

**The Impact of Soil Moisture, Nectar Production, and Spectral Reflectance on
Hummingbird Visitation Patterns to the Scarlet Gilia (*Ipomopsis aggregata*)**

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Abstract:

Climate change has brought intense disruptions to the natural patterns of growth and reproduction in sub-alpine ecosystems, generating changes to the food sources that Broad-tailed hummingbirds (*Selasphorus platycerus*), which breed in the Rocky Mountains, depend on. A hotter, dryer climate results in changing levels of soil moisture, which may impact floral traits such as color and sugar concentration in nectar. In order to find out how variations in these traits impact hummingbird visitation rates to certain flowers, we used data from HummerFlowerWatch, a long-term study gathering camera trap data (documenting every time a hummingbird visits a chosen flower) and transect data (measuring flower abundance), we collected specimens of the native *Ipomopsis aggregata* flower in Gothic, CO, and we noted the trail and measured soil moisture, glucose concentration in nectar, and spectral reflectance to determine correlations between these variables. We predicted higher soil moisture in Stupid Falls, causing higher sugar concentration in nectar, high general nectar output, and higher intensity of reflected red and UV wavelengths. We then expected current trends in visitation data to reflect higher visitation in Stupid Falls. In contrast, we predicted lower soil moisture in Judd Falls, causing lower sugar concentration in nectar, lower general nectar output, and lower intensity of reflected red and UV wavelengths. We also expected current trends in visitation data to reflect lower visitation in Judd Falls. After a 20-day research period for nectar studies, the data supports our hypothesis about Stupid Falls having higher soil moisture and nectar output, indicates a significant positive correlation between soil moisture and nectar output, and indicates a significant negative correlation between soil moisture and nectar concentration. No significant conclusions can be drawn about nectar concentration differences by trail. After a net data set of seven days of spectroscopy, our results show that reflectance does vary significantly by trail (Stupid Falls flowers reflected with greater intensity), but with a small effect size; there is still a lot of overlap between the spectral shape of flowers from both sites. Analysis also showed that *Ipomopsis* tubes reflect UV and red wavelengths at a higher intensity than the petals do. Overall, hummingbird visitation rates from 2022-2024 indicate a significant difference between trails, favoring Stupid Falls as the more highly visited site possibly caused by greater nectar output and reflectance.

Introduction:

Climate change has had drastic effects on the phenology of vital food sources for the Broad-tailed hummingbirds (*Selasphorus platycerus*) that breed in the Rocky Mountains. Earlier snowmelt has resulted in the earlier flowering of certain flora, which has led to an increased exposure of flowers to frost (damaging their reproductive capabilities), and to a mismatch between when migratory birds arrive in certain regions and when traditional food sources become available and reach peak availability in that region. Decreases in floral size, number of flowers, and nectar volume per flower have also appeared as a result of reduced water availability (Kuppler & Kotowska 2021). Importantly, changes in the amount of flowers present later in the season may imply a drastic change in the amounts of nectar that hummingbirds have access to. Due to higher rates of regional warming, a loss of snowpack, and the general sensitivity of alpine species, high-elevation ecosystems are more vulnerable to climate change. This vulnerability emphasizes the importance of studying the effects of climate change on such ecosystems (Pepin et al. 2015, Musselman et al. 2021, Körner 2003, Oldfather 2025).

Seeking to understand the correlation between nectar resources and altitude, researchers have compared hummingbird perching frequency to nectar sugar concentration at altitudes ranging from 3,000 to 5,000 m above sea level (Carpenter 1978). Previously, most studies had been performed on lowland hummingbird communities. Researchers discovered that high altitude communities represent an extreme case of resource limitation (Carpenter 1978): when hummingbirds feed at high altitudes, there is a greater energetic output per amount of glucose obtained, since plants at high altitudes often produce less glucose-concentrated nectar than at lower elevations (Wolf and Gill 1986). Since floral traits such as nectar production and flower color have been shown to play an important role in facilitating pollinator visitation, plasticity in these traits can impact pollen transfer and thus fitness (Lam et al. 2023). Additionally, plants growing in soil with lower moisture levels were associated with reduced flower size, nectar production, and decreased local diversity (Cha et al. 2025). Although this study placed its emphasis on *Primula florindae* in a higher elevation location (14,000 feet), the impact that soil moisture has on many of this flower's traits points to a necessity to study this relationship in more depth. As a whole, these studies indicate the importance of continuing to investigate the impact of soil moisture on nectar production of more flowers in high-altitude ecosystems.

Tied inextricably to nectar concentration, flower color plays a large role in being a major attractor for pollinators (Melendez-Ackerman et al. 1997). The fact that past studies have determined hummingbirds' clear preference for red-colored flowers leads us to ask whether variations in (the intensity of) the redness of a flower also contributes to their preferences (Melendez-Ackerman et al. 1997). When presented with *Ipomopsis* flowers differing in discrete color categories (red and white) but experimentally keeping the nectar reward constant between the flowers, hummingbirds demonstrate a spontaneous preference for red *I. aggregata* over white *I. tenuituba* (Melendez-Ackerman et al. 1997). They also preferred flowers which were painted red over flowers of the same species that were painted white (Melendez-Ackerman et al. 1997). This result is due to hummingbirds' past experience of red *I. aggregata* as the flower with the highest nectar reward (Melendez-Ackerman et al. 1997). Indeed, when scientists varied the nectar concentration in the flowers such that the white flowers had a higher nectar concentration, the hummingbirds quickly changed their preference to the white *Ipomopsis* (Melendez-Ackerman et al. 1997), demonstrating that color-preference is based on past reward abundance. This change of preference highlights the importance of investigating the effects of possible changes in nectar concentration brought about by drier soil resulting from climate change. If circumstances are not conducive to flowers producing the amounts of nectar they once did, how will hummingbird preferences for certain flowers change? Will the colors that they associate with the highest nectar rewards shift, and will the color of flowers differ significantly between sites with different visitation rates to those flowers?

Specifically for the migratory *S. platycerus*, the Rocky Mountains have been a yearly stop full of plentiful wildflowers for their feeding, and a warmer location for their breeding. In return, the hummingbirds pollinate and spread local populations such as *Delphinium nuttallianum* and *Delphinium barbeyi*, *Aquilegia elegantula* and *Aquilegia caerulea*, *Mahonia repens*, *Mertensia ciliata* and *Mertensia fusiformis*, etc. throughout the summer months. However, this symbiotic relationship may be disrupted by changes in abiotic factors brought about by climate change, causing a change in the usual abundance of flowers over time and subsequently a mismatch in feeding availability for hummingbirds. As snowmelt occurs earlier on in the season, the moisture content in the soil increases earlier on as well. With less presence of snow and higher moisture content, floral bloom can begin earlier, leading to the earlier presence of high

nectar concentration. As the season progresses, and floral abundance of certain species diminishes, nectar availability for hummingbirds may diminish earlier on in the season. As climate change progresses, areas like Gothic, Colorado are also seeing more frequent droughts, corresponding to less water availability for flowers. Therefore, how does earlier snowmelt and differing soil moisture affect the color and nectar content of flowers in different regions in Gothic, and what is the effect of these variables on hummingbird visitation rates?

To narrow in on this question, we studied the flower *Ipomopsis aggregata*, a bright red tubular flower which is also known by the common name of “Scarlet Gilia,” or “Skyrocket,” (Phlox family). The color their five petals bear can range from red, and pink, to salmon, and white (Nagy 2013). These flowers can grow to a height of three feet, and have curly, hairy leaves close to the stalk (Nagy 2013). *I. aggregata* is predominantly found in rocky soils, open areas, and dry meadows, flowering between the spring and fall (Nagy 2013). The presence of *I. aggregata* in large abundance in two sites within Gothic, namely Stupid Falls and Judd Falls, made the flower an ideal representative species for our question. At the Rocky Mountain Biological Laboratory (RMBL), the blooming time of this species of *Ipomopsis* is early June to late August, matching up with the breeding season of a migratory population of *S. platycerus*.

The RMBL ecosystem is a subalpine meadow ecosystem, making it a host to a series of pollinators which include these migratory hummingbirds. Given that higher elevation presents resource limitations, this ecosystem is a valuable site to study hummingbird feeding patterns. Highlighting the importance of studying our chosen flower, data from 2018 indicates the *I. aggregata* as a flower that is both one of the most highly visited species and one that grows in various locations around RMBL, thus allowing us to analyze differences due to location (HummerFlowerWatch). Overall, we investigated the visitation patterns of the migratory *S. platycerus* to the seasonally blooming wildflower, *I. aggregata*, to discover whether there is a significant correlation between trail, soil moisture, flower color, and nectar production. Wanting to know why hummingbirds visit the flowers that they do, our central question was: How does the amount of moisture in the soil where the *I. aggregata* grow affect their nectar concentration and hummingbirds’ preference for certain spectra (within the range of red and UV wavelengths) that are linked to higher reward experiences?

Our hypothesis was that differences in soil moisture significantly affect spectral reflectance and nectar concentration in *I. aggregata*. Our null hypothesis was that there is no significant correlation between spectral reflectance, nectar abundance, and soil moisture. We predicted higher hummingbird visitation rates to *I. aggregata* in the Stupid Falls trail site, due to higher soil moisture, higher nectar concentration, and higher general reflectance of red wavelengths. On the contrary, we predicted lower hummingbird visitation rates to *I. aggregata* in the Judd Falls trail site, due to lower soil moisture, lower nectar concentration, and lower general reflectance of red wavelengths.

Methods:

I. Study design

We are part of HummerFlowerWatch, an ongoing long-term research project initiated by the Stoddard Lab of Princeton University. HummerFlowerWatch scientists collect camera trap data from several sites at RMBL to determine the frequency of hummingbird visitation to different species of wildflowers in the area. Scientists also collect transect data from eight transects in the area to be able to determine how the

abundance of those hummingbird-visited species varies over the summer (specifically June-August). We used an Ocean Optics spectrophotometer to determine differences between the reflectance spectra of flowers in higher moisture (mainly in Stupid Falls) and lower moisture (mainly in the Judd Falls trail) locations. Using microcapillary tubes and a handheld Fisher refractometer, we measured glucose content to determine differences in glucose percentage in higher moisture (Stupid Falls trail) and lower moisture (Judd Falls trail) locations. We used a moisture meter to measure the soil moisture at the location of each of the flowers. One moisture meter also provided soil temperature, soil pH, and sun exposure data at the time of measurement. Additionally, data on rainfall was collected, indicating the presence or lack of rain.

II. Flower Collection/Nectar Production measurements

Starting on July 7th, we collected flowers from five different random locations along the Judd Falls Trail on one day, and from five different random locations along Stupid Falls on the next day. We alternated days between Judd and Stupid Falls to allow for nectar accumulation for testing. The first day of isolation was July 5th, and the last day of collection was July 26th. We chose two stems at each of the ten sites and randomly selected and isolated three flower buds. Plastic drinking straws (cut in approximately one inch long segments) were placed over the buds and tied with twist ties to deter pollinators from obtaining the nectar and get an objective measurement of nectar production and sugar concentration for each of the flowers. The general production of nectar in the topmost flowers on an *I. aggregata* stalk does not differ much from that of the flowers near the bottom, maintaining uniform nectar production throughout the plant (Diane Campbell, pers. comm.). Small tins were used to place the nectar-designated flowers.

In order to measure nectar production, the length of the nectar column (mm) was multiplied by $10\mu\text{L} / (2 \text{ days} \times 32 \text{ mm})$. The formula changed according to microcapillary volume. The two days is indicative of the time of isolation, and the 32 mm was also subject to change according to microcapillary tube length. For nectar concentration, the contents of the microcapillary tube were transferred to a handheld refractometer and percent sugar was recorded per flower (Lam, Powers, Campbell 2023).

We had a total of 30 nectar concentration measurements each day (5 sites, 2 stems, 3 flowers from each stem), with a total of 10 days of collection per site. This yielded about 300 data points for nectar in each site. A few of the samples dried out or had dead flowers, but the collection remained relatively consistent throughout the 20 days.

Nectar sugar concentration in the *I. aggregata* is highest in the afternoon and lowest in the early morning; therefore collection time was written down and all collections were done in the period from 9:00 AM to 4:00 PM (Pleasants 1983).

III. Flower Collection/Flower Color Measurements

In addition to nectar measurement, we had flowers chosen for spectroscopy. Even though spectral reflectance measurements had been taken of two blooming flowers from every stem from which we had taken flowers for nectar measurement, the files from July 12th through the 26th did not save, leaving us only with the reflectance measurements for

flowers collected from July 7th through the 11th. Therefore, flowers were also collected for spectroscopy on July 30th and 31st to increase the sample size.

From July 7th through the 11th, flowers were collected from each of the two stalks at the five different randomly chosen sites from which we collected flowers for nectar. This yielded a total of 100 spectroscopy flowers for the first five days. On July 30th, flowers were collected from ten different randomly chosen sites on the Stupid Falls trail, and then from ten different randomly chosen sites on the Judd Falls trail. Two stalks were chosen at each site, and two blooming flowers were taken from each stalk. On July 31st, flowers were collected from ten different random sites at Judd Falls and then ten different sites from Stupid Falls, with two stalks per site and two blooming flowers per stalk. This yielded a total of 160 spectroscopy-designated flowers from July 30th through July 31st. All flowers were placed in small plastic tins to ensure they remained fresh and were taken back to the lab.

Within 2.5 hours of collection, the flowers chosen for spectroscopy were placed in front of a black velvet background. Using an Ocean Optics spectrometer, a fiber-optic probe was used to direct light onto the flower at various locations and collect the reflected light. The spectrophotometer then measured the intensity of each wavelength of light that was reflected from the petals' surface, using a diffraction grating to separate each wavelength. We kept the lights off and the curtains closed when using the spectrometer to ensure that the only light reaching the petals was from the light source.

From July 7-8 and 30-31, reflectance was measured at two locations on each flower (July 7-8 and 30-31). Location 1 was on the petals. The first "petals" measurement was done by directing the light onto the center of the inside of the flower, where hummingbirds face when extracting nectar. The second "petals" measurement was a couple of millimeters away from the first measurement, still on the inside of the petals. Location 2 was on the middle part of the outside of the flower's tube. The tube was held flat on the velvet with two fingers. The second "tube" measurement was done a few millimeters from the first. From July 9-11, reflectance was also measured at a third location: the lower end of the tube. On July 7-8, only one reflectance measurement was taken per location.

Plastic gloves were used to avoid getting any oils or debris on our specimens or on the fiber optic probe. We used the OceanView software, which shows graphs of the spectra (and their corresponding intensity) reflected off the flowers once illuminated by the light source. Each spectral reflectance measurement was saved as a .txt file, holding the information of the reflectance intensity (in percent reflectance) at each wavelength (from 300 to 700 nm in increments of 1 nm). Each file name indicated the date of collection, whether the flower was collected on the Judd Falls or Stupid Falls trail, the site number (1-5 for July 7-11 and 1-10 for July 30-31), the stalk number (1-2), the flower number (1-2), and the location on the flower ("petals," "tube" or "tube mid," or "tube-low").

This procedure was conducted on a total of 260 flowers, yielding a total of 1,080 reflectance measurements. Below is a table summarizing the net spectrometry flower collection and reflectance measurements taken each day.

Day	Total Stalks	Total Flowers	Spec Locations/ Flower	Measurements/ Location	Total Measurements That day
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July 7 (JF)	10	20	2	1	40
July 8 (SF)	10	20	2	1	40
July 9 (JF)	10	20	3	2	120
July 10 (SF)	10	20	3	2	120
July 11 (JF)	10	20	3	2	120
July 30 (SF&JF)	40	80	2	2	320
July 31 (JF&SF)	40	80	2	2	320

IV. Soil moisture measurements

From July 9-26, we took one soil moisture measurement with a YAMRON Moisture meter at the soil by each of the chosen stalks (2 trails (Judd and Stupid), 10 sites, 2 stalks per site, 1 soil measurement per stalk) on the day of collection. From July 7-13 and July 30-31, we used a different moisture meter whose values ranged from 1 (driest) to 10 (wettest). Four moisture measurements were taken per location on the first seven days, and two measurements were taken per location on the last two days.

The moisture meters were inserted at a distance of an inch or less of where the *Ipomopsis* stalk emerged from the ground. The moisture meters were inserted ~9cm into the soil, except for the times when the soil was impenetrable for more than 4-5cm (when it was pure rock). When we could not get the moisture meter in, the soil moisture was entered as dry (-). In addition to measuring soil moisture in percent water, the YAMRON Moisture Meter measured the temperature of the soil in degrees Celsius, the pH, and the sunlight intensity (in Lux) reaching the soil. This yielded 10 YAMRON moisture measurements per day, or a total of 80 moisture measurements per location, giving a total of 160 YAMRON moisture readings for this study.

V. Weather Data

To account for the presence of the confounding variable of rain/weather difference, an additional column was added to the data set indicating the presence of rain. On the day of collection, weather conditions from the previous night were identified. Weather data is not considered for days of isolation.

VI. Hummingbird visitation/Floral Abundance

Data for the 2025 HummerFlowerWatch was continuously being collected this season using 7 Brinno TLC200 cameras on the Stupid Falls trail and 8 Brinno TLC200 cameras on the Judd Falls trail, in addition to having 4 transect data plots in Judd Falls and 4 transect data plots on the Stupid Falls trail.

For floral abundance, the 4 transects on each site were 50 meters long along the traditional trail path, and 2 meters to the left and 2 meters into the meadow. The presence

and abundance of 26 known hummingbird visited flowers were recorded each time and catalogued into a spreadsheet. Specifically, data on the number of stalks and inflorescences on each stalk were recorded. Transects were done every 6 days on each site. Finally, data was uploaded into RStudio to indicate the changing presence of *I. aggregata* throughout the summer and indicated when to isolate data from DeepMeerkat. Only 2025 data was used to graph *I. aggregata* presence.

Cameras were on a 3 day rotation: SD cards, batteries, and camera locations were changed every three days per site. SD footage was transferred onto a hard drive and uploaded to an AI platform called DeepMeerkat for analysis. A positive hummingbird identification is shown in *Figure 4*. To answer our hypothesis, data from 2018 - 2024 was used that identified hummingbirds feeding on the *I. aggregata* on Judd Falls trail and Stupid Falls trail.

VII. GPS Mapping

We mapped the location of each stalk designated for spectroscopy and nectar measurement using a TRIMBL GPS, with an accuracy of 2cm or 60cm depending on which specific GPS was used. The mapping was done during the time of flower bud isolation, and at the time of collection on July 30 and 31. These data were used to create maps of our research sites, allowing us to see topographical variation between our sites. One map is of the sites flowers were collected for nectar examination, and the other is of the sites where flowers were collected for spectroscopy.

VIII. Data analysis

To quantify *I. aggregata* abundance through the season, we used the HummerFlowerWatch transect data collected in 2025. To quantify hummingbird visitation rates, we compiled records from 2021 - 2024 and isolated visitations to Judd Falls and Stupid Falls using the GPS coordinates for the camera placements. This data was recorded on Google Sheets.

Our independent variables in this study included: our 10 location sites (5 from Judd Falls and 5 from Stupid Falls), the randomly selected individual *I. aggregata* stems and flowers. Our dependent variables included: reflectance and intensity of reflected wavelengths per flower, nectar volume per flower, glucose concentration in nectar per flower, soil moisture measurements, and hummingbird visitation rates. Our points of reference included the floral abundance data from the transects and the number of times hummingbirds visited each flower (from the camera videos).

Statistical analyses were performed using R Studio. First, normality tests were done using soil moisture percentage, total nectar output, and average sugar concentration of nectar to determine subsequent analyses. Next, these same variables were tested against trail (SF or JF) as well as separated into four different analyses adding weather as a variable: SF Dry, SF Rain, JF Dry, and JF Rain. Wilcoxon-rank sum tests or Welch's T-tests were done to determine statistical relationships. To assess relationships between soil moisture and each of the nectar traits, as well as nectar sugar concentrations vs. nectar output, simple linear regressions were performed. Spearman and Pearson correlation tests were done in addition to the linear regressions to find statistical relationships. Weather and trail were additional variables added to these analyses as well.

To analyze visitation data and floral abundance, Rstudio was used to create graphs that represented the relationships present longitudinally. These graphs were compared to each other and no additional statistical analyses were performed for these data sets.

IX. Spectral Data Analysis Specifics

The analysis of spectral reflectance data was conducted using the pavo package in R (Maia R. et. al, 2019). Each .txt file (measurement of spectral reflectance data) had been saved under a folder depending on what day the measurement was taken. Each folder was imported as a different dataset into R. Since two measurements had been taken per flower location on July 9-11 and 30-31, we averaged these files by two (aggspec) for these days. All spectra were then smoothed using LOESS regression with a smoothing factor of 0.2 to remove noise and preserve the spectral shape. All files were given consistent names (which included the date they were taken) and combined into one large dataframe with wavelengths from 300 - 700 nm as the first column (with intervals of 1 nm) and reflectance of flower measurement n in column n. This dataset was then transposed and variables were named as wavelength bins to prepare for running a Principal Component Analysis (PCA) .

The PCA is a linear dimensionality reduction method which lets us capture the major axes of variation in our data while reducing the dimensionality. The PCA takes the original spectra and finds new summary variables (principal components, or PCs). The resulting standard deviation refers to how much variation is captured by each PC, and the proportion of variance refers to what fraction of the total variance is explained by a PC. PC scores were then extracted. New columns based on the Date, Trail, Site, Stalk, Flower Number, and Flower Area (location on the flower the reflectance measurement was taken) were added to the pcscore dataset. Note that we only used measurements of the petals and tube, and tube-low was excluded from the analyses.

To find out whether there was a statistically significant difference in the soil moisture in Judd vs. Stupid Falls at the stalks of all flowers collected for spectroscopy from July 7-11 and July 30-31, we ran a Welch Two sample t-test comparing the averaged soil moisture at a stalk's location to the Trail.

We ran four t-tests to test to determine whether Flower Area had a significant effect on PC1, on PC2, on PC3, and on PC4. We ran four t-tests to determine whether Trail had a significant effect on PC1, PC2, PC3, and PC4.

Results: Nectar, Soil Moisture, Visitation

Test	P-value	Significant?	Subsequent test	Results
Av. Glucose - general	W = 0.96364, p-value = 0.0009365	Y		
Av. Glucose - JF	W = 0.94188, p-value = 0.005529	Y	Wilcoxon	W = 2669, p-value = 0.2328
Av. Glucose - SF	W = 0.93826, p-value = 0.001004	Y	Wilcoxon	

Av. Glucose - JF Rain	W = 0.90668, p-value = 0.259	N	Welch's T-Test	W = 32, p-value = 0.01216
Av. Glucose - SF Rain	W = 0.9215, p-value = 0.1783	N	Welch's T-Test	
Av. Glucose - SF Dry	W = 0.92528, p-value = 0.001135	Y	Wilcoxon	W = 1982.5, p-value = 0.02249
Av. Glucose - JF Dry	W = 0.93722, p-value = 0.008647	Y	Wilcoxon	

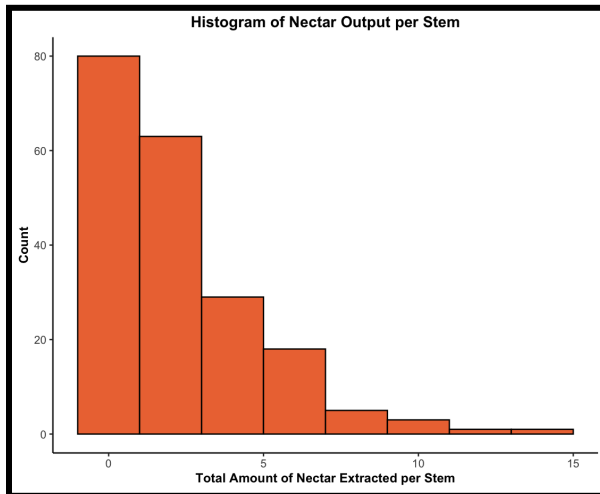
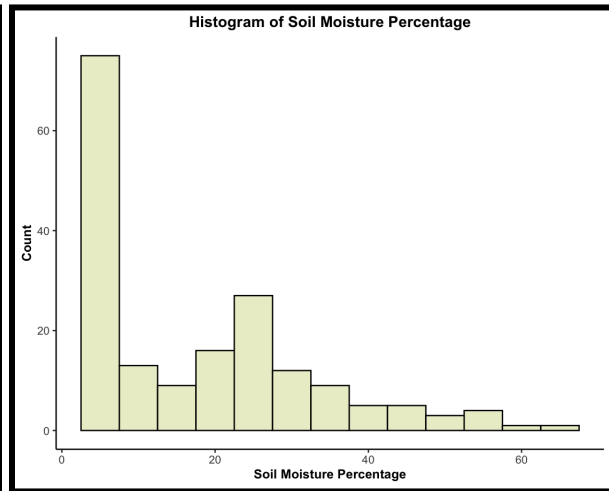
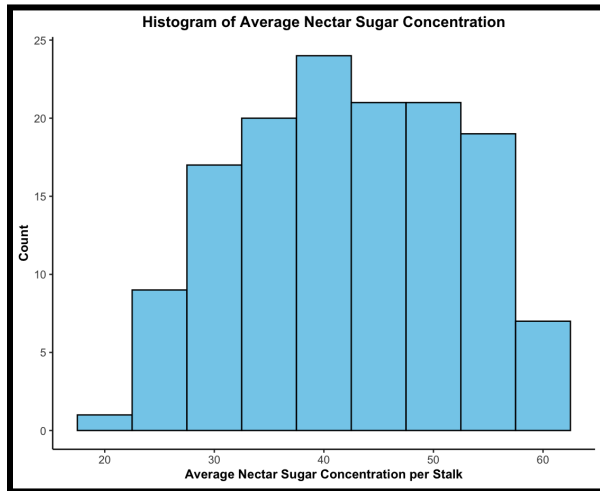
Table 1: Normality Statistics for Average Glucose

Test	P-value	Significant?	Subsequent test	Results
Nectar Out. - general	W = 0.84355, p-value = 2.113e-13	Y	Wilcoxon	
Nectar Out. - JF	W = 0.85814, p-value = 2.399e-08	Y	Wilcoxon	W = 4149, p-value = 0.03749
Nectar Out. - SF	W = 0.84576, p-value = 8.292e-09	Y	Wilcoxon	
Nectar Out. - JF Rain	W = 0.71406, p-value = 5.814e-05	Y	Wilcoxon	W = 3184, p-value = 0.9578
Nectar Out. - SF Rain	W = 0.87426, p-value = 0.01397	Y	Wilcoxon	
Nectar Out. - SF Dry	W = 0.8384, p-value = 6.734e-08	Y	Wilcoxon	W = 42, p-value = 1.937e-05
Nectar Out. - JF Dry	W = 0.88608, p-value = 3.3e-06	Y	Wilcoxon	

Table 2: Normality Statistics for Nectar Output

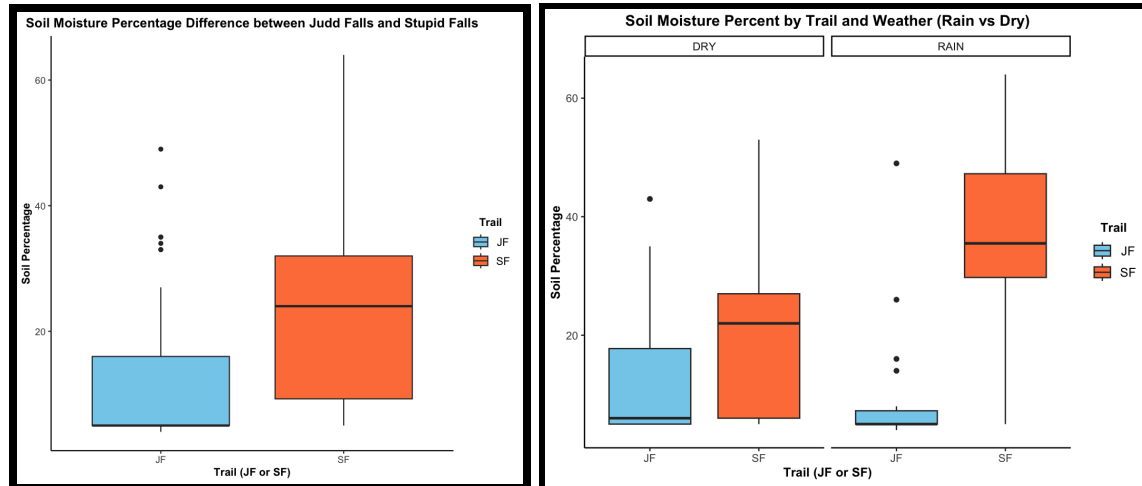
Test	P-value	Significant?	Subsequent test	Results
Soil % - general	W = 0.84507, p-value = 1.501e-12	Y		
Soil % - JF	W = 0.70748, p-value = 4.245e-12	Y	Wilcoxon	W = 1948.5, p-value = 7.979e-10
Soil % - SF	W = 0.93285, p-value = 0.000169	Y	Wilcoxon	
Soil % - JF Rain	W = 0.51138, p-value = 4.036e-07	Y	Wilcoxon	t = -6.3724, df = 33.728, p-value = 2.942e-07
Soil % - SF Rain	W = 0.95511, p-value = 0.4513	N	Welch's T-Test	
Soil % - SF Dry	W = 0.90839, p-value = 8.449e-05	Y	Wilcoxon	W = 1473.5, p-value = 3.194e-05
Soil % - JF Dry	W = 0.74552, p-value = 1.094e-09	Y	Wilcoxon	

Table 3: Normality Statistics for Soil Moisture Percentage



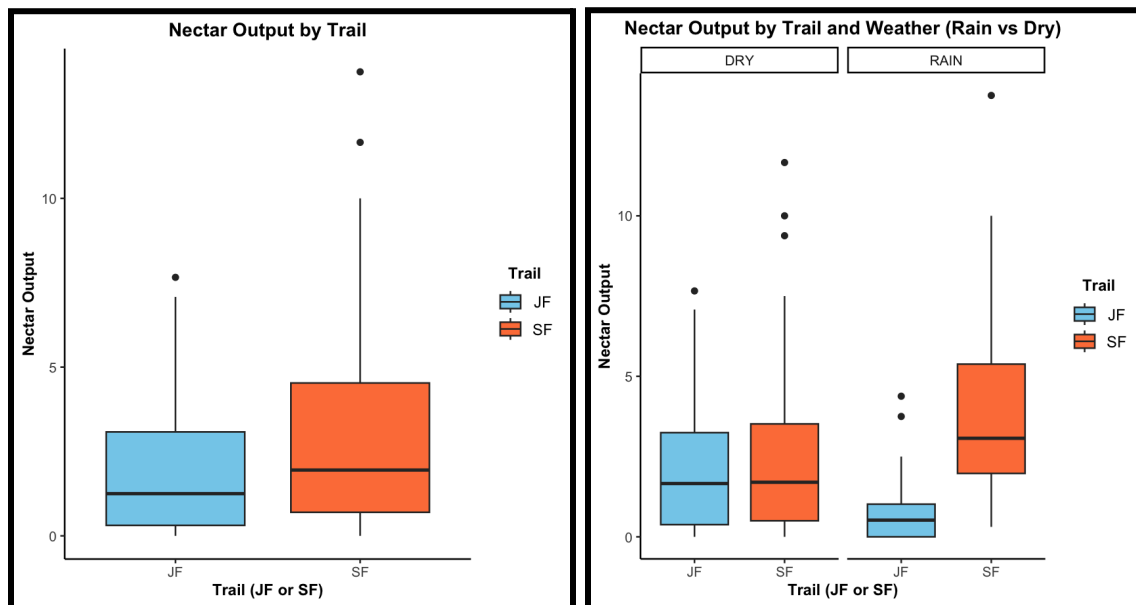
Graphs 1, 2, 3: Exploratory Data Analysis - Soil Moisture Percentage, Nectar Output, and Nectar Sugar Concentration

A Shapiro-Wilkins test was done and determined non-normality for Soil Moisture Percentages (Table 3), Nectar Output (Table 2), and Average Nectar Sugar Concentration (Table 1).



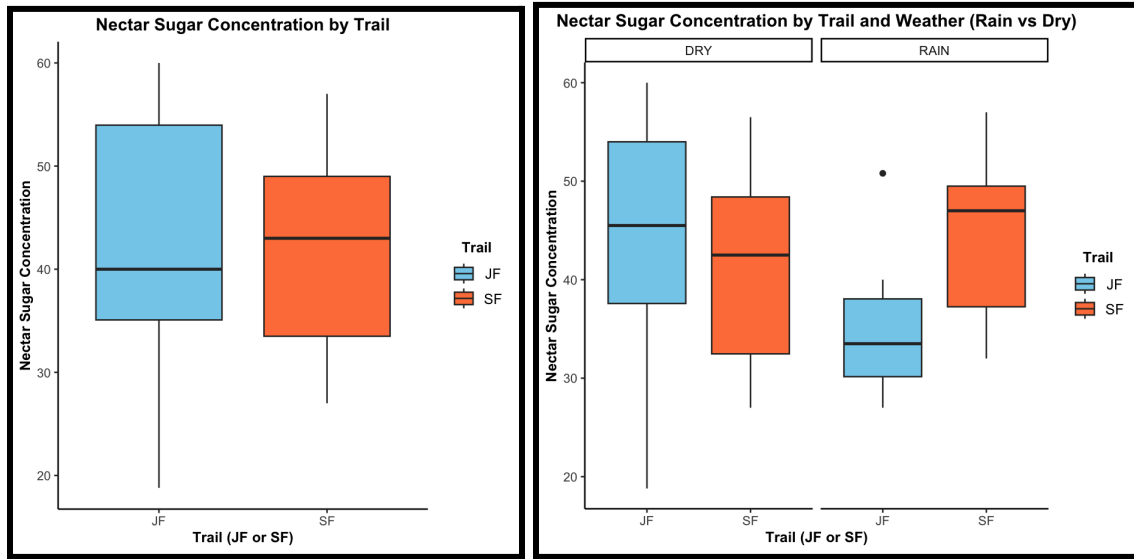
Graph 4 and 5: Soil Moisture vs. Trail (With and Without Weather Adjustments)

Soil moisture differed significantly between Judd and Stupid Falls (Wilcoxon rank sum test, $W = 1948.5$, $p\text{-value} = 7.979\text{e-}10$, Table 3). To examine how soil moisture varied between the two sites under different weather conditions, we compared soil moisture on days classified as “dry” vs “wet”. Under dry conditions, soil moisture differed significantly between the two sites (Wilcoxon rank sum test, $W = 1473.5$, $p\text{-value} = 3.194\text{e-}05$, Table 3). Under wet conditions, soil moisture differed significantly between both sites (Welch’s t-test, $t = -6.3724$, $df = 33.728$, $p\text{-value} = 2.942\text{e-}07$, Table 3)



Graph 6 and 7: Nectar Output (Total Per Stalk) vs. Trail (With and Without Weather Adjustments)

Nectar output differed significantly between Judd and Stupid Falls (Wilcoxon rank sum test, $W = 4149$, $p\text{-value} = 0.03749$, Table 2). To examine how nectar output varied between the two sites under different weather conditions, we compared nectar output on days classified as “dry” vs “wet”. Under dry conditions, nectar differed significantly between the two sites (Wilcoxon rank sum test, $W = 42$, $p\text{-value} = 1.937\text{e-}05$, Table 2). Under wet conditions, soil moisture did not differ significantly between the two sites (Wilcoxon rank sum test, $W = 3184$, $p\text{-value} = 0.9578$, Table 2).



Graph 8 and 9: Nectar Concentration(Average per Stalk) vs. Trail (With and Without Weather Adjustments)

Nectar sugar concentration did not differ significantly between Judd and Stupid Falls (Wilcoxon rank sum test, $W = 2669$, $p\text{-value} = 0.2328$, Table 1). To examine how nectar output varied between the two sites under different weather conditions, we compared nectar output on days classified as “dry” vs “wet”. Under dry conditions, nectar differed significantly between the two sites (Wilcoxon rank sum test, $W = 1982.5$, $p\text{-value} = 0.02249$, Table 1). Under wet conditions, soil moisture differed significantly between the two sites (Welch’s t-test, $t = -2.9507$, $df = 20.632$, $p\text{-value} = 0.007729$, Table 1).

Test	Non-normally distributed data?	Subsequent test	Results
Nectar Con. vs. Soil Moisture	Y/Y	Spearman	$S = 376085$, $p\text{-value} = 0.01818$, $\rho = -0.2126908$
Nectar Con. vs. Soil Moisture (SF)	Y/Y	Spearman	$S = 64497$, $p\text{-value} = 0.2892$, $\rho = -0.1284591$
Nectar Con. vs. Soil Moisture (JF)	Y/Y	Spearman	$S = 27275$, $p\text{-value} = 0.4778$, $\rho = -0.09963973$
Nectar Con. vs. Soil Moisture (SF RAIN)	N/N	Pearson	$t = 0.34915$, $df = 8$, $p\text{-value} = 0.736$, $\text{cor} = 0.1225134$
Nectar Con. vs. Soil Moisture (SF DRY)	Y/Y	Spearman	$S = 33743$, $p\text{-value} = 0.03593$, $\rho = -0.2861654$
Nectar Con. vs. Soil Moisture (JF RAIN)	N/Y	Pearson	$t = -0.6734$, $df = 14$, $p\text{-value} = 0.5117$, $\text{cor} = -0.1771282$
Nectar Con. vs. Soil Moisture (JF DRY)	Y/Y	Spearman	$S = 16845$, $p\text{-value} = 0.0778$, $\rho = -0.2718673$

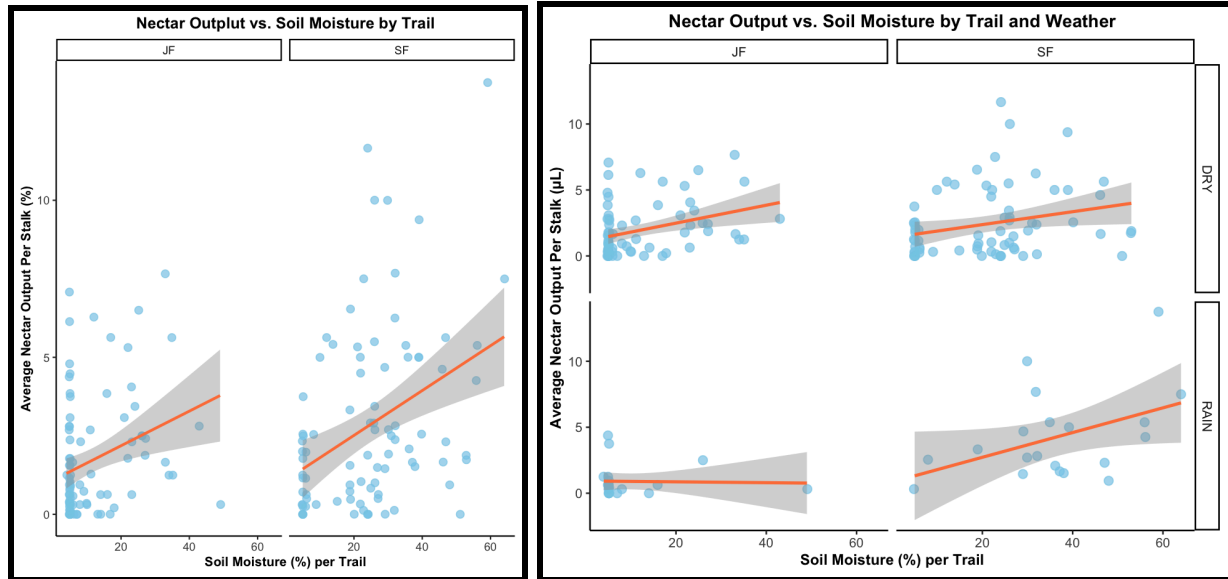
Table 4: Correlation Test Values for Nectar Concentration vs. Soil Moisture

Test	Non-normally distributed data?	Subsequent test	Results
Nectar Output vs. Soil Moisture	Y/Y	Spearman	S = 596433, p-value = 8.46e-08 rho = 0.3863668
Nectar Output vs. Soil Moisture (SF)	Y/Y	Spearman	S = 75717, p-value = 0.0002523 Rho = 0.3767353
Nectar Output vs. Soil Moisture (JF)	Y/Y	Spearman	S = 90392, p-value = 0.0149 Rho = 0.2559379
Nectar Output vs. Soil Moisture (SF RAIN)	Y/N	Pearson	t = 2.0526, df = 18, p-value = 0.05494, cor = 0.4355157
Nectar Output vs. Soil Moisture (SF DRY)	Y/Y	Spearman	S = 42039, p-value = 0.02693 Rho = 0.2644784
Nectar Output vs. Soil Moisture (JF RAIN)	Y/Y	Spearman	S = 1572.8, p-value = 0.4411 Rho = -0.1825587
Nectar Output vs. Soil Moisture (JF DRY)	Y/Y	Spearman	S = 39667, p-value = 0.009997 Rho = 0.3059706

Table 5: Correlation Test Values for Nectar Output vs. Soil Moisture

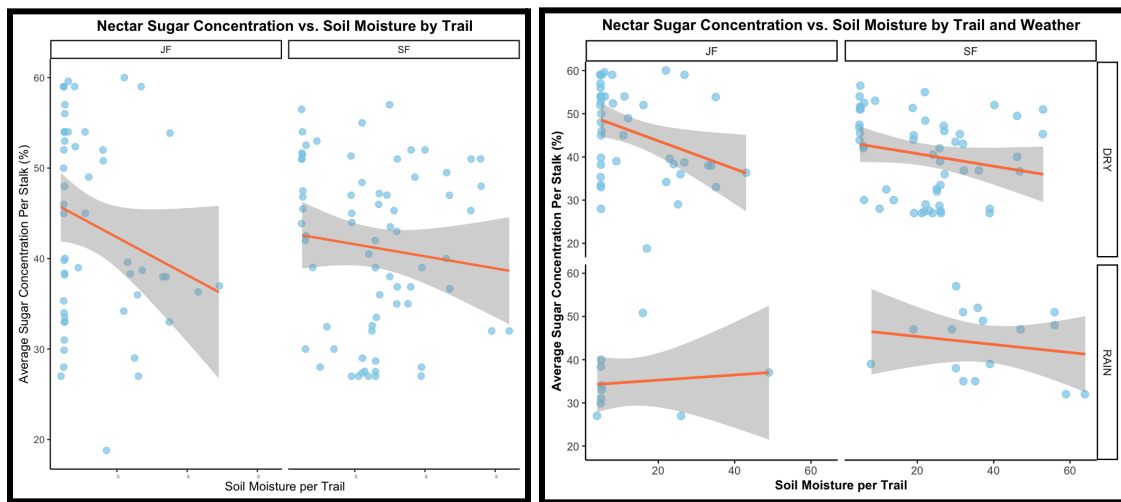
Test	Significant values?	Subsequent test	Results
Nectar Con. vs. Nectar Output	Y/Y	Spearman	S = 624838, p-value = 1.394e-06 Rho = -0.3960365
Nectar Con. vs. Nectar Output (SF)	Y/Y	Spearman	S = 113262, p-value = 6.468e-06, Rho = -0.488806
Nectar Con. vs. Nectar Output (JF)	Y/Y	Spearman	S = 51312, p-value = 0.02122 Rho = -0.2921454
Nectar Con. vs. Nectar Output (SF RAIN)	Y/N	Pearson	t = -3.974, df = 14, p-value = 0.001385, cor = -0.7280726
Nectar Con. vs. Nectar Output (SF DRY)	Y/Y	Spearman	S = 57330, p-value = 2.09e-05 Rho = -0.5158764
Nectar Con. vs. Nectar Output (JF RAIN)	Y/N	Pearson	t = -1.384, df = 8, p-value = 0.2037, cor = -0.4395248
Nectar Con. vs. Nectar Output (JF DRY)	Y/Y	Spearman	S = 33831, p-value = 0.0009725, Rho = -0.4441455

Table 6: Correlation Test Values for Nectar Concentration vs. Nectar Output



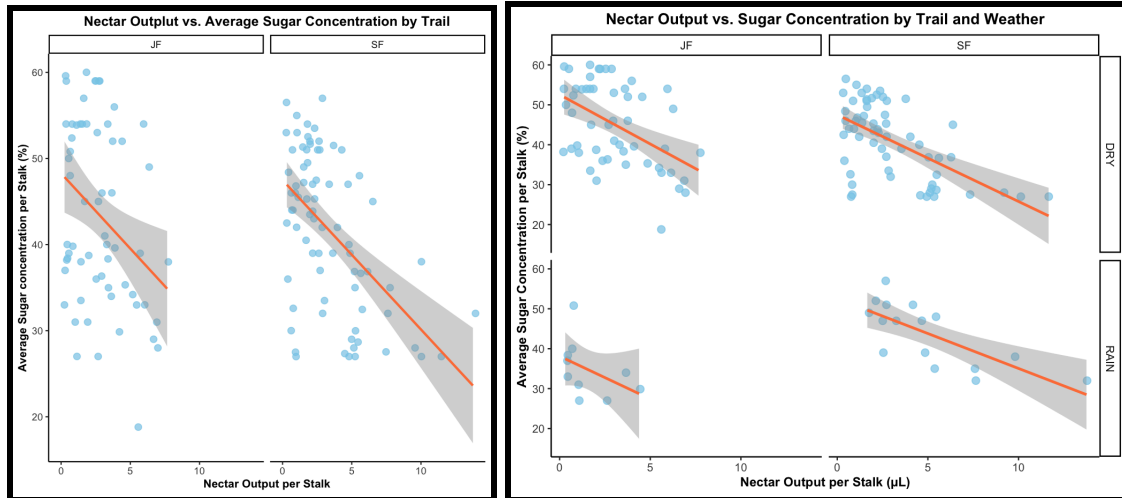
Graph 10 and 11: Nectar Output (Average per Stalk) vs. Soil Moisture (Percentage) (With and Without Weather Adjustments)

A positive relationship was found between soil moisture and nectar output (Linear regression, $p = 0.01352$, Multiple R-squared = 0.2046, Table 10).



Graph 12 and 13: Nectar Concentration(Average per Stalk) vs. Soil Moisture (With and Without Weather Adjustments)

A negative relationship was found between soil moisture and nectar sugar concentration (Linear regression, $p < 2e-16$, Multiple R-squared = 0.03645, Table 11).



Graph 14 and 15: Nectar Output (Average per Stalk) vs. Nectar Sugar Concentration (%) (With and Without Weather Adjustments)

An overall strong negative correlation is indicated between nectar output and nectar sugar concentration (Spearman Correlation, $S = 624838$, $p\text{-value} = 1.394\text{e-}06$, $Rho = -0.3960365$, Table 6). Further separating this data into Judd Falls and Stupid Falls, a strong negative correlation remains between the variables (Spearman Correlation, $S = 113262$, $p\text{-value} = 6.468\text{e-}06$, $Rho = -0.488806$; $S = 51312$, $p\text{-value} = 0.02122$, $Rho = -0.2921454$, Table 6). Taking into account “dry” and “wet” weather conditions, a negative correlation remains present for Stupid Falls and Judd Falls dry data (Spearman Correlation, $S = 57330$, $p\text{-value} = 2.09\text{e-}05$, $Rho = -0.5158764$; Spearman Correlation, $S = 33831$, $p\text{-value} = 0.0009725$, $Rho = -0.4441455$, Table 6). A negative correlation is present in Stupid Falls Rain data (Pearson Correlation, $t = -3.974$, $df = 14$, $p\text{-value} = 0.001385$, $cor = -0.7280726$, Table 6). No significant correlation is present for Judd Falls Rain data (Pearson correlation, $t = -1.384$, $df = 8$, $p\text{-value} = 0.2037$, $cor = -0.4395248$, Table 6). However, there is a limited scope for the Rain data in general.

Model #1	Estimate Values	P-values
Variables	Nectar Output ~ Soil %	
Intercept	1.00976	0.000284
Soil %	0.07031	1.31e-08
Multiple R-squared	0.1664	
Adjusted R-squared	0.1617	
F-statistic p-value	1.315e-08	

Table 7: Nectar Output vs. Soil Percent - Linear Model 1

Model #2	Estimate Values	P-values
Variables	Nectar Output ~ Soil % + Trail	

Intercept	0.95315	0.00104
Soil %	0.06632	1.26e-06
TrailSF	0.25711	0.50194
Multiple R-squared	0.1685	
Adjusted R-squared	0.1591	
F-statistic p-value	8.053e-08	

Table 8: Nectar Output vs. Soil Percent - Linear Model 2

Model #3	Estimate values	P-values
Variables	Nectar Output ~ Soil % * Trail	
Intercept	1.083353	0.00354
Soil %	0.055056	0.02196
TrailSF	0.003269	0.99557
Soil % : TrailSF Estimate	0.016310	0.56995
Multiple R-squared	0.1701	
Adjusted R-squared	0.1559	
F-statistic p-value	3.381e-07	

Table 9: Nectar Output vs. Soil Percent - Linear Model 3

Model #4	Estimate values	P-Values
Variables	Nectar Output ~ Soil % * Trail * Weather	
Intercept	1.12913	0.00912
Soil %	0.06791	0.01352
TrailSF	0.26671	0.68870
WeatherRAIN	-0.20235	0.80238
Soil % : TrailSF	-0.01898	0.57709
Soil % : WeatherRAIN	-0.07111	0.20272
TrailSF : WeatherRAIN	-0.34522	0.83241
Soil % : TrailSF : WeatherRAIN	0.11590	0.09013
Multiple R-squared	0.2046	

Model #4	Estimate values	P-Values
Adjusted R-squared	0.1722	
F-statistic p-value	1.297e-06	

Table 10: Nectar Output vs. Soil Percent - Linear Model 4

Model #1	Estimate Values	P-values
Variables	Nectar Concentration ~ Soil %	
Intercept	44.85618	< 2e-16
Soil %	-0.12568	0.0344
Multiple R-squared	0.03645	
Adjusted R-squared	0.02848	
F-statistic p-value	0.03442	

Table 11: Nectar Sugar Concentration vs. Soil Percent - Linear Model 1

Model #2	Estimate Values	P-values
Variables	Nectar Concentration ~ Soil % + Trail	
Intercept	45.21284	<2e-16
Soil %	-0.11000	0.0901
TrailSF	-0.18668	0.5476
Multiple R-squared	0.03936	
Adjusted R-squared	0.02335	
F-statistic p-value	0.08989	

Table 12: Nectar Sugar Concentration vs. Soil Percent - Linear Model 2

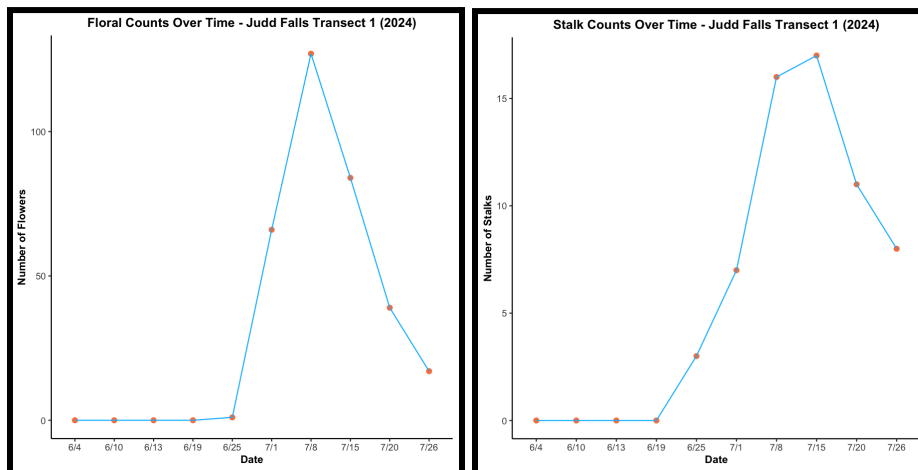
Model #3	Estimate values	P-values
Variables	Nectar Concentration ~ Soil % * Trail	
Intercept	46.5239	<2e-16
Soil %	-0.2086	0.0758
TrailSF	-3.6107	0.2453
Soil % : TrailSF Estimate	0.1420	0.3117
Multiple R-squared	0.04762	

Model #3	Estimate values	P-values
Adjusted R-squared	0.02361	
F-statistic p-value	0.1202	

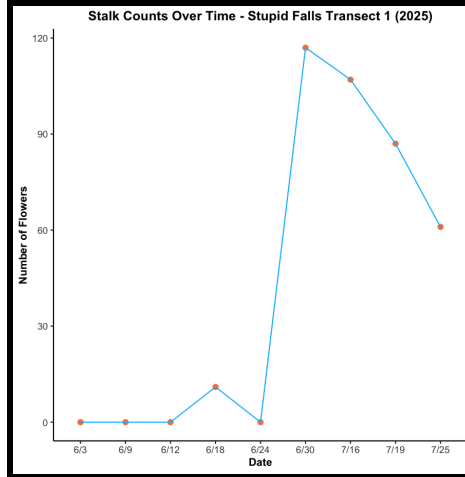
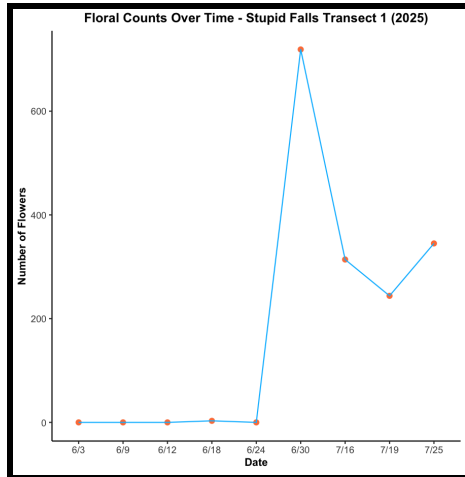
Table 13: Nectar Sugar Concentration vs. Soil Percent - Linear Model 3

Model #4	Estimate values	P-Values
Variables	Nectar Concentration ~ Soil % * Trail * Weather	
Intercept	50.2058	< 2e-16
Soil %	-0.3245	0.013415
TrailSF	-6.5567	0.050936
WeatherRAIN	-16.1493	0.000604
Soil % : TrailSF	0.1797	0.264502
Soil % : WeatherRAIN	0.3845	0.125504
TrailSF : WeatherRAIN	19.7188	0.019977
Soil % : TrailSF : WeatherRAIN	-0.3325	0.287636
Multiple R-squared	0.1723	
Adjusted R-squared	0.1219	
F-statistic p-value	0.002337	

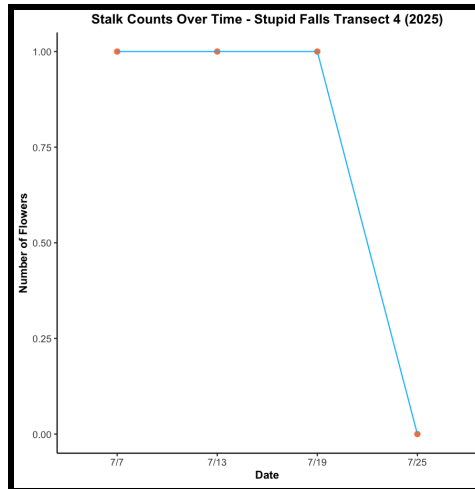
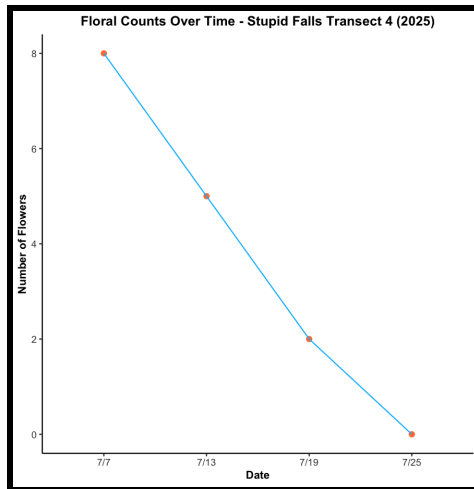
Table 14: Nectar Sugar Concentration vs. Soil Percent - Linear Model 4



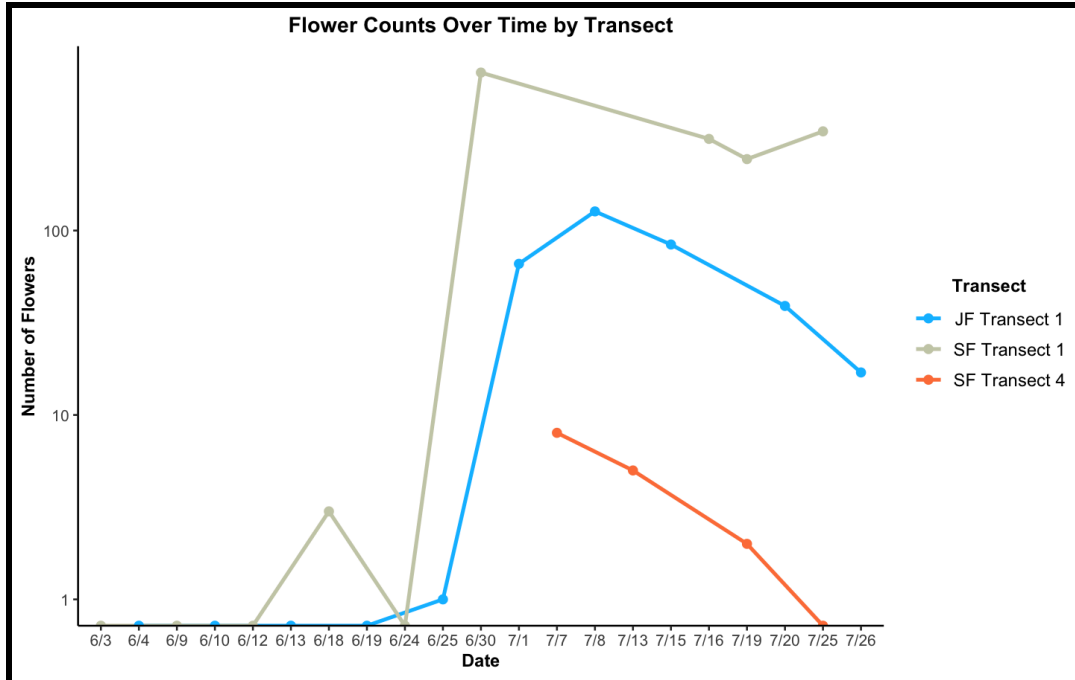
Graphs 16 and 17: Judd Falls Transect 1 - Floral Counts and Stalk Counts over the Summer



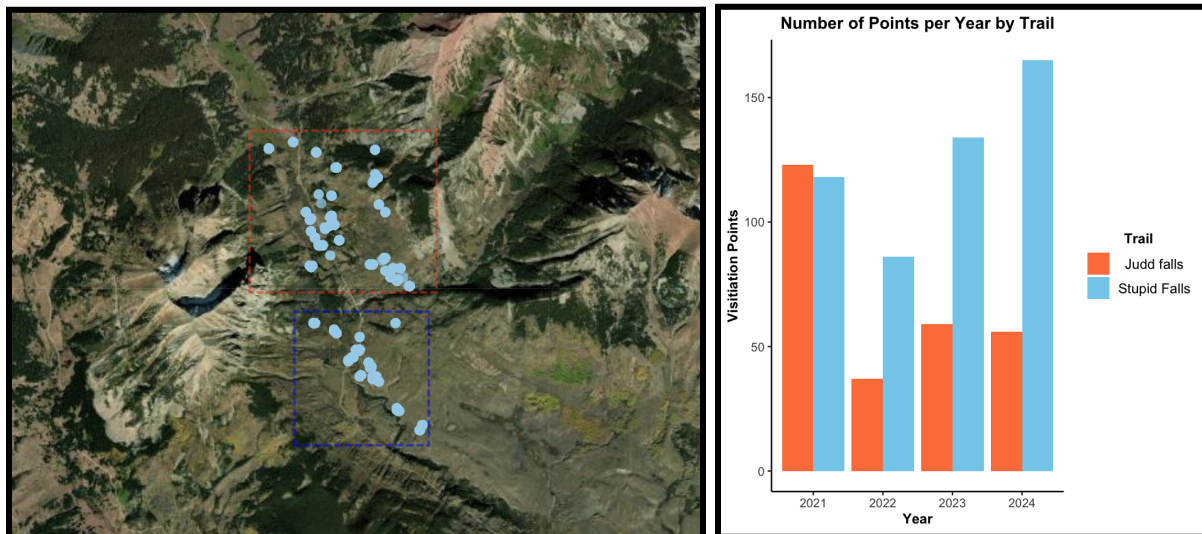
Graphs 18 and 19: Stupid Falls Transect 1 - Floral Counts and Stalk Counts over the Summer



Graphs 20 and 21: Stupid Falls Transect 4 - Floral Counts and Stalk Counts over the Summer



Graph 22: All Transects Overlapping - General *Ipomopsis* presence - 2025
Ipomopsis aggregata were present on only three of our transects.



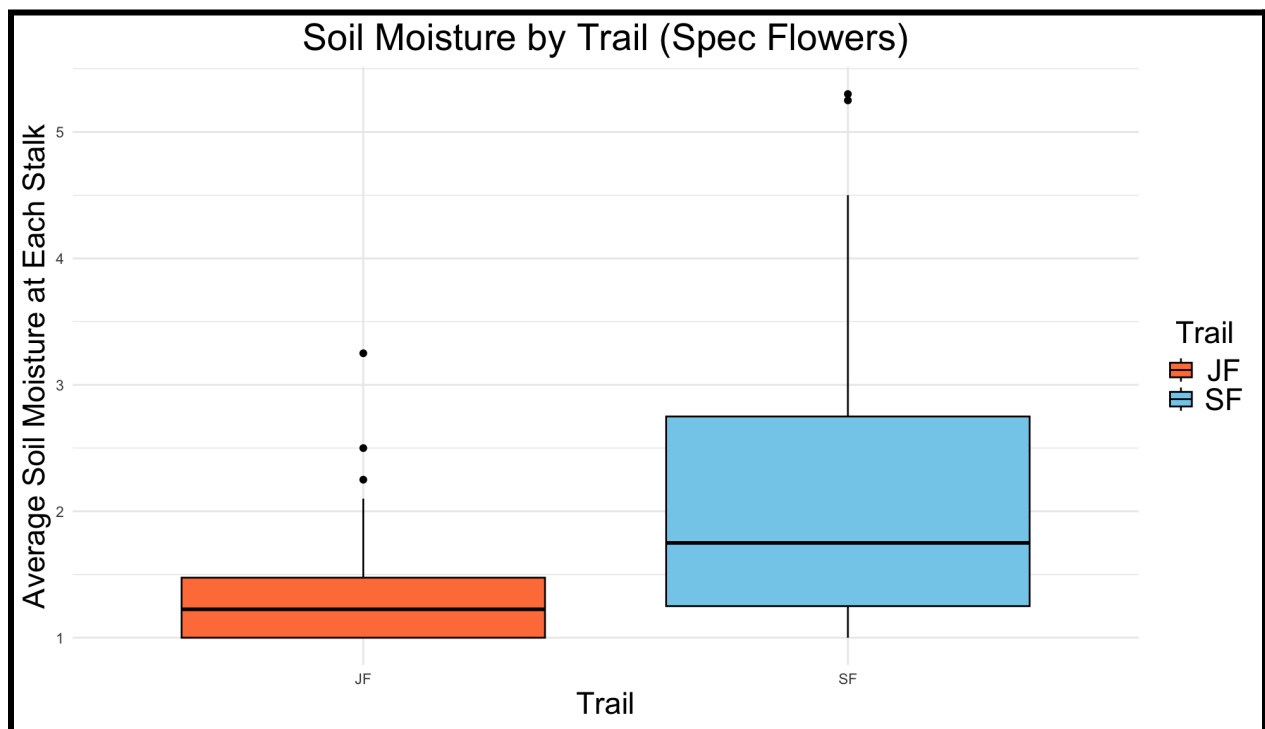
*Hummingbird visitation points to the *I. aggregata* from the years 2021-2024*
*Blue = Stupid Falls Hummingbird *Ipomopsis* visitation 2021-2024*
*Red = Judd Falls Hummingbird *Ipomopsis* visitation 2021-2024*
Graph 23: Points per year by Trail - Map analysis

After transect collection throughout the summer, *I. aggregata* presence was only indicated in Judd Falls Transect 1, Stupid Falls Transect 1, and Stupid Falls Transect 4. Judd Falls Transect 1 only showed *I. aggregata* presence starting on June 25th, peaked on July 8th, and has been in decline ever since. Stupid

Falls Transect 1 only showed *I. aggregata* presence starting June 18th, peaked on June 30th, and has been in decline ever since. There has been an increase starting July 19th, but is expected to decrease from then on. Stupid Falls Transect 4 began later than the other Transects, but peaked in the beginning, on July 7th, and had fallen ever since. Overall, the *I. aggregata* presence in 2025 has spanned June 12th to July 26th. (Reference graphs 16-22)

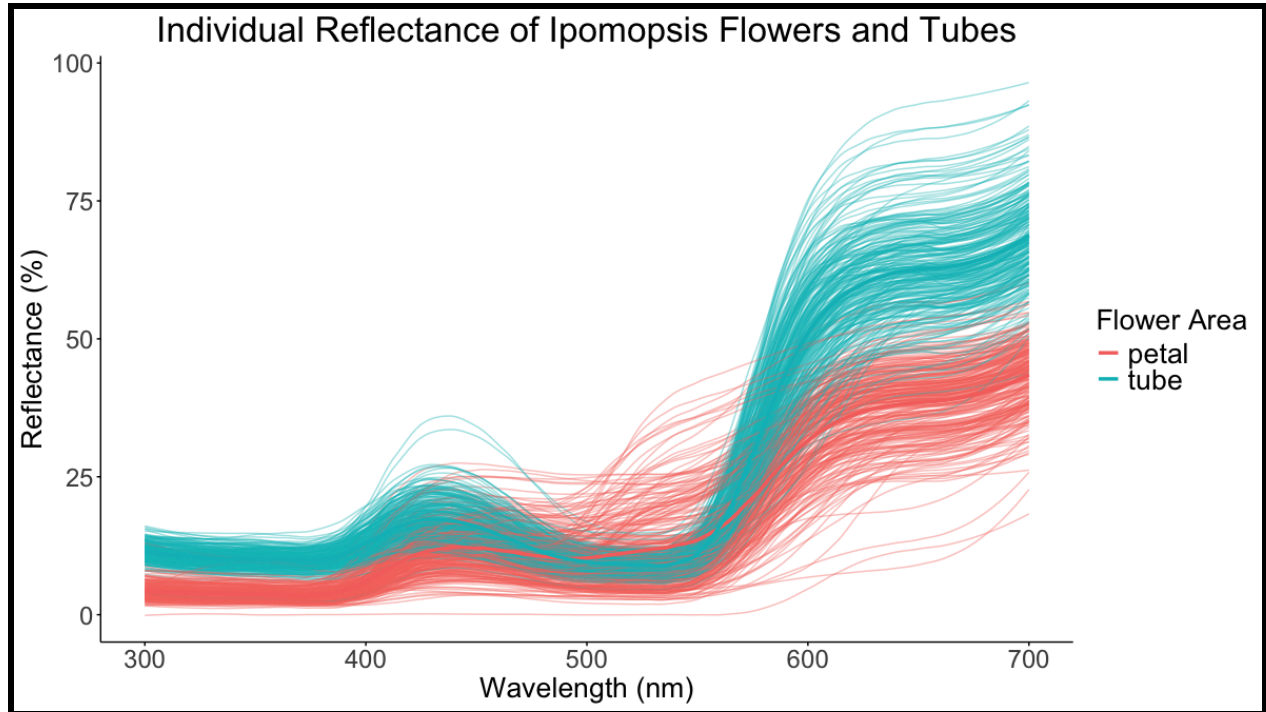
Using this duration of time, broad-tailed hummingbird visitation was narrowed into the *I. aggregata* from years 2021 -2024, specifically from the days of June 12th to July 26th. We see that in the years 2022-2024, there was considerably more visitation to JF *I. aggregata*.

Results: Color and Soil Moisture



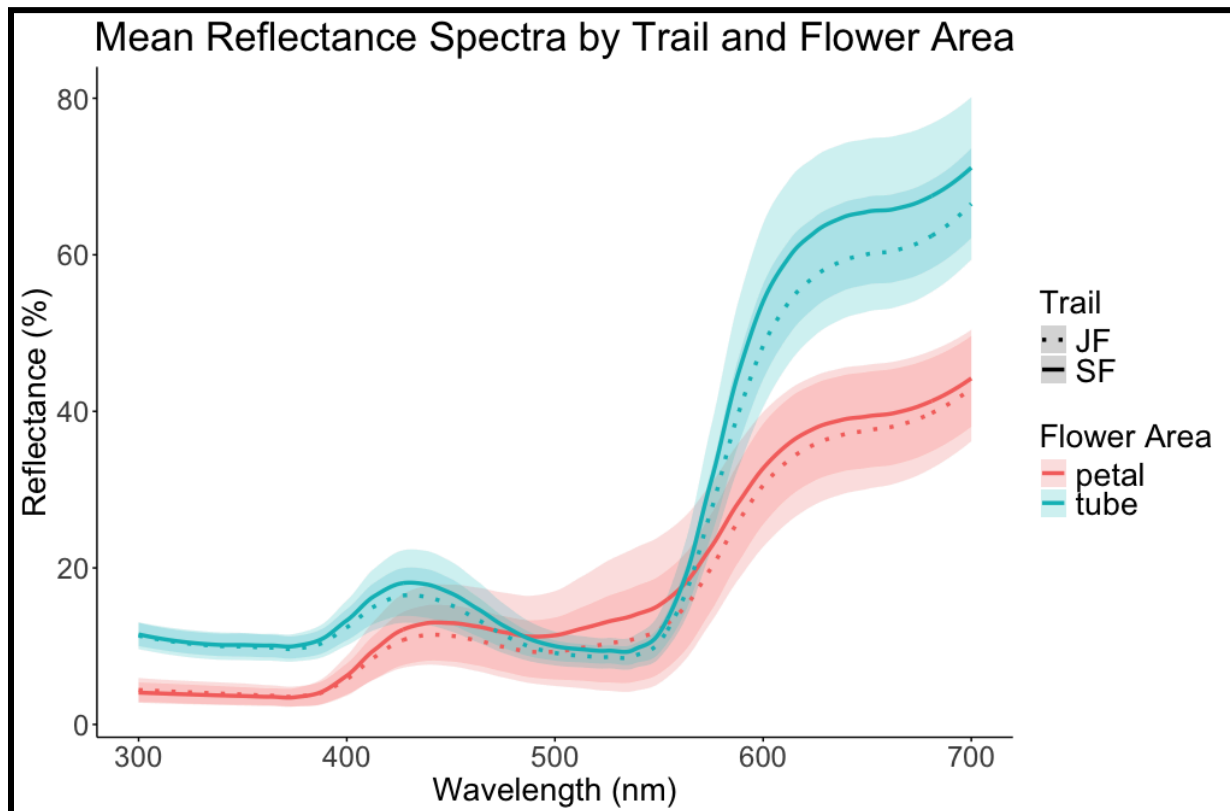
Graph 24: Soil Moisture by Trail (Judd vs Stupid Falls) for July 7-11 and 30-31

Soil moisture varied significantly for flowers collected between Judd and Stupid Falls (two-sample t-test, $t = -5.6734$, $p = 2.5e-07$, $df = 74.874$). The mean soil moistures were found to be 1.3 and 2.2 in Judd and Stupid Falls respectively (on the 1(driest) through 10 (wettest) scale).



Graph 25: Individual Reflectance of Ipomopsis Flowers and Tubes or July 7-11 and 30-31

Percent reflectance was measured for light of every wavelength between 300 and 700 nm in increments of 1 nm.



Graph 26: Mean Reflectance Spectra by Trail and Flower Area for July 7-11 and 30-31

The mean percent reflectance was measured for all flowers for Judd vs Stupid Falls at the petals vs the tube. The highlighted sections denote the standard deviation.

The Principal Component Analysis showed that the first four PCs account for 98.3% of the variance. Examining the loadings of the PC scores against the wavelengths, we determined that PC1 (64% of variance) accounts for reflectance intensity, PC2 (25% of variance) accounts for more UV and long wavelength reflectance (>550 nm), PC3 (5% of variance) accounts for more reflectance above 500 nm, and PC4 (3% of variance) accounts for peaks in UV and 500 - 600 nm.

Principal Components	PC1	PC2	PC3	PC4
Standard Deviation	16.1141	10.0742	4.6233	3.41885
Proportion of Variance	0.6475	0.2531	0.0533	0.02915

Table 15: Principal Component Analysis Results

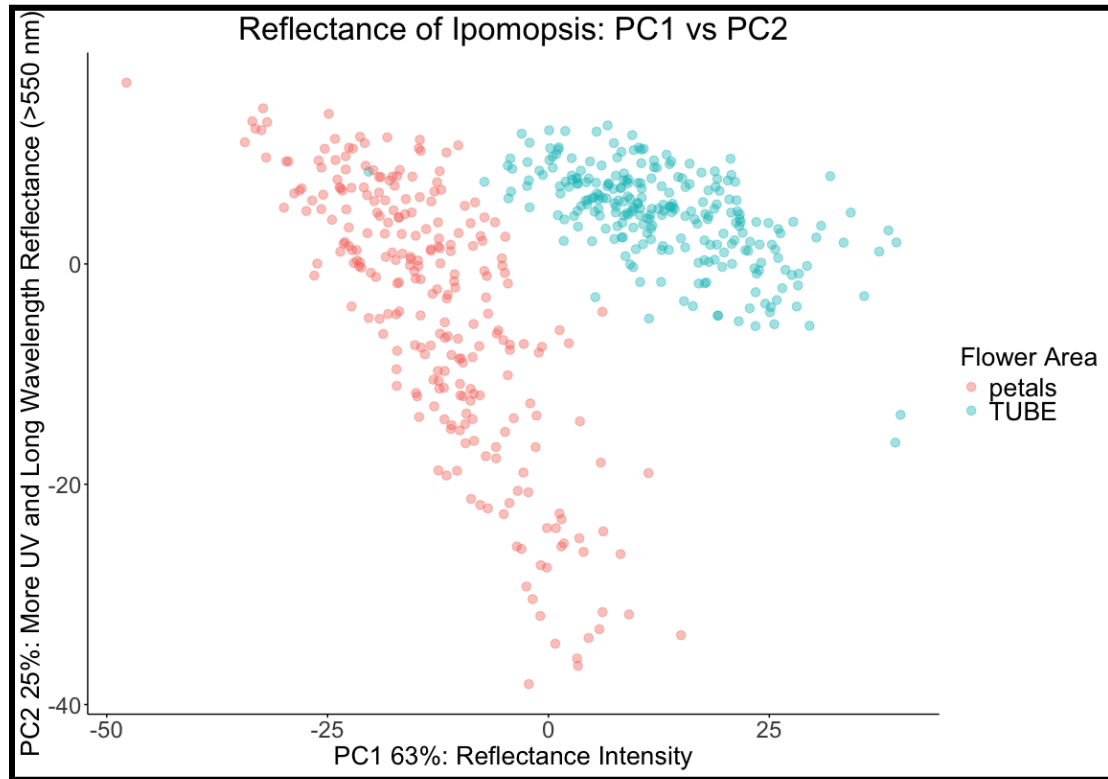
Test Variables	Effect on:	T-Test results	Cohen's D test (D = number of standard deviations)
Flower Area	PC1	$t = 30.62$, $df = 506.8$, $p < 2.2e-16$	$d = 2.71$
Flower Area	PC2	$t = 10.954$, $df = 318.94$, $p < 2.2e-16$	$d = 0.97$
Flower Area	PC3	$t = -0.38564$, $df = 434.74$, $p = 0.7$	$d = -0.03$
Flower Area	PC4	$t = -3.6896$, $df = 506.99$, $p = 0.000249$	$d = -0.33$

Table 16: Effect of Floral Area on Principal Component Scores

Test Variables	Effect on:	T-Test results	Cohen's D test (D = number of standard deviations)
Trail	PC1	$t = 2.8196$, $df = 483.61$, $p = 0.005005$	$d = 0.25$
Trail	PC2	$t = -4.0754$, $df = 451.74$, $p = 5.426e-05$	$d = -0.37$
Trail	PC3	$t = -1.8243$, $df = 470.34$, $p = 0.06874$	$d = -0.16$

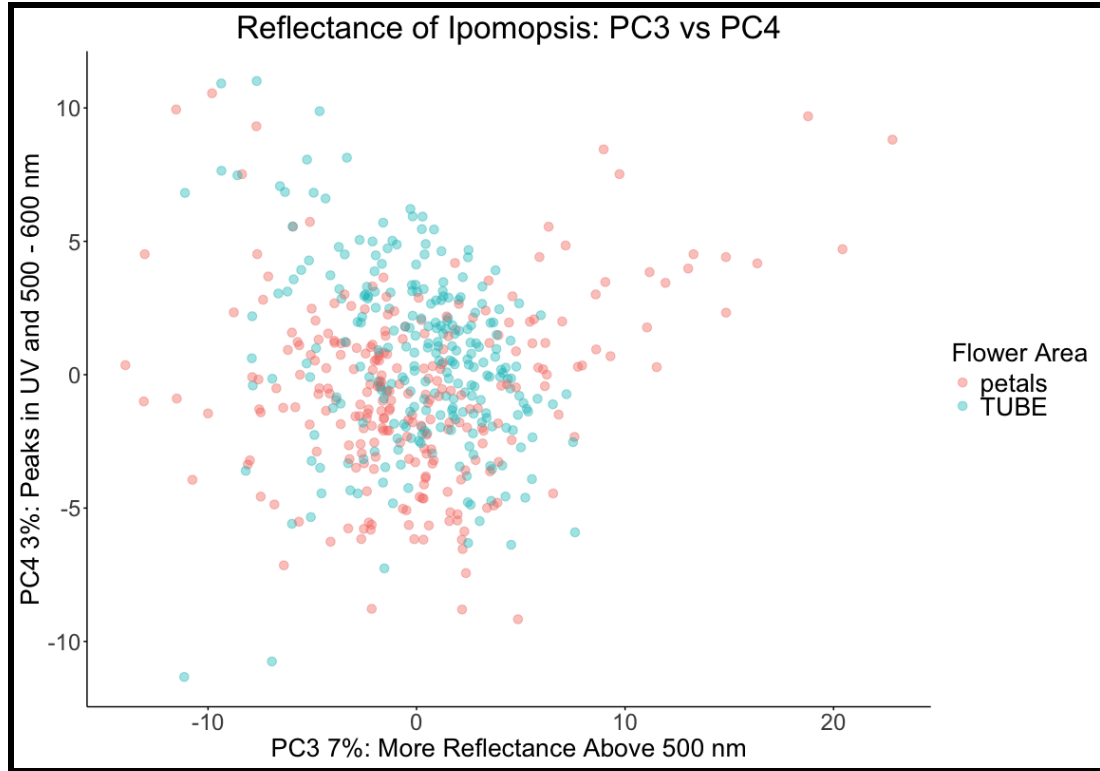
Trail	PC4	$t = 3.3037$, $df = 506.65$, $p = 0.001022$	$d = 0.29$
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Table 3: Effect of Trail on Principal Component Scores



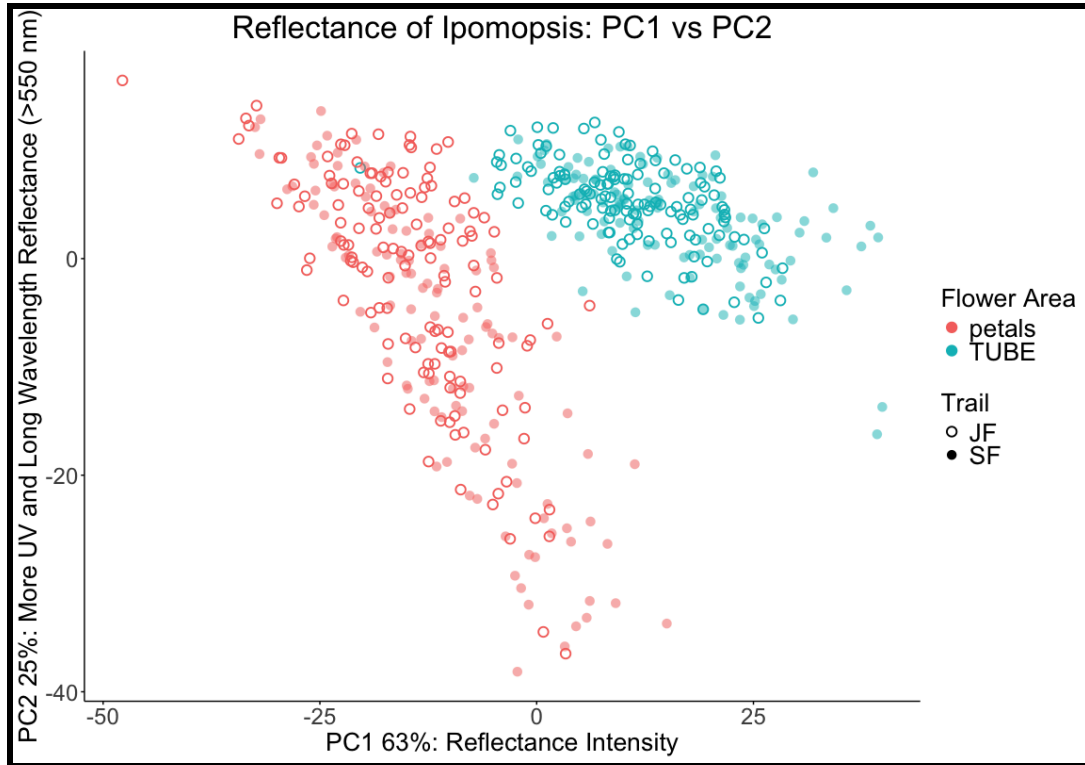
Graph 27: Do PC1 and PC2 Vary by Flower Area?

PC1 scores varied significantly between petals and tube (t-test, $t = 30.62$, $df = 506.8$, $p < 2.2e-16$). The difference in PC1 between petals and tube is 2.71 standard deviations apart (Cohen's d , $d = 2.71$). PC2 varied significantly between petals and tube (t-test, $t = 10.954$, $df = 318.94$, $p < 2.2e-16$). The difference in PC2 between petals and tube is 0.97 standard deviations apart (Cohen's d , $d = 0.97$).



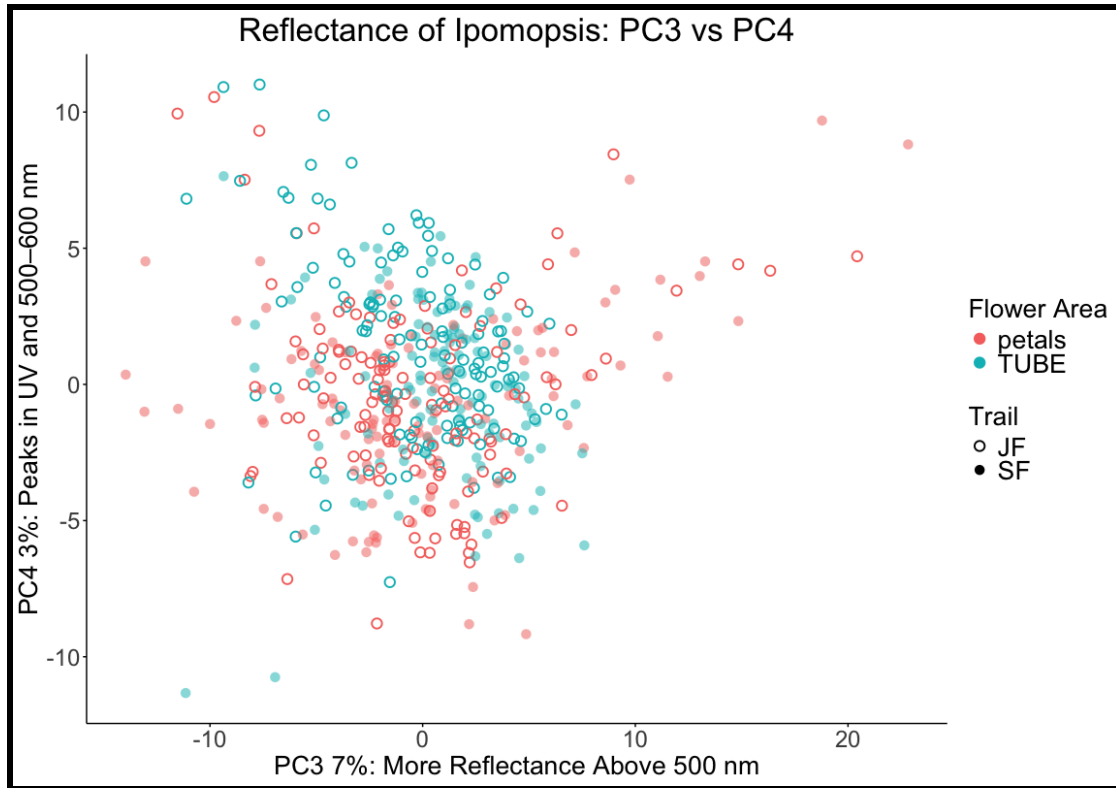
Graph 28: Do PC3 and PC4 Vary by Flower Area?

PC3 scores did not vary significantly between petals and tube measurements (t-test, $t = -0.38564$, $df = 434.74$, $p = 0.7$). The difference in PC3 between petals and tube is 0.03 standard deviations apart (Cohen's d , $d = -0.03$). PC4 scores varied significantly between petals and tube (t-test, $t = -3.6896$, $df = 506.99$, $p = 0.000249$). The difference in PC4 between petals and tube is 0.33 standard deviations apart (Cohen's d , $d = -0.33$).



Graph 29: Do PC1 and PC2 Vary by Trail?

PC1 scores varied significantly between trail (t-test, $t = 2.8196$, $df = 483.61$, and $p = 0.005005$). The difference in PC1 between trails is 0.25 standard deviations apart (Cohen's d , $d = 0.25$). PC2 scores varied significantly between trail (t-test, $t = -4.0754$, $df = 451.74$, $p = 5.426e-05$). The difference in PC2 between trails is 0.37 standard deviations apart (Cohen's d , $d = -0.37$).



Graph 30: Do PC3 and PC4 Vary by Trail?

PC3 scores did not vary significantly between trail (t-test, $t = -1.8243$, $df = 470.34$, $p = 0.06874$). The difference in PC3 between trails is 0.16 standard deviations apart (Cohen's d , $d = -0.16$). PC4 scores varied significantly between trail (t-test, $t = 3.3037$, $df = 506.65$, $p = 0.001022$). The difference in PC4 between trails is 0.29 standard deviations apart (Cohen's d , $d = 0.29$).

Discussion:

Soil Moisture:

When looking at the results from the Two-Sample t-tests comparing the average soil moisture at each stalk that spectroscopy and nectar flowers were taken from, we see that Stupid Falls (SF) and Judd Falls (JF) differ significantly in soil moisture, both from July 7-26 and from July 7-11 and 30-31, with higher soil moisture at SF. From July 7-11 and July 30-31 specifically, the mean soil moisture was 1.3 and 2.2 at Judd and Stupid Falls respectively (on the 1 (driest) through 10 (wettest) scale). This means that the *Ipomopsis* flowers were taken for spectroscopy and nectar testing from locations which were both generally very dry but nevertheless varying significantly in soil moisture. We had predicted this difference due to SF's lower general elevation and closeness to the East River. This result is fundamental to understanding the landscape differences between JF and SF, as SF presents as more of a meadow ecosystem with

more consistent floral abundance and grass varieties, compared to JF's rockier areas with sparse floral varieties and visually distinct microecosystems. At JF, we encountered both rocky slopes and muddy patches, although the *Ipomopsis* were found only at the rockier regions. Looking at whether this relationship persists across weather differences, we see that Stupid Falls continued having higher average soil moisture readings compared to Judd Falls during the 16 days of dry data and the 4 days of rain data (Reference Graphs 4 and 5). This result shows that despite weather differences, the soil moisture differences remain consistent between sites. However, the lack of specificity in rain data leaves a gap in our understanding of these sites, and therefore calls for taking more exact precipitation data for the study days.

Nectar:

Our hypothesis that nectar output is significantly different between sites is supported by the fact that Stupid Falls flowers had more nectar output per stalk on average. Adding weather as an additional factor, we see that dry conditions indicate a significant relationship, with SF having greater output than JF. Rain data, however, was not significant, but this lack of significance was perhaps due to the lack of a large data set, as there were only 4 days of rain data. With these findings, we can begin thinking about the possible relationship between soil moisture and nectar output, as SF exhibits both the higher soil moisture and higher nectar output compared to JF. A linear regression is appropriate to expose relationships between these variables. Running linear regression to understand the correlation between soil moisture and nectar output, we see an overall strong positive correlation by trail without weather adjustments: as soil moisture is increasing, nectar output is also increasing. The model is significant, but the R^2 indicates that only 17% of the data is explained by this relationship, indicating a possible gap in the data that needs to be filled. Another possible variable to consider might be the impact of soil pH or more precise precipitation data. Exposing the role of additional variables in this relationship, adding trail indicated the same relationship between soil moisture and nectar output. Nectar output and trail as an additional relationship was not significant. Adding this variable led to even less of the data fitting into this relationship, decreasing the model's fit from 17% to 15%. Finally, adding trail and weather as additional interaction variables, we see that the relationship between nectar output and soil moisture stays strong, meanwhile all other variable considerations are insignificant. Therefore, although a specific relationship between nectar output, soil moisture, weather, and trail isn't necessarily indicated by this data set, it gears us in a few directions. First, it tells us that a larger data set might be needed, compared to the 20 day data set used. It also tells us that there is a void, a possible other variable that might indicate a stronger relationship. But finally, it tells us that a strong positive correlation is present between soil moisture and nectar output, highlighting the importance of moisture levels in the development of the *I. aggregata* as a population in Gothic, and also the importance of moisture in regulating energy sources for local pollinators.

We also looked at nectar concentration, or the amount of sugar found in a nectar sample. Refractometers tests for general refraction, so "sugar" is the best term for this situation, as nectar is made of many different types of sugar that cannot be determined without chemical analysis.

The first test we ran was the relationship between nectar concentration and trail, finding that nectar concentration does not differ significantly between trails. However, when weather was taken into account, we see significant results for both rain and dry data. Dry data indicates JF as having higher average nectar concentration. On the other hand, rain data indicates SF as having higher average nectar concentration. The dry data's result probably holds more weight than the rain data because there are more dry days to compare to, but the nuance from each of these data sets indicates a need for more specific tests to be run.

In order to deal with this discrepancy, we ran linear regression to see the relationship between nectar concentration and soil moisture. When comparing nectar concentration and soil moisture by trail, we see a strong negative relationship, indicating that as soil moisture increases, nectar concentration decreases. The first linear regression model, which compared only nectar concentration and soil moisture, was significant and indicated a strong significant relationship between the two variables. Taking into account trail as an additional variable, the data became less and less significant. However, when nectar concentration, soil moisture, trail, and weather were all taken into account, the relationship accounted for by this model was 17% compared to the 3.6% accounted for by the first model. This fourth model maintains the strong relationship between soil moisture and nectar concentration, indicating once again a negative correlation between them. This model also accounts for a significant relationship between nectar concentration and trail, indicating variance in nectar concentration by site. An additional significant strong relationship is present between weather data and nectar concentration, indicating variance by weather. The last significant relationship identified by this model was between trail, weather, and nectar concentration as an interaction, indicating how there is variance in the data when all these variables are dependent on each other. This indicates that there is a strong relationship between soil moisture and nectar concentration, but again, the lack of relationship certainty between these models indicates this data as a good start in indicating relationships between nectar and soil moisture, but not the final picture.

Lastly, we look at the correlation between nectar output and nectar sugar concentration. Since higher soil moisture led to greater nectar output, and higher soil moisture led to lower nectar concentration, we can predict that an increase in nectar output would correspond to a decrease in nectar concentration, due to nectar dilution for wetter sites and evaporation of water in drier sites. The correlation present indeed indicated a negative correlation between these two variables: as nectar output increased per stalk, the refractometer indicated lower sugar concentration. This relationship remained significant even when weather was added as an additional variable.

Overall, we see a strong correlation between soil moisture and nectar output, soil moisture and nectar concentration, and nectar concentration and nectar output. We see that SF has greater average soil moisture values and greater average nectar output values. However, how can we use this information as a possible explanation of hummingbird visitation patterns?

Using the time frame indicated by floral bloom of the *I. aggregata* (Graph 22), we see that from the years of 2022-2024, Stupid Falls had significantly more visitation to the *I.*

aggregata. A possible reason for this visitation is the higher nectar output values possibly caused by soil moisture at SF. Due to greater satiation from higher nectar volumes, as well as lower energetic output to reach these flowers (because SF is relatively flat compared to JF), hummingbirds might be choosing to feed in Stupid Falls meadows at a greater rate.

Knowing all this about nectar's relationship to soil moisture, we see how climate change may be impacting hummingbirds in the long run. Specifically, we are interested in how climate change changes water resources and therefore nectar. As snow melts earlier in the season, and more drought periods are expected, a likely effect of this might be less nectar availability for incoming hummingbird populations. This leads to less energy resources for a larger hummingbird population over time. This might mean that hummingbirds will need to go beyond the bounds of Gothic, CO for feeding despite concentrating in the area before. Judd Falls visitation might be in greater decline as a whole due to the lack of rain in the area. Therefore, visitation to Stupid Falls may be the predominant feeding location due to greater overall floral abundance and energy resources, showing an increasing trend over these last years and possibly over the next few years. This exposes the even greater need for understanding climate change's effects in the area and how hummingbirds make decisions based on nectar. Climate change may be a big driver of this change.

Spectroscopy:

When light hits a surface, part of it is transmitted, and part is reflected. The different wavelengths of this light which are reflected correspond to different colors, and different species perceive these wavelengths differently depending on the specific photoreceptors in their eyes. Each wavelength stimulates a photoreceptor a certain amount. Hummingbirds are able to perceive more wavelengths than humans can, including UV wavelengths. The spectrometer's ability to encompass a wider range of wavelengths therefore made it very useful. With the spectrometer, we measured the wavelengths that were reflected as well as their intensity (a higher reflectance percentage corresponds to a higher intensity). Focusing on our initial question of the variation of spectral reflectance, we encounter an interesting and nuanced result telling us that certain aspects of color do vary between flower area and between trails.

The graph "Do PC1 and PC2 Vary by Flower Area?" combined with the t-test and Cohen's d together reveal that flower area (tube or petals) significantly affects the reflectance intensity, the amount of reflected UV wavelengths, and the amount of reflected long wavelengths. Specifically, we see that most of the tube measurements are clustered further to the right on the x axis, and most of the petals measurements are clustered further to the left on the x axis. This means that the *Ipomopsis* tube reflects wavelengths of light at a higher intensity than the petals do. The first graphs depicting all of the reflectance spectra for every flower at the petals and tube also visually emphasizes this result, since most of the blue lines (denoting tube measurements) are above the red lines (denoting petals measurements). It is only in the 480-560 nm range that we encounter an interesting reversal of this fact, with more petals measurements reaching higher reflectance than the tube measurements. This 480-560 nm range corresponds to

colors that we see as blue, blue-green, and yellow. Despite this greater reflectance of such colors, the reason why the *Ipomopsis* petals do not appear blue, blue-green, or yellow to us is that many of the colors which we see are mixtures of various wavelengths, and there truly are many additional wavelengths being reflected in this case. It is important to note that the petals were sometimes (but not always) thinner than the tube, and this difference in thickness might have influenced the intensity measurements. There were also sometimes small pieces of yellow pollen on the *Ipomopsis* petals. This pollen was not removed for spectroscopy in order to provide an accurate representation of colors visible to hummingbirds when they go to the flowers.

Furthermore, we see in this graph that there is more variation in how much UV and long (>550 nm) wavelengths are reflected in the petals region than in the tube region, since the petals data points are more spread out along the y axis, as opposed to the way that the tube data points are clustered in the upper half of the y axis. It is especially clear when looking at the “Mean Reflectance Spectra by Trail and Flower Area” graph that the standard deviation of the petals measurements is much greater than that of the tube measurement for the 550-600 nm range, with the mean reflectance plus and minus the standard deviation of the petals even containing that of the tube for 550-580 nm range. This result reflects the fact that sometimes the petals were bright red, while other times they were a paler red or bore traces of yellow and white. The longer wavelengths correspond to more red hues, the amount of which truly did vary between the petals of flowers in both regions.

Moving on to the next Principal Components, the graph “*Do PC3 and PC4 Vary by Flower Area?*” together with the t-test and Cohen’s d show that flower area does not significantly affect the amount of reflectance of wavelengths above 500 nm. This result may be due to the fact that petals tend to reflect higher in the 500-570 nm range, while tubes tend to reflect higher in the 570-700 nm range, but when simply referring to the 500+ nm range, we cannot say which flower area would have a higher reflectance. The analyses also revealed information about the significance of the spectral shape, showing that flower area does significantly affect the peaks in UV and 600 nm reflected wavelengths. Although petals and tubes both showed spectral peaks in these areas, tubes had a significantly higher reflectance than petals, as demonstrated by the “Mean Reflectance Spectra by Trail and Flower Area” graph. Our “Mean Reflectance Spectra by Trail and Flower Area” graph adds to the visualization of these results, as there is distance between the lower bound of the tubes’ percent reflectance and the upper bound of the petals’ percent reflectance for UV and long wavelengths, but standard deviations overlap for the 400-600 nm range.

These results bring up several questions. What is it, exactly, that birds look for when foraging for food? What information do different sections of the *Ipomopsis* flower reveal? Does the significant difference in color between flower area have to do with evolutionary reasons? What areas of *Ipomopsis* flowers are hummingbirds attracted by the most, and how may the changes to floral anatomy (the size of certain flower areas, specifically) brought about by changes in resources due to climate change affect the amount of attractive color that

hummingbirds perceive? How may these changes be impacting the color of the petals and the tubes of *Ipomopsis* over longer periods of time?

We also looked at PCs varying by trail, to see if there was a significant difference in flower color depending on the geography of the individual stalks. The graph “Do PC1 and PC2 Vary by Trail?” combined with the t-test reveal that the trail has a statistically significant effect on the reflectance intensity, and on the amount of UV wavelengths and long wavelengths that are reflected. Referring back to the “Mean Reflectance Spectra by Trail and Flower Area” graph, we see that Stupid Falls flowers have a higher general reflectance in comparison to more than Judd Falls flowers. However, Cohen’s d indicates a small effect size. This result suggests that while a difference exists, the two groups (SF and JF flowers) still show a fair amount of overlap in their spectral shapes. We therefore do not know if these differences are able to be distinguished by the hummingbirds. Finally, the graph “Do PC3 and PC4 Vary by Trail?” combined with the t-test and Cohen’s d reveal that the trail had a significant effect on the peaks in UV and 500-600 nm wavelengths, but trail did not have a significant effect on the amount of reflectance above 500 nm. It is important to note that these reflectance measurements were taken with the only light source being the fiber optic probe. Although we observed that the *Ipomopsis* generally grow in areas that are very exposed to the sun, it would be interesting to study possible differences pertaining to the light reaching the *Ipomopsis* at Stupid falls vs the light reaching them in the regions where they grow along the Judd Falls trail.

Finding these differences in color between trails leads us to ask the following: if these are the differences we see between flowers from trails whose mean soil moisture is 1.3 and 2.2 (on the 1 through 10 scale) respectively, what is the difference in color between the flowers today and those of many years ago, when Gothic was not undergoing a drought and the overall soil moisture was undoubtedly higher? Changes brought about by climate change may be strongly influencing hummingbirds’ visual ecology and their memory of what sites, hues, and color intensities correspond with the greatest rewards. Although we have found both higher reflectance this year and higher visitation over the past couple of years in Stupid Falls, we cannot yet determine whether the reflectance is a factor contributing to the visitation. Indeed, the overlap in spectral shape between the sites poses the great question of whether the difference is even perceptible to hummingbirds.

In the end, although we found correlations between some of the variables that we measured, it is still unknown whether the greater visitation rates in Stupid Falls are due to differences in these variables between trails. Thus, we believe a larger dataset collected over a longer period of time and encompassing more variables can shed more light on our question.

Future Directions:

To answer future questions and seek specificity, nectar methods in the future could analyze specific precipitation data and determine if total volume of rainfall is an additional variable or if rain timing plays a bigger role (i.e. does rain 1, 2, or 3 days before collection impact soil moisture retention more?). Larger sample sizes and studies done outside of a

drought-occurring summer would likely yield different results. Finally, studying soil moisture in a more controlled setting, either through potting or plot manipulation, would yield more specific results regarding relationships between nectar output and soil moisture.

Regarding the spectral analysis, given that PCs were found to vary significantly by trail but with a small effect size, the great amount of overlap in spectral shape between flowers from Judd and Stupid Falls raises the question of biological significance. Are hummingbirds able to distinguish between these differences? Therefore, the next steps in the spectral reflectance analysis would be to model these reflectance spectra using avian visual models, to test whether the color differences we have found are discriminable to hummingbirds. It would be interesting to look at how much each of the hummingbirds' four photoreceptors are stimulated by each spectra, which can be done by generating quantum catch data (the estimated amount of light absorbed by each photoreceptor) using Pavo. Furthermore, it would be good to run controlled experiments to test these visual models to see whether any perceived differences are enough to warrant changes in hummingbird choice of flowers. It would also be good to run controlled experiments varying only soil moisture and keeping all other variables constant, to see whether moisture has a direct impact on flower color.

Since PCs were found to vary significantly by flower area (with tubes generally reflecting wavelengths at higher intensities than petals), it would be interesting to delve further into the visual ecology of birds by seeking to understand what part of the flower is the most visible to them when they are foraging. When Dr. Kristina Fialko showed us a hyperspectral image of a meadow and directed our attention to an *Ipomopsis aggregata* flower in that meadow and the spectral shape associated with it at that moment, we recognized the spectral shape as belonging to the tube, with high spectral peaks at around 440 and 600 nm. Further studies that divide the flowers into more parts than just the center of the petals and the middle of the tube would also be useful for further exploring variations in reflectance within the flower.

In general, studies that combine reflectance spectra and nectar calculations could determine possible correlations between flower color and nectar.

Finally, it would be interesting to study several additional factors which we think might contribute to visitation. We would like to look at the hummingbird territories (which other members of our lab have collected data on) in relation to visitation data, to see whether the regions with higher visitation also have a greater density of hummingbirds present per unit of land squared. While collecting flowers, we noticed that their petals sometimes bore white or yellow streaks. We would like to conduct studies that investigate what causes this occasional streaking, to see whether there is any correlation between this streaking and the variables we measured. It would also be beneficial to see if there is a correlation between pH and the other variables which we measured. We have not done statistical analyses focusing on pH as a variable, but we did notice that flowers which tended to have lower soil moisture tended to have a lower pH than flowers whose soil was more moist.

Figures:



Figure 1: Nectar Isolation

Using small straws and twist ties, *I. aggregata* flowers were isolated from their stem. This isolation lasted 2 days each time.



Figure 2: Soil Moisture Meter

A small YAMRON moisture meter was used to take data points on soil temperature, soil moisture, soil pH, and sun exposure.



Figure 3: HummerflowerWatch Camera

Small BRINNO cameras are placed on individual hummingbird-visited flowers throughout three trail sites to collect visitation data.



Figure 4: Positive hummingbird identification

After SD cameras are brought back from the field, a platform called DeepMeerkat determines if a hummingbird visited that flower with a camera. Above is a positive identification from that software.



Figure 5: Collection tins

During collection, *I. aggregata* flowers collected for both spectroscopy and nectar extraction are placed in these small tins. They allow for less exposure to sun and air, maintaining the freshness of the flowers.



Figure 6: Nectar Extraction Equipment

At the top of the image is the Fisher Refractometer, which measures the percent sugars in a nectar sample. Below it is the collection tin, and to the right is a tube of microcapillary tubes. These microcapillary tubes with the small plunger on the side collect nectar and can be safely placed within the refractometer glass plate for sugar determination. Finally, the small paper towel on the right is used to wipe off the refractometer plate to obtain objective results.

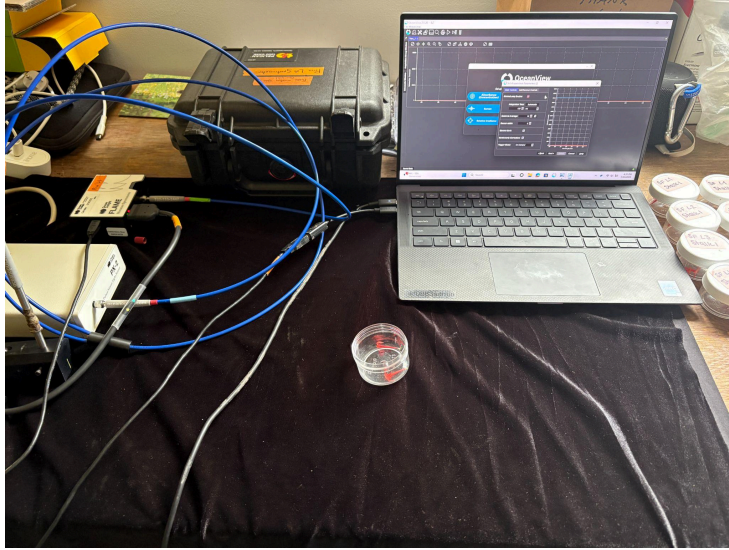


Figure 7: Spectrometer

Pictured is the OceanOptics spectrophotometry setup. A power source and light source are attached to a fiber-optic probe, which directs light onto flowers and collects reflected light. Graphs of the reflected spectra ranging from 300-700 nm show up on the computer screen. Flowers are placed on a black velvet background, the lights are turned off, and curtains are closed to provide an unreflective background and ensure objective measurements.

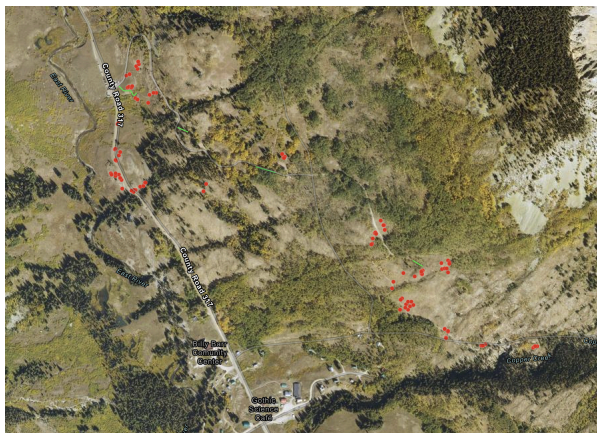


Figure 8: Birds-eye view of Judd Falls Trail with *I. aggregata* points

Birds-eye view of the Judd Falls Trail with *I. aggregata* points isolated in RED and four transects identified in NEON GREEN. Apparent features include a mixed landscape: trees, desert, meadows are all present on this trail; higher elevation site; greater variable elevation; and greater flower-pockets: more spread out concentration of *I. aggregata*, with some sites showing no presence at all of the flower.



Figure 9: Birds-eye view of Stupid Falls Trail with *I. aggregata* points

Birds-eye view of the Stupid Falls Trail with *I. aggregata* points isolated in RED and four transects identified in NEON GREEN. Apparent features include a consistent landscape: meadow ecosystem; bordering of the East River; and lack of flower-pockets: more consistent presence of the flower throughout the landscape

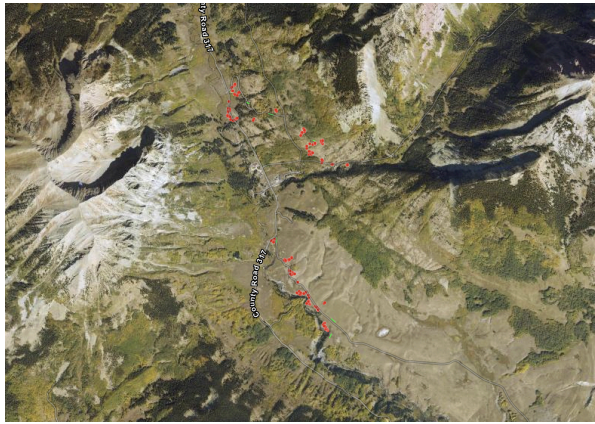


Figure 10: Gothic Map with All *I. aggregata* points

Birds-eye view of Greater Gothic with both trails visible, Judd Falls points aggregated towards the top and Stupid Falls points aggregated towards the bottom.

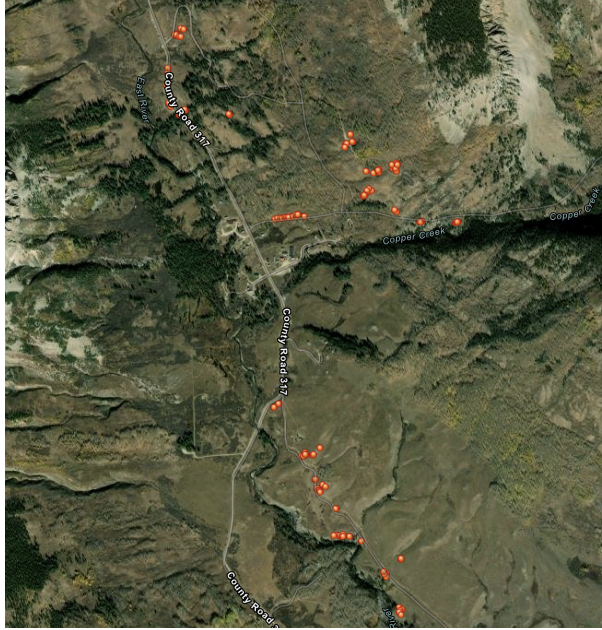


Figure 11: Gothic Map with Spectrosopy *I. aggregata* points

Birds-eye view of Greater Gothic with both trails visible, Judd Falls points aggregated towards the top and Stupid Falls points aggregated towards the bottom.



Figure 12: *Ipomopsis* size variability

An additional factor that was not tested was general flower size, tube size, or flower base size/color. After about 300 extractions, it was noticed that the lighter colored base (more white and less yellow) indicates higher nectar capacity. We were able to extract greater nectar from these flowers in general. The size of the flower, however, is less indicative of the level of nectar. We found both large flowers with absolutely no nectar to extract and large flowers with an abundance of extractable nectar.

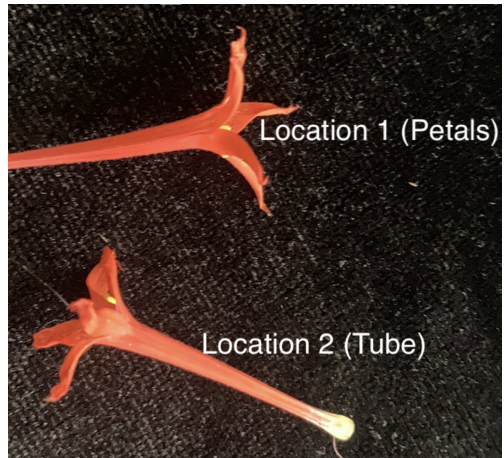


Figure 13: Location of Reflectance Measurements on Flowers

The first location of reflectance measurement was done directly on the innermost part of the petals. The second location was on the middle of the tube (where “Location 2” touches the flower). The third measurement (taken on three days but not considered for data analysis) was near the end of the tube.

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