Plasticity in *Ipomopsis* flower color and nectar production over space and time in response to water availability

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ABSTRACT

Climate change is altering water availability in subalpine ecosystems. Shifts in snowmelt timing and summer monsoonal precipitation, which alter soil moisture, have the capacity to elicit plastic responses in plants. Plasticity in floral traits such as nectar production and flower color can impact pollen transfer and thus fitness in *Ipomopsis aggregata*, since these traits have been shown to play an important role in facilitating pollinator visitation. Understanding how these traits vary spatially and temporally in response to changes in water availability will better inform how *I. aggregata* will persist and be distributed in the future. Two studies were conducted to assess variation in nectar production and flower color: a long-term study analyzed temporal variation and a short-term study assessed spatial variation within populations. Snowmelt date and total summer precipitation were used as a proxy for water availability in the long-term study, and soil moisture was used in the short term study. Results from the short-term study suggest there are no significant effects of soil moisture on either flower color or nectar production. Conversely, the long-term study indicates both measures of water availability interacted to impact nectar production and flower color. These results indicate that floral traits respond to variation in water availability across years, however, it is unclear whether these shifts impact pollinator visitation.

INTRODUCTION

Climate change is projected to impact a number of abiotic factors within subalpine habitats, including water availability. In subalpine ecosystems, water availability is determined by two sources: snowmelt and monsoonal summer rains. Earlier snowmelt dates and increasingly arid summers are expected to result in decreased soil moisture in early summer (Blankinship et al., 2014, Seager et al., 2013). These shifts in water availability may result in climate-induced plasticity (environment-dependent variation in phenotypic expression) in plants. Plastic changes in floral traits may have significant consequences for the abundance and distribution of plant populations since floral traits play an important role in facilitating successful pollination events and increasing plant fitness (Knight et al., 2005). *Ipomopsis aggregata* is an abundant subalpine species which occupies a large range across the western United States. *Ipomopsis aggregata* is a monocarpic perennial that produces trumpet-shaped red flowers and is primarily pollinated by hummingbirds. At our study sites in Poverty Gulch, Gunnison County, the species usually grows in the valley bottom at elevations of 2900 m or below. It is able to hybridize with a closely related species, *Ipomopsis tenuituba*, which generally occupies higher elevations (around 3200 m). Previous studies have shown that some floral traits in *I. aggregata* respond to reduced soil moisture (Powers et al., 2021). Further, studies of other species have shown that effects of water on floral traits impact fitness (Gallagher and Campbell, 2017). Since seed production is pollen limited in *I. aggregata* (Campbell et al. 2022), plastic changes in floral traits in *I. aggregata* in

response to changes in water availability may have important implications for its distribution and abundance in the future.

Nectar production and flower color are two floral traits which may impact *I. aggregata* fitness if their expression varies in response to soil moisture. Nectar is important in attracting pollinators because it acts as an incentive for visitation (Mitchell 1993). As such, the quality (concentration) and quantity (volume) of nectar plays an important role in plant fitness, since successful pollinator visits are required for *I. aggregata* to produce offspring (they are self-incompatible; Campbell et al. 2022b). A previous study conducted in a controlled lab setting demonstrated that nectar production varied under water and temperature stress (Villareal and Freeman, 1990). However, field experiments designed to assess variation in *I. aggregata* nectar production within the context of their environment have been limited, particularly as it relates to changes in water availability. Flower color also plays a role in facilitating visits from pollinators. Flower color has been shown to act as a floral signal, indicating to pollinators that there is a reward associated with flower visitation (Elam and Linhart, 1988). Both nectar production and flower color have been established as floral traits involved in mediating pollinator behavior, impacting plant fitness. Both traits have been shown to have some effect on seed production in *Ipomopsis* (Campbell et al. 2022b).

Given that floral traits including nectar production and flower color play significant roles in facilitating pollination in flowering plants such as *Ipomopsis aggregata*, two studies were conducted to address how phenotypic expression of these traits vary across space and time in response to changes in water availability. First, in order to assess how nectar production and flower color vary spatially, these traits were measured at three study sites and soil moisture was recorded at each plant throughout the season. Microsites with greater soil moisture were expected to produce nectar that is greater in both quantity and concentration. This is because reduced water stress was expected to enable a plant to invest more resources into producing rewards to encourage pollinator visitation. Similarly, flower color was expected to vary between microsites, specifically, plants in areas with greater soil moisture were anticipated to produce flowers that are more red due to increased resources. Second, in order to analyze how these floral traits have varied over time, 13 years of nectar production and flower color data from each site were analyzed against annual snowmelt date and total summer precipitation.

METHODS

I. Study design

Nectar production and flower color were investigated in observational studies. The studies were conducted at three sites. One area was located near the RMBL townsite at Vera Falls (V). The other two were located at Poverty Gulch, Gunnison County, CO, USA: the low elevation site (L) and the hybrid site (H). The H site consists of *Ipomopsis aggregata* and *Ipomopsis tenuituba* hybrids and corresponds to site I in Campbell et al. 1997. Plants that were at least 1m away from any other previously marked plants were tagged (plants with single rosettes were preferentially selected). All tagged flowering plants that were not browsed by deer were sampled at each of the three locations. Each of the floral traits were sampled throughout the summer

(from early July to early August) in order to account for changes in soil moisture throughout the season. Soil moisture was recorded for each plant the day flowers were collected for flower color. For nectar production two soil moisture measurements were averaged: one the day the flower was strawed to exclude pollinators and the second the day the nectar was extracted In addition to the data collected in 2023, long term data on nectar production and flower color of *I. aggregata* and hybrids, snowmelt date, and total summer precipitation were analyzed. The long-term data included data described in Campbell et al. (2022) in addition to data from the natural populations in 2019-2022.

II. Nectar production measurements

Nectar was collected from two flowers per plant (if not browsed). Straws were placed over the flower in order to prevent pollinators from consuming the nectar. Microcapillary tubes were used to extract the nectar produced by a single flower over a 48-hour period. In order to measure the nectar production rate, the length of the nectar column (mm) was multiplied by 5 µL / (2 days x 32 mm). In order to determine the concentration of the nectar, the contents of the microcapillary tube were transferred to a handheld refractometer. The percent sucrose concentration was recorded for each flower sampled.

III. Flower color measurements

Corolla samples were collected from two flowers per plant (if not browsed). Color was analyzed using an Ocean Optics (Ocean Optics Inc., Dunedin, FL, USA) Red Tide USB650 reflectance spectrometer with a LS-I light source. The reflectance spectrometer was calibrated using a white reflectance standard, and a fiber optic probe held at a 45° angle. Corollas were measured on a black background, and their reflectances were recorded. In order to determine the degree of redness of each sample, the reflectance in the green (476-550 nm) was subtracted from the reflectance in the red (626-700 nm). This value was then divided by the total reflectance (401-700 nm). This produced a value (the degree of redness) between 0 and 1, where 0 is a purely white sample and 1 is a purely red sample.

IV. Long-term water availability data

Long-term data for nectar production and flower color were collected by the Campbell lab group at the H and L sites between 2010 and 2022 (including some data from common gardens), and at the V site from 2017-2022. The long term floral trait data were analyzed against annual snowmelt date and total summer precipitation which served as metrics of water availability. Snowmelt date for each year of sample collection was determined using long-term satellite data(NASA/USGS Landsat; Breckheimer et al., in prep.; https://www.rmbl.org/scientists/resources/data-catalog/data-catalog-entry/?catalog-id=230). Precipitation was recorded at the Gothic, CO EPA CASTNET weather station at the Rocky Mountain Biological Laboratory Research Meadow (https://www3.epa.gov/castnet/site_pages/GTH161.html). Missing

data from 2012-2022 were filled using the normal ratio method (https://github.com/jmpowers/RMBLweather) from the Crested Butte, CO weather station (https://www.ncdc.noaa.gov/cdo-web/datasets/GHCND/stations/GHCND:USC00051959/detail). The weather station is approximately 5 miles east of the Poverty Gulch sites, due to the distance, the weather station likely receives a different amount of total summer precipitation compared to the two Poverty Gulch sites. However, there is no known long-term precipitation data that has been collected closer to Poverty Gulch than the Research Meadow weather station dataset. Consequently, the data collected from the Rocky Mountain Biological Laboratory Research Meadow is the best (albeit imprecise) approximation of precipitation for all sites. Total summer precipitation was summed across June and July (the months most floral measures were recorded).

V. Data analysis

For the short term spatial variation data, a linear mixed effects model was used to account for replicate samples from the same plant. Soil moisture (volumetric water content, VWC) and site were the independent variables and the given floral trait (nectar concentration, 24 hour nectar volume, or flower redness) was the dependent variable. At each site, the slope indicates the change in phenotype in response to soil moisture. For the long-term data, site, snowmelt date and total summer precipitation were the independent variables. The mean floral trait per plant (nectar production or flower color) was the dependent variable. A linear model was run with all interactions between variables. General conclusions concerning the direction and magnitude of effects and interactions were made using three dimensional plots of the data and curved regression planes.

RESULTS

For the short-term study, 94 flowering plants were sampled: 22 from the H site, 46 from the L site, and 29 from the V site. All plants were sampled for color and 81 were sampled for nectar. Between one and four replicates were collected for each floral trait for a total of 179 color measurements and 152 nectar measurements. Nectar volume (figure 1) and concentration (figure 2) did not correlate significantly with soil moisture, nor did the effect of soil moisture differ by site (Table 1). Flower redness differed among sites (with the H site having whiter flowers on average) and there was no significant effect of soil moisture on color overall. However, there was a significant interaction between soil moisture and site. Specifically, further visual analysis of the data (figure 3) showed that the effect of soil moisture on redness for the H site was negative whereas the effects for both the L site and the V site were close to zero.

In the long-term study there were a total of 635 flowering plants sampled over 13 years: 198 at the H site, 281 at the L site, and 156 at the V site. Nectar production (figure 4) was affected by snowmelt date and total summer precipitation (Table 2). There were also significant ($P<0.04$) interactions between snowmelt and precipitation, site and precipitation, and snowmelt, site and precipitation. Flower color (figure 5) was affected by snowmelt date, total summer precipitation, and site (Table 2). There were significant ($P<0.04$) interactions between every

combination of variables: snowmelt and site, snowmelt and precipitation, site and precipitation, and snowmelt and site and precipitation. In order to better understand the interactions between these variables, three dimensional plots were produced for each site and trait (figure 6). For example, at the V site nectar production is greatest with a combination of early snowmelt and high summer precipitation and is lowest with late snowmelt and low summer precipitation (figure 6a).

DISCUSSION

Contrary to what was predicted, there was no significant overall effect of soil moisture on either nectar production, nectar concentration,or flower color in the short-term study. However, redness decreased with soil moisture at the hybrid site (Figure 2). These null results may be explained by drought conditions throughout the sample collection period. It is possible that, since the summer of 2023 had exceptionally low summer precipitation, there was not enough variability in soil moisture among plants to detect a difference in traits within sites. Previous experiments using other species have demonstrated that stem color varies in response to water stress (Warren and Mackenzie, 2001). Further, additional studies have shown that flower pigmentation varies in response to changes in soil moisture (Arista et al., 2013) and drought stress (Wettberg et al., 2010).

Conversely, the long-term study revealed floral plasticity to climate variables across years As was expected, nectar production increased as snowmelt was later and total summer precipitation increased (both associated with increasing soil moisture). These results are consistent with what has been observed in previous research concerning the relationship between water availability and nectar production in *Ipomposis* (Powers et al., 2021). Additional *Ipomopsis* studies have suggested that nectar production (and shifts in its expression in response to drought) impact pollinator success (Waser and Price, 2016). The two climate variables also had interactive effects on nectar production and their effects differed by site. Across all sites, nectar production increased as total summer precipitation increased (figure 4). Similarly, nectar production increased at all sites as snowmelt date got later. All sites had similar linear regression slopes, however, the line for the H site was shifted downwards. This is consistent with previous observations of hybrid plants producing less nectar than *I. aggregata* plants.

Likewise, flower color responded to both climate variables. However, the direction of change contradicted our predictions: at the hybrid site, redness decreased with later snowmelt timing. This negative relationship between flower redness and increased water availability is consistent between the long-term and short-term studies at the hybrid site (although the change was not found to be significant in the short-term data). A possible mechanism for this trend is that hybrids may perform differently in years with late or early snowmelt or more arid or mesic summers depending on which species (*I. aggregata* or *I. tenuituba*) they are more closely related to. For instance, it is possible that plants more closely related to *I. tenuituba* (which are thus more white) are more likely to produce flowers in years where the mean snowmelt date is later. An additional mechanism behind this observed trend is that it is possible hybrid plants were over represented in earlier years due to transplants being surveyed for another experiment. Consequently, it is possible that there happened to be more white flowers in earlier years, years

that generally had later snowmelt dates. Further analysis should be conducted to rule out whether this is a confounding variable.

Previous research has demonstrated that plants under water stress produce more pigmented stems (Warren and Mackenzie, 2001). Stem and flower pigmentation have comparable mechanisms, therefore flower color could be expected to respond similarly to water stress. This finding is consistent with our results at the hybrid site, as flower redness increased with earlier snowmelt dates. In the future, the mechanism for why the effect of snowmelt date depended on site could be investigated. Since the long-term study was observational, it is not possible to attribute a causal relationship between flower color and changes in snowmelt date or total summer precipitation. Therefore, a manipulative experiment could be designed in order to determine whether the effect is attributable to plastic differences or genetic differences.

Other future directions could be to incorporate additional long-term climate data such as temperature and the length of the summer drought period. This would enable the model to incorporate a more detailed representation of the conditions experienced by plants at a given site in a given year. An additional area of interest is how nectar sugar content (nectar volume x concentration) may change with water availability. Further, investigating the magnitude of each effect size and whether these trends have an impact on pollinator behavior would provide additional insight into the implications for plant reproduction. A future direction for the short-term study is to replicate the study and analysis in another year which may have greater variability in soil moisture throughout the season. This may enable researchers to either detect a significant effect or collect additional evidence that these traits do not change significantly in response to changes in soil moisture across space.

Given that there were significant interactions between both floral traits and water availability, these results (particularly in the long-term data) suggest that there is a relationship between the two. Although a causal relationship cannot be established due to the non manipulative nature of the study, the results have made clear that there is variability in nectar production, flower color, and water availability both across sites and years. Previous research has demonstrated that these floral traits play an important role in facilitating pollinator visitation through signaling (Elam and Linhart, 1988) and reward (Mitchell 1993). Consequently, plasticity in the expression of these traits have the potential to disrupt plant-pollinator interactions. For species like *Ipomopsis aggregata*, being both self incompatible and pollen limited in seed production, disruptions to pollinator visitation can have especially significant impacts on their ability to produce offspring. Climate change is anticipated to increase variability in weather patterns, including annual precipitation. Consequently, since water availability and nectar production and flower color are related, expression of these floral traits may become more variable across years. These trends have the capacity to impact not just the persistence and distribution of pollinator-dependent plant species, but also species of pollinators or herbivores. Changes in plant populations in response to shifts associated with climate change (such as more variable snowmelt dates and summer precipitation) have the potential to have cascading effects.

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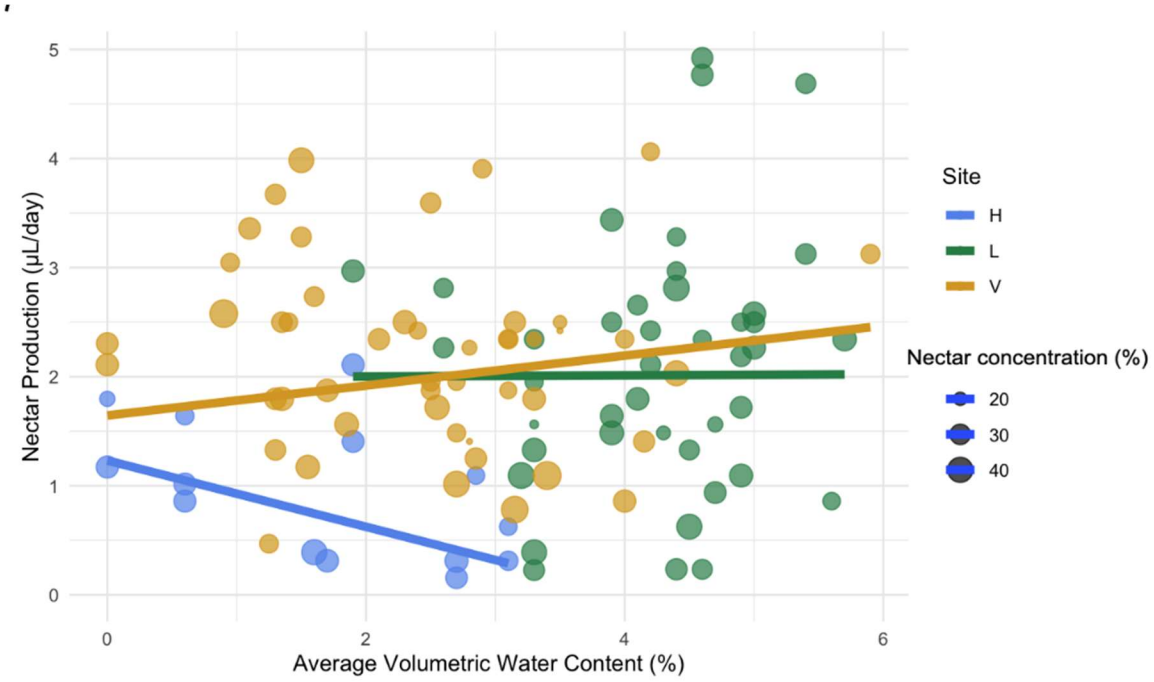
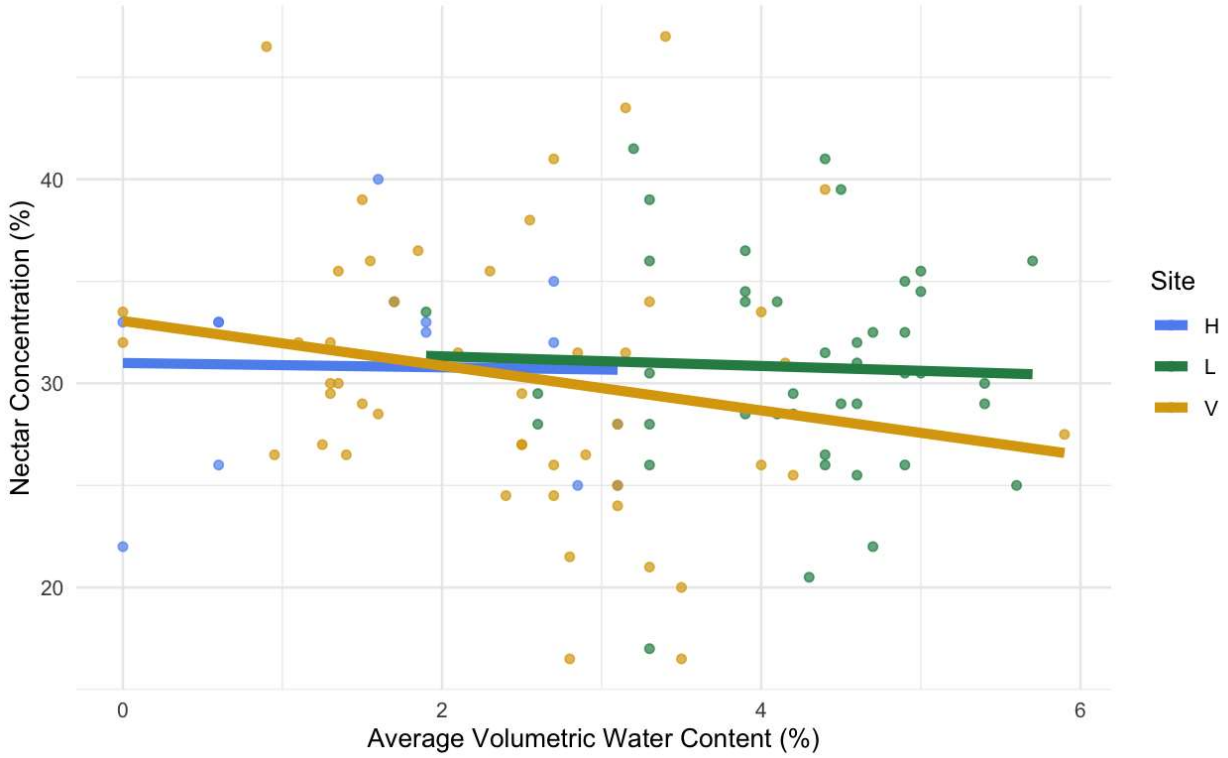
FIGURES

Figure 1



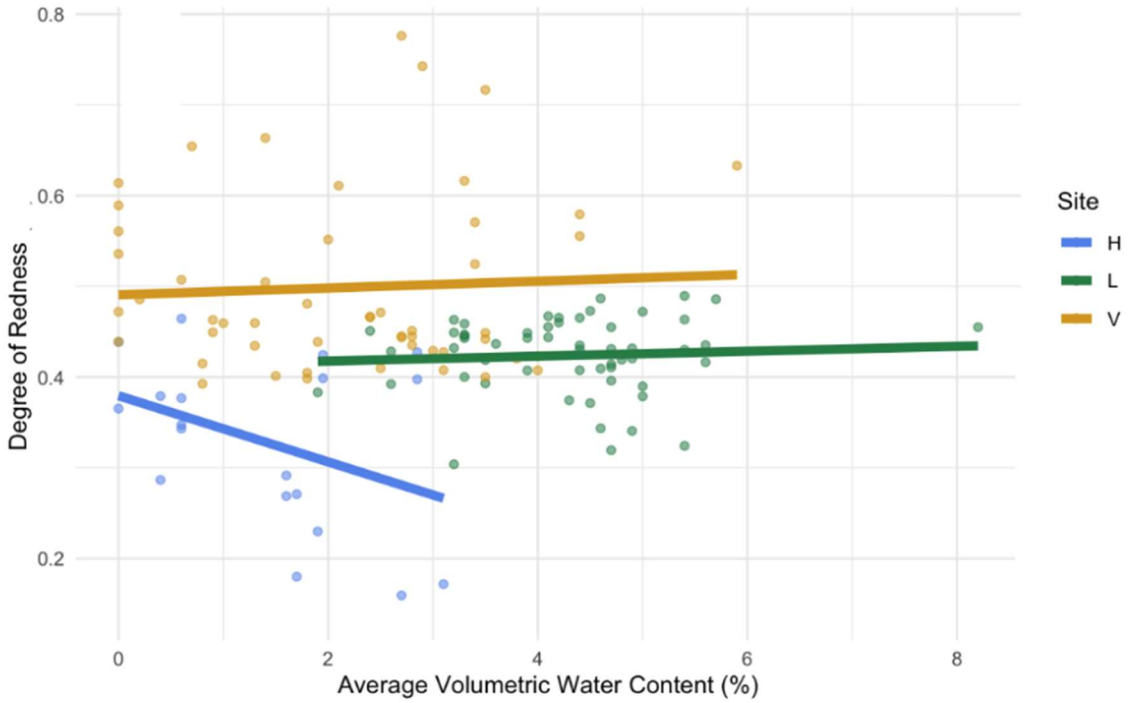
Raw data of nectar production (µL/day) for each plant plotted against average VWC.

Figure 2



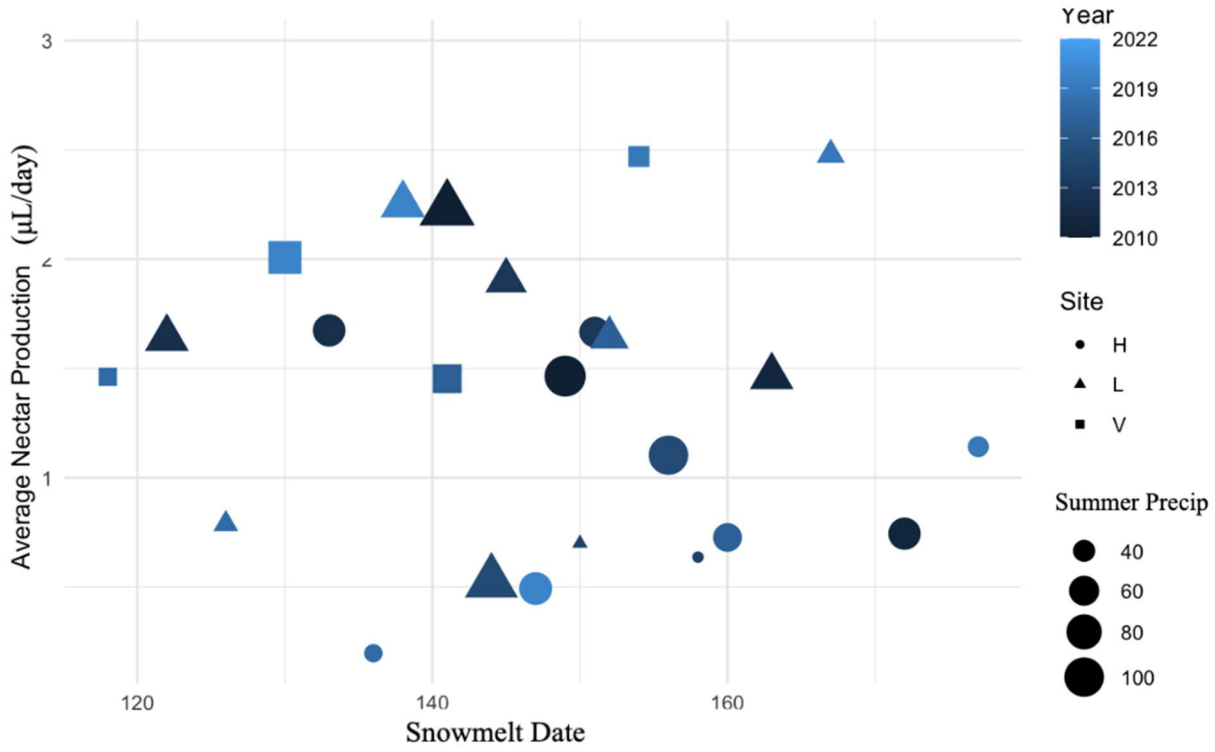
Raw data for nectar concentration (%) for a given plant plotted against average VWC.

Figure 3



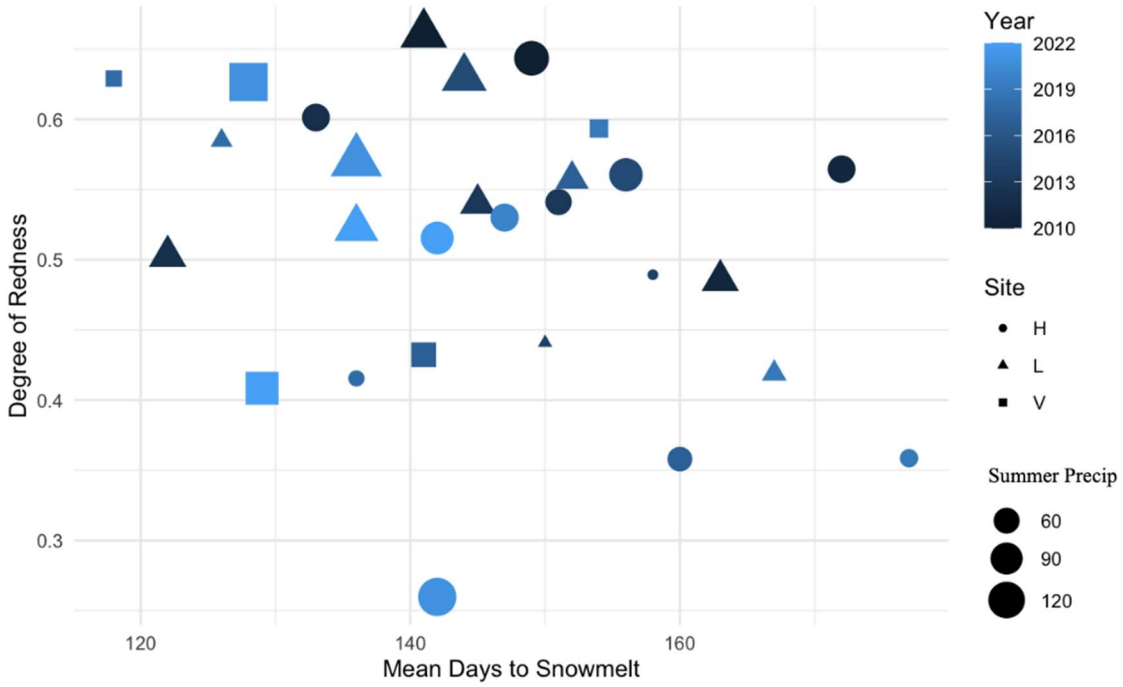
Raw data for the degree of redness of a given plant plotted against average VWC.

Figure 4



Average nectar production for a given site and year plotted against snowmelt date. The size of each point corresponds to the total summer precipitation for that year (larger value corresponds to greater total).

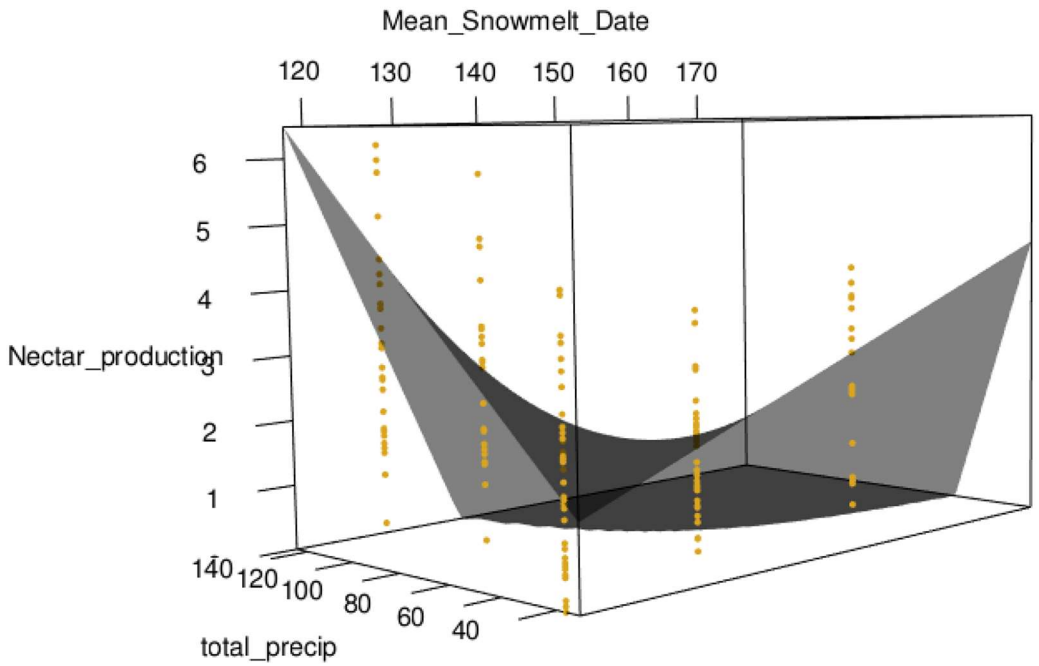
Figure 5



Average degree of redness for a given site and year plotted against mean days to snowmelt. The size of each point corresponds to the total summer precipitation for that year (larger value corresponds to greater total).

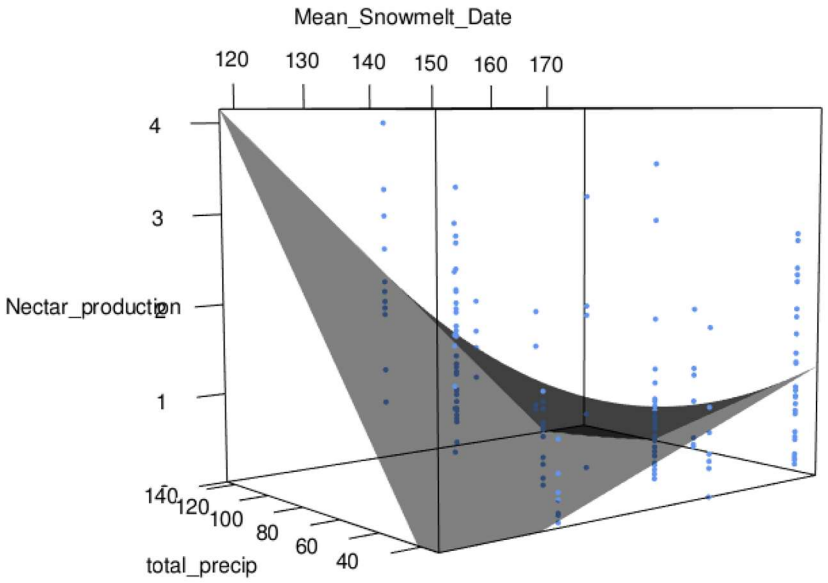
Figure 6

a) Nectar production – V site



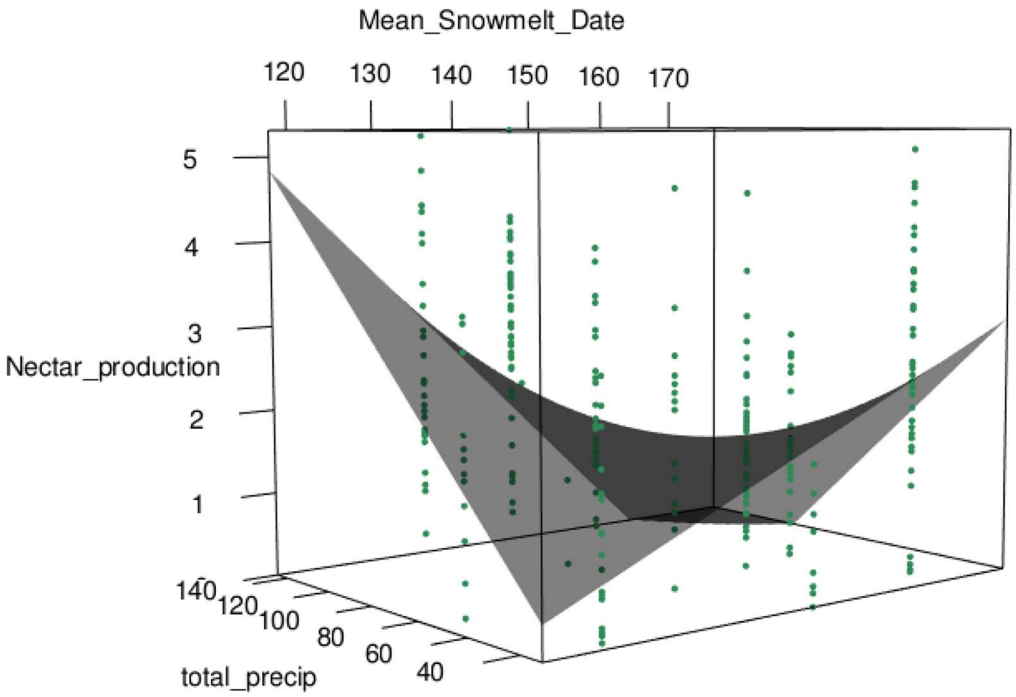
Regression plane for 24 hour nectar production (µL/day) in response to snowmelt date and total summer precipitation at the V site.

b) Nectar production – H site



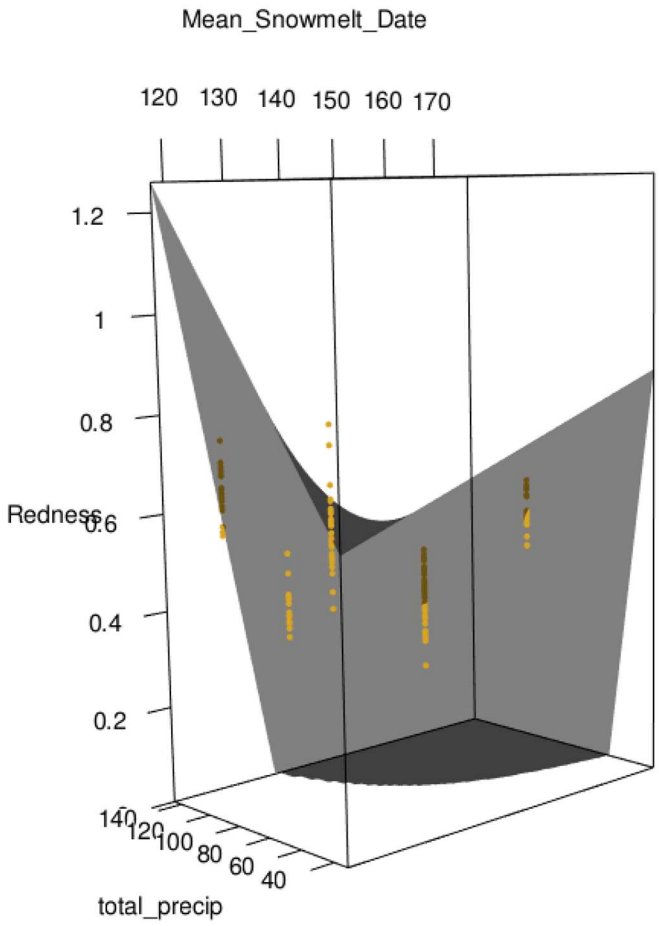
Regression plane for 24 hour nectar production (µL/day) in response to snowmelt date and total summer precipitation at the H site.

c) Nectar production – L site



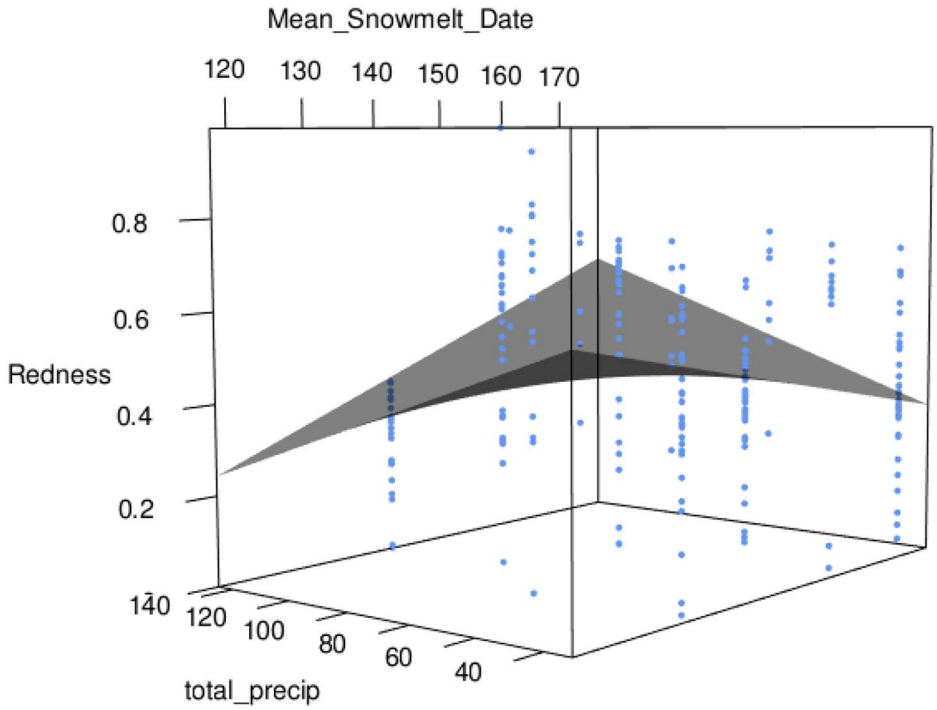
Regression plane for 24 hour nectar production (µL/day) in response to snowmelt date and total summer precipitation at the L site.

d) Flower color – V site



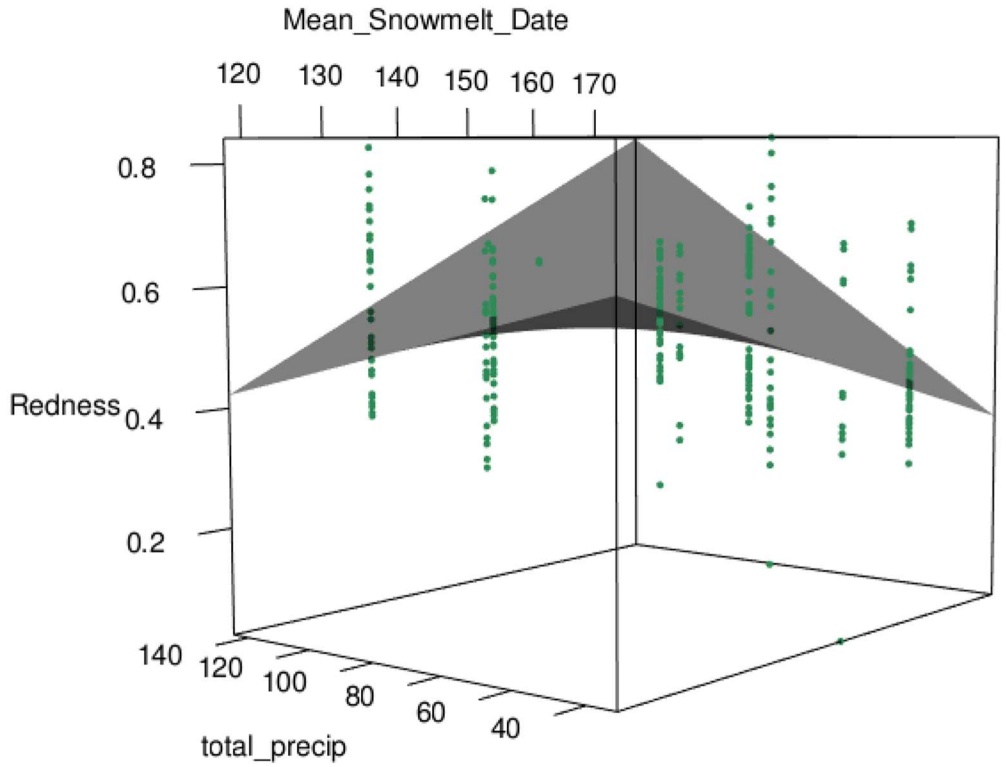
Regression plane for degree of redness in response to snowmelt date and total summer precipitation at the V site.

e) Flower color – H site



Regression plane for degree of redness in response to snowmelt date and total summer precipitation at the H site.

f) Flower color – L site



Regression plane for degree of redness in response to snowmelt date and total summer precipitation at the L site.

TABLES

Table 1

Trait	Interaction	Sums of Squares	Mean Squares	Degrees of freedom	Den Degrees of freedom	F Value	P Value
Nectar Production (volume)	**VWC_Averaged**	**0.09026**	**0.09026**	**1**	**70.661**	**0.1312**	**0.7183**
	Site	**0.45442**	**0.22721**	**2**	**68.165**	**0.3303**	**0.7199**
	VWC_Averaged:Site	**2.21959**	**1.10980**	**2**	**71.875**	**1.6132**	**0.2064**
Nectar Concentration	**VWC_Averaged**	**16.069**	**16.0690**	**1**	**55.758**	**0.6946**	**0.4081**
	Site	**9.192**	**4.5960**	**2**	**57.408**	**0.1987**	**0.8204**
	VWC_Averaged:Site	**12.498**	**6.2492**	**2**	**57.549**	**0.2701**	**0.7642**
Redness	**VWC_Averaged**	**0.0042957**	**0.0042957**	**1**	**74.274**	**1.0444**	**0.3101**
	Site	**0.0189815**	**0.0094907**	**2**	**72.020**	**2.3076**	**0.1068**
	VWC_Averaged:Site	**0.0271345**	**0.0135672**	**2**	**78.726**	**3.2987**	**0.0421**

Results of a mixed effects ANCOVA where nectar production (volume and concentration) and redness were the response variables. The covariates were site, total summer precipitation, and mean snowmelt date.

Table 2

Trait	Interactions	Sums of Squares	Degrees of freedom	F Value	P Value
Nectar Production	**Mean Snowmelt Date**	**12.49**	**1**	**11.5265**	**0.0007331**
	Site	**4.68**	**2**	**2.1612**	**0.1161181**
	Total Precipitation	**12.66**	**1**	**11.6891**	**0.0006729**
	Mean Snowmelt Date : Site	**7.58**	**2**	**3.4960**	**0.0309603**
	Mean Snowmelt Date : Total Precipitation	**10.50**	**1**	**9.6875**	**0.0019467**
	Site : Total Precipitation	**7.18**	**2**	**3.3156**	**0.0370040**
	Mean Snowmelt Date : Site : Total Precipitation	**8.38**	**2**	**3.8675**	**0.0214538**
Redness	**Mean Snowmelt Dat**	**0.2528**	**1**	**11.2212**	**0.0008558**
	Site	**0.7650**	**2**	**16.9785**	**6.510e-08**
	Total Precipitation	**0.1371**	**1**	**6.0876**	**0.0138713**
	Mean Snowmelt Date : Site	**0.7671**	**2**	**17.0262**	**6.222e-08**
	Mean Snowmelt Date : Total Precipitation	**0.1282**	**1**	**5.6905**	**0.0173445**
	Site : Total Precipitation	**0.8500**	**2**	**18.8656**	**1.089e-08**
	Mean Snowmelt Date : Site : Total Precipitation	**0.8400**	**2**	**18.6436**	**1.344e-08**

Results of a mixed effects ANCOVA where nectar production and redness were the response variables. The covariates were site, total summer precipitation, and mean snowmelt date.