in the slope of WUE on snowmelt. WUE tended to increase with earlier snowmelt date at the hybrid site only—at both Vera Falls and Poverty Gulch site “L”, WUE trended higher in years with later snowmelt timing.

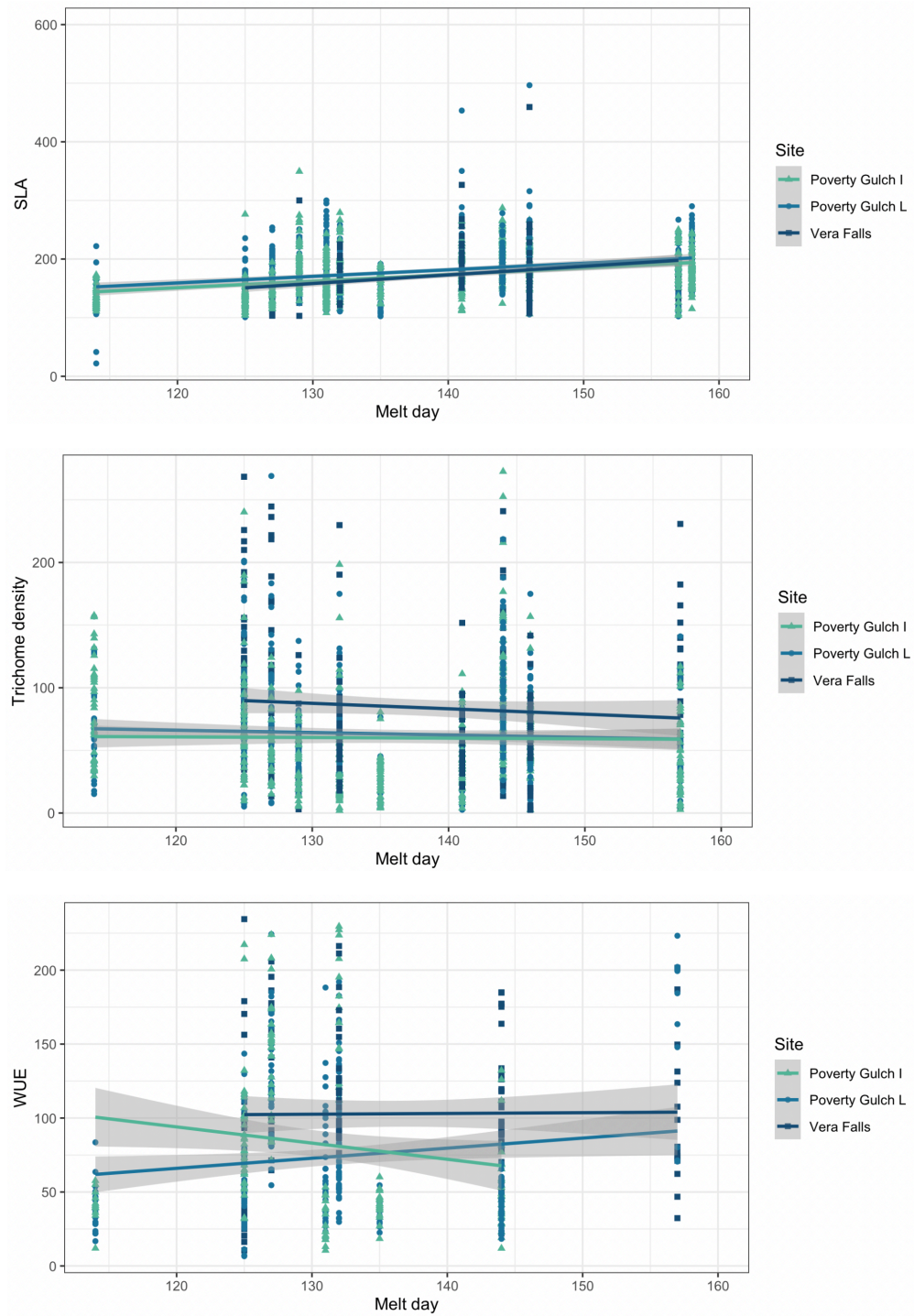


Figure 4. Temporal comparisons between leaf traits and snowmelt, historical data. **(a)** SLA increases with later snowmelt ($p < 2e-16$). **(b)** Trichome density decreases with later snowmelt ($p = 0.0664$). **(c)** WUE differs by site, and there is an interaction between snowmelt date and site ($p < 0.01$).

DISCUSSION

We did not find a statistically significant relationship between soil moisture and the 2024 variables of SLA, trichome density, or WUE, suggesting that these traits did not vary based on differences in soil moisture that year. This result contrasts with an earlier study which found decreases in trichome density and WUE with higher precipitation (Navarro et al. 2022), a difference that might be explained by the relatively higher values for soil moisture observed in this study (up to 15% or more compared to < 8% in the earlier study). In a year with more rain during June (such as 2024, which had the highest amount in all years of record keeping; <https://www.gothicwx.org/rainfall-and-total-precipitation.html>), variation in these traits might be better explained by additional environmental factors which differ both between and within sites.

Conversely, the statistically significant relationship found between the variables of soil moisture and leaf VWC indicates that, across all sites, leaf mass contains a higher proportion of water in wetter areas. When examining the sites individually, however, the data collected from Poverty Gulch site “L” show an opposite trend: as soil moisture increased, leaf VWC decreased. This may be consistent with previous findings of a leaf water content threshold in plants such as maize (Zhou et al. 2021)—a value of soil VWC above which leaf VWC failed to increase. Future study would be needed in order to identify a threshold value in *Ipomopsis* populations.

Our identification of plasticity in the historical expression of SLA is consistent with the findings of Navarro et al. (2022), which demonstrated adaptive plasticity in this trait when snowmelt was manipulated. Furthermore, the relationship found in this study between snowmelt date and SLA supports our initial prediction that SLA would increase with later snowmelt dates. Contrary to predictions, we found no significant evidence for plasticity in the historical expression of trichome density, and for WUE in only one of three populations.

The differences in historical WUE between sites can be identified as an area for future research, as the relationship between site, expression, and snowmelt date remain unexplained. Our prediction of increased WUE as a result of earlier snowmelt was seen only in data from Poverty Gulch site “I”, with the remaining sites exhibiting a trend that may be counter-adaptive. A potential factor which could account for this result is genetics, as the “I” site contains a hybrid population (with *Ipomopsis tenuituba*, one of the contributing species, adapted to the comparatively dry, higher-elevation conditions; Campbell and Waser 2007).

Placing these results in the context of climate change, we might expect to observe local *Ipomopsis* populations respond to earlier snowmelt—and, in turn, decreased soil moisture—by

expressing a lower SLA and leaf VWC in future years. We do not have sufficient evidence to support the prediction of similar shifts in expression of trichome density or WUE, and further study is needed to determine whether this might negatively impact populations. This might involve an examination of traits' effects on plant fitness in these populations, in addition to analysis of trait expression and differences in soil moisture while plants are experiencing drought stress.

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