

Investigating the Effect of Drought on Floral Traits Mediating Pollinator Interactions

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Abstract

Understanding the effects of water stress on flowering plants is crucial for ecosystem sustainability. Changing water availability due to climate shifts and persistent drought conditions have significant implications for the reproduction and survival of subalpine plants and their pollinators. This paper addressed two key questions: (1) Do changes in soil moisture levels have an effect on floral height and display size? (2) What is the relationship between floral traits and pollinator visitation? Field data was collected from three subalpine meadows in Gunnison County, Colorado, including measurements of floral traits, soil moisture, and pollinator visitation spanning three years. Our research team measured the floral display size, height, and abundance of two focal species: *Linum lewisii*, and *Potentilla pulcherrima*. The outcomes revealed a series of correlations: higher soil moisture was associated with taller plants but diminished displays. Pollinator visitation showed no significant correlations with floral traits.

Introduction

Studying the effects of water stress on flowering plants is crucial as these changes have cascading impacts on the ecosystems they support, especially their pollinator partners. In the Rocky Mountains, subalpine plants typically rely on spring snowmelt and summer monsoon rains for their water supply. However, the mid-season dry period has become prolonged due to a combination of rising temperatures, diminished snowpack, and earlier spring snowmelt (Sloat et al., 2015). Since 2019, Colorado has been contending with persistent drought conditions (*RMBL Snowpack Data*, 2021). Changing water resources for plants can have large impacts on floral development and plant-pollinator interactions.

Pollination by pollinators is essential for the reproduction of flowering plants and is a crucial source of food for these pollinators. The modern global decline of pollinators poses a significant threat to global ecosystems. Reduction in pollination services can significantly reduce the productivity of natural communities (Potts et al., 2010). Therefore, the conservation of wild pollinators plays a crucial role in sustaining the health within natural systems. Gaining a deeper understanding of the plants upon which pollinators depend on for food and resources will aid our ability to preserve wild pollinators, even in the midst of drought.

In response to limited water resources, plants display adaptive mechanisms to withstand drought stress. These responses to drought can instigate changes in floral characteristics, as plants shift their focus towards water preservation and survival, rather than extravagant floral appearance (Lambrecht et al., 2017). This holds significant implications for the pollinators that rely on floral cues.

The morphology of flowers plays a pivotal role in guiding pollinators' visitation choices, and alterations due to drought may have a large impact on the foraging behavior of pollinators. More prominent displays signal greater floral rewards to pollinators and typically are associated with more visitation from pollinators (Schiestl and Johnson, 2013; Barragan-Fonseca et al., 2020). Previous research has demonstrated the responsiveness of floral height and display to drought conditions (Lambrecht et al., 2017), and their consequential effects on pollinator visitation (Rowe et al., 2020), rendering them valuable traits to study the interactions between drought, plants and pollinators. Literature suggests flower height and display size increase with soil

moisture (Kuppler, 2021), thereby elevating pollinator visitation rates (Schiestl and Johnson, 2013). Changes in flower morphology have a cascading effect on pollinator visitation patterns and ultimately influence the overall success of both the plants and pollinators (Descamps et al., 2021).

Although studies examining both the effect of drought on floral traits and the effect of floral traits on pollinator visitation have been well documented (Suni et al., 2023; Conner & Rush, 1996), studies linking the two effects have seen less publication. Previous studies exploring the association between drought, floral traits, and visitation have predominantly employed small-scale greenhouse experiments with controlled manipulation (Kuppler et al., 2021; Höfer et al., 2021). However, unlike controlled greenhouse experiments, field site experiments provide a realistic and dynamic environment that better represents the complex interactions found in natural ecosystems which may elucidate novel interactions and effects not captured in greenhouse experiments.

In this study, we made the vital connection between drought, floral morphology, and pollinator interactions. Our research aimed to address the following key questions: (1) Do changes in soil moisture levels have an effect on floral height and display size? (2) What was the relationship between floral traits and pollinator visitation? We hypothesized that in high soil moisture conditions, we would observe an increase in both floral display size and height, which would entice more visitation from pollinators. By investigating these research questions, we sought to enhance our understanding of the intricate dynamics between drought, floral traits, and plant-pollinator interactions.

Methods

Field Methods

We collected data at three subalpine meadows (Avery Picnic, Virginia Basin, Gothic Town) located in Gunnison County, CO, as part of the Brosi lab's projects at the Rocky Mountain Biological Laboratory (Figure 1). Each site contained four transects measuring 20m by 1m and divided into ten segments. Each site was visited once per week to collect data on floral abundance and traits, soil moisture, and pollinator visitation.

We collected data on floral abundance by counting the number of blooming flowers of our study species rooted within the transects at each site. This data allowed us to identify the average pollinator visitation to a floral species by normalizing the number of pollinator visits by the number of available and receptive flowers.

We used observational transect sampling to record visitation from pollinators. Data collectors were equipped with nets and assigned random transect segments to survey. Each segment was observed for a total of 3 minutes, 1:30 minutes on each side. When pollinators came into contact with a blooming flower's reproductive parts, they were captured and their visit was recorded. For each pollination event we record the flower species and assign a gross ID category for the pollinator.

We measured flower morphology by recording the floral display size and flower height. We calculated floral display size as length by width measuring from petal apex to petal gap

measured using digital calipers. Flower height was measured as the distance from the ground to the tallest part of the flower without adjusting the flower measured using a ruler. We gathered trait data from a minimum of five haphazardly selected individuals within the transect for each focal species. Measurements were taken after 12pm to allow flower displays to fully open. Our focal species, *Linum lewisii*, and *Potentilla pulcherrima*, were selected based on their presence and abundance at all 3 field sites, high visitation from pollinators, blooming times that matched our field season and sufficient data collected in previous years.

We used a soil moisture probe at each site to obtain location and time-specific moisture data. We took two measurements of soil moisture from each site, following a consistent protocol that was established for collecting soil moisture data in previous years. The soil moisture probe was inserted into the soil at the easternmost end of the plot, and another measurement was recorded 10 meters to the west of that point.

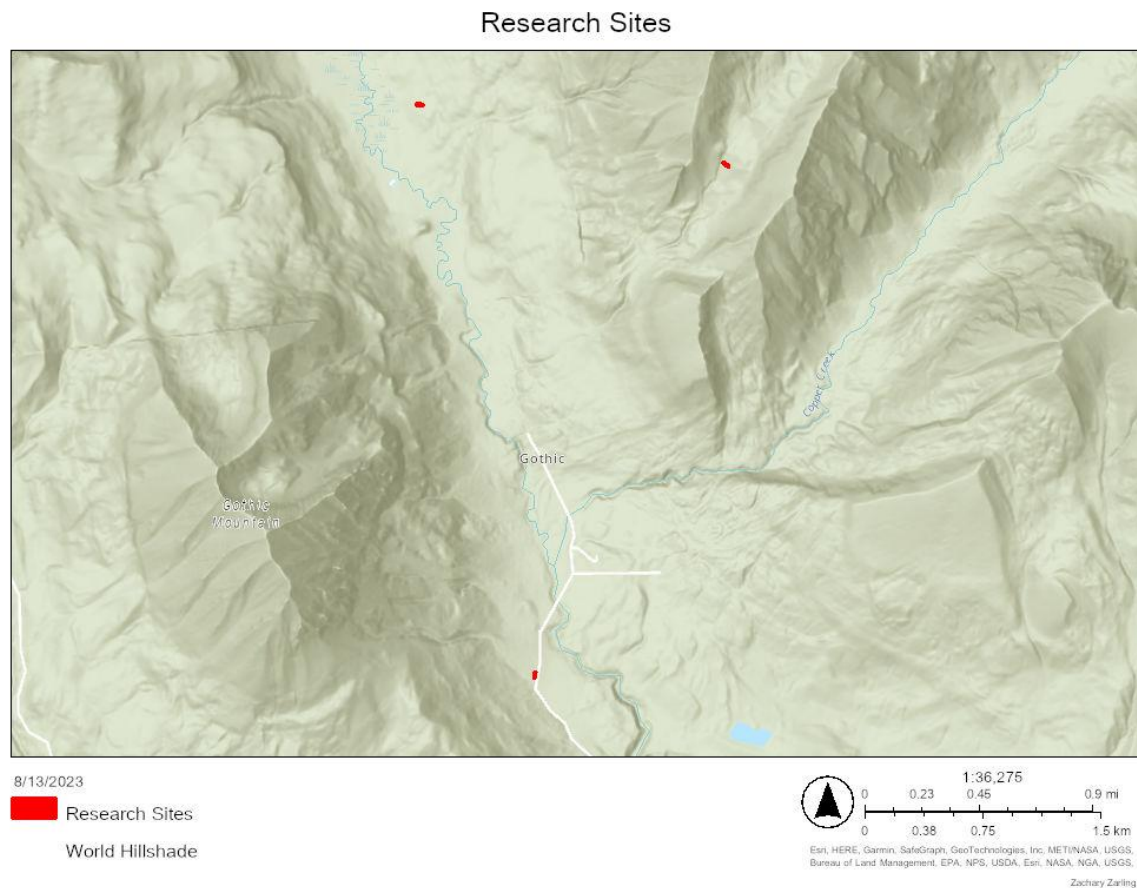


Figure 1.
Map of Three Study Sites in Gunnison County, CO

Data Analysis

In this study, we aimed to explore the impact of moisture on floral traits and the reciprocal effect of these traits on pollinator visitation. To achieve this, we utilized mixed effect models (MEMs), to account for the random effects of site since we collected our data at three different sites. This approach allowed us to derive more meaningful and reliable conclusions from our study.

Given our limited sample size and scope of complexity for this project, all MEMs were computed as random intercept models. To address the two research questions, which focused on two plant traits, we developed four general types of models: (1) soil moisture vs. flower height, (2) soil moisture vs. display size, (3) flower height vs. pollinator visitation, and (4) display size vs. pollinator visitation. To accommodate the limited sample size, an individual MEM was used for each species, resulting in a total of 8 graphs.

To ensure the validity of our analyses, we visually examined the residuals of the mixed effect models to ensure there were no underlying nonlinear patterns. No patterns emerged in the residuals of the relationship between soil moisture and plant traits, as well as display size and visitation. However, there was a trend in the residuals of the flower height and visitation relationship, indicating a nonlinear trend which violates the assumptions of a linear mixed effect model. While there are alternative models that could be employed to better capture the nonlinear relationship, due to the complexity and time constraints of this project we were unable to pursue further analysis at this stage.

We conducted our analysis in RStudio (Posit Team, 2023) using the “lme4” and “lmerTest” packages for mixed effect modeling (Bates, 2015; Kuznetsova, 2017). For analysis of the relationships between soil moisture and floral traits, soil moisture was set as a fixed effect and site was set as a random effect. Analysis of floral traits and pollinator visitation set average height or display as a fixed effect and site was set as a random effect. Graphs with dashed lines indicate statistically insignificant results, and those with red lines indicate an overfitted model.

A graph showing the relationship between soil moisture over time was also created to understand trends in soil moisture over our three flowering seasons. This graph was used to identify potential ecological drivers of observed data patterns in our other studied relationships. For each year, the data was grouped based on research site, and linear regression analyses were used to visualize trends in the data.

Results

Here are our findings detailing the intricate interplay between soil moisture and various floral traits, along with their impact on pollinator visitation. The data comprises 166 observations for floral traits and 73 for visitation events. These data were collected across a span of three distinct sites and three consecutive years, resulting in a dynamic portrayal of ecosystem dynamics and interactions.

Soil Moisture and Time

Our assessment of soil moisture variations across seasons and sites revealed minimal significant overall trends, with the exception of a slight mid-season drought (Figure 2). We employed linear regression to visually represent the moisture trends across various sites and years. We found that only in 2023 did soil moisture decline for all three sites.

Soil Moisture and Floral Traits

In our investigation into the relationship between flower height and soil moisture, we observed a negative correlation for both flower types. In *Linum Lewisii* flowers, our analysis unveiled a statistically significant relationship between height and soil moisture. The application of a mixed effect model yielded a slope of -0.1606 ($p = 0.960661$) and an intercept of 366.9732 ($p = 0.000218$) (Figure 3). *Potentilla Pulcherrima* flower height had a statistically insignificant relationship with soil moisture with a slope of -7.062 ($p = 0.00307$) and an intercept of 314.543 ($p = 1.56e-12$) (Figure 4).

In the analysis of display size and soil moisture, we observed a positive correlation for both flower types. *Linum Lewisii* flower height had a statistically significant relationship with soil moisture with a slope of 21.687 ($p = 0.00244$) and an intercept of 562.939 ($p = 0.00016$) (Figure 5). *Potentilla Pulcherrima* flowers showed a statistically insignificant relationship between display and soil moisture with a slope of 2.201 ($p = 0.232$) and an intercept of 212.062 ($p = 12.68e-05$) (Figure 6).

Floral Traits and Pollinator Visitation

The analysis of the impact of floral traits on pollinator visitation revealed no correlation for either flower, whether in terms of height or display. Both models for *Linum Lewisii* were overfitted due to low sample size (Figure 7 & 9). Height analysis of *Linum* yielded a slope of a slope of 0.0003217 ($p = 0.0287$) and an intercept of -0.0448504 ($p = 0.2981$) (Figure 7). Display saw a slope of $5.420e-05$ ($p = 0.322$) and an intercept of $2.147e-02$ ($p = 0.630$) (Figure 9). *Potentilla Pulcherrima* flowers yielded statistically insignificant results for both height and display. Height analysis of *Potentilla* yielded a slope of a slope of -0.001153 ($p = 0.606$) and an intercept of 0.507594 ($p = 0.369$) (Figure 8). Display saw a slope of -0.002501 ($p = 0.212$) and an intercept of 0.819264 ($p = 0.112$) (Figure 10).

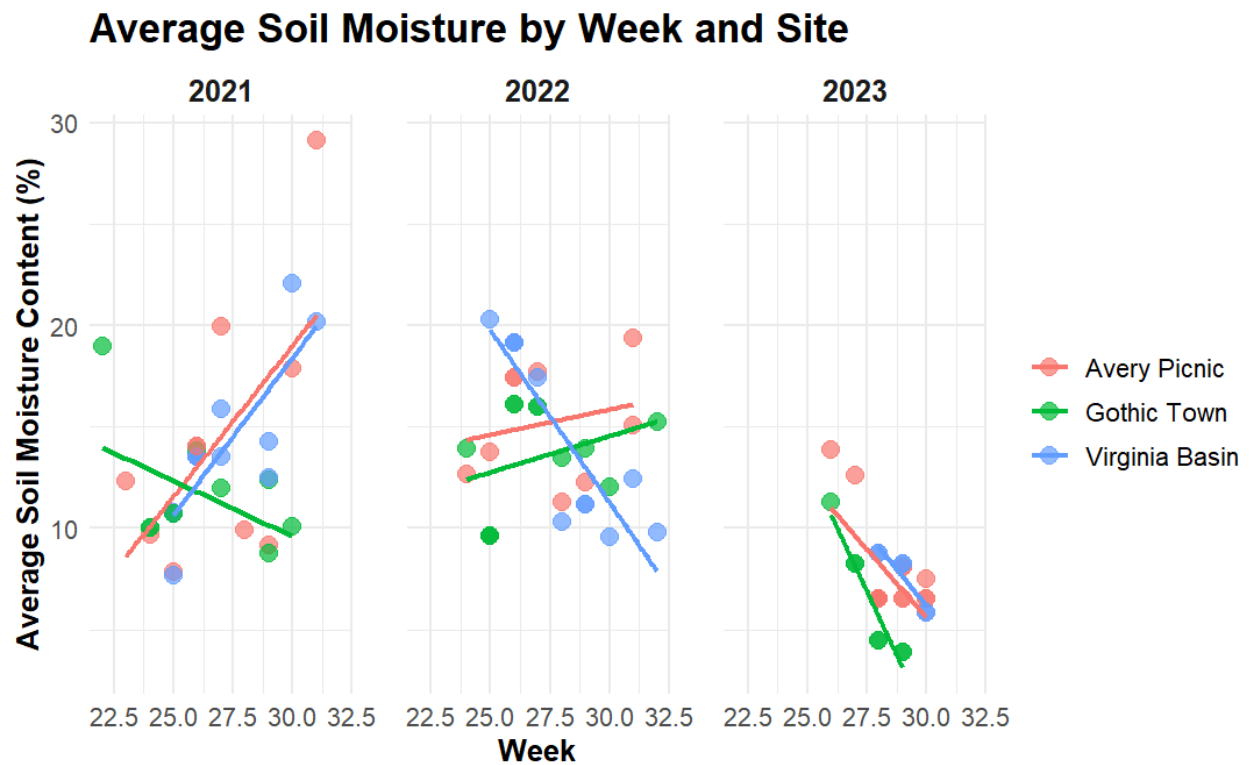


Figure 2.

Temporal Variations in Soil Moisture Across Three Successive Seasons. Analyzed using Linear Regression.

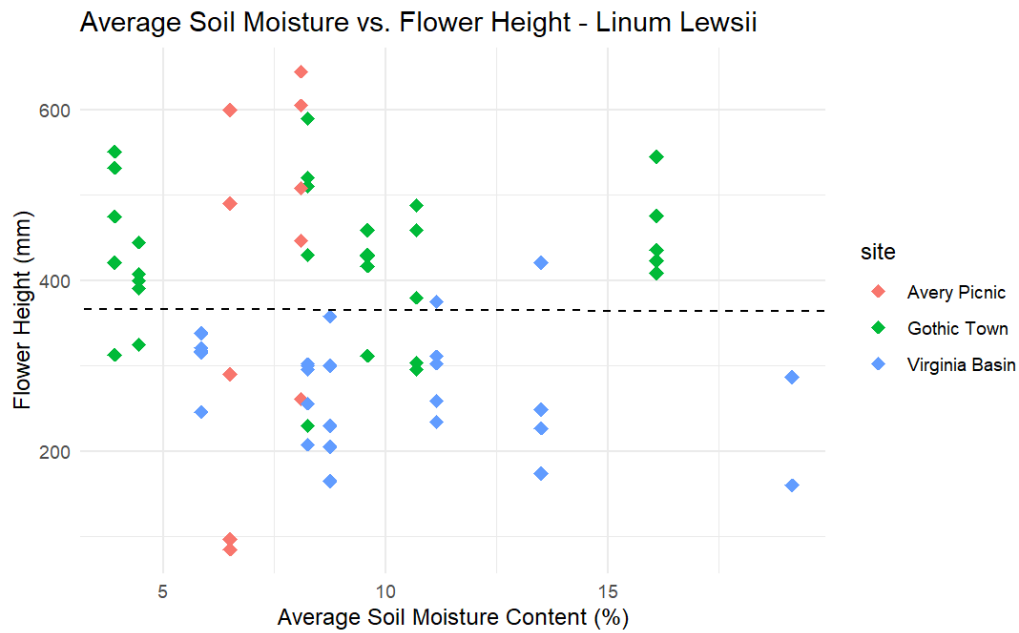


Figure 3.

*Flower height of *Linum Lewisii* and associated average daily soil moisture content. Analyzed using a mixed effect model, results are statistically insignificant.*

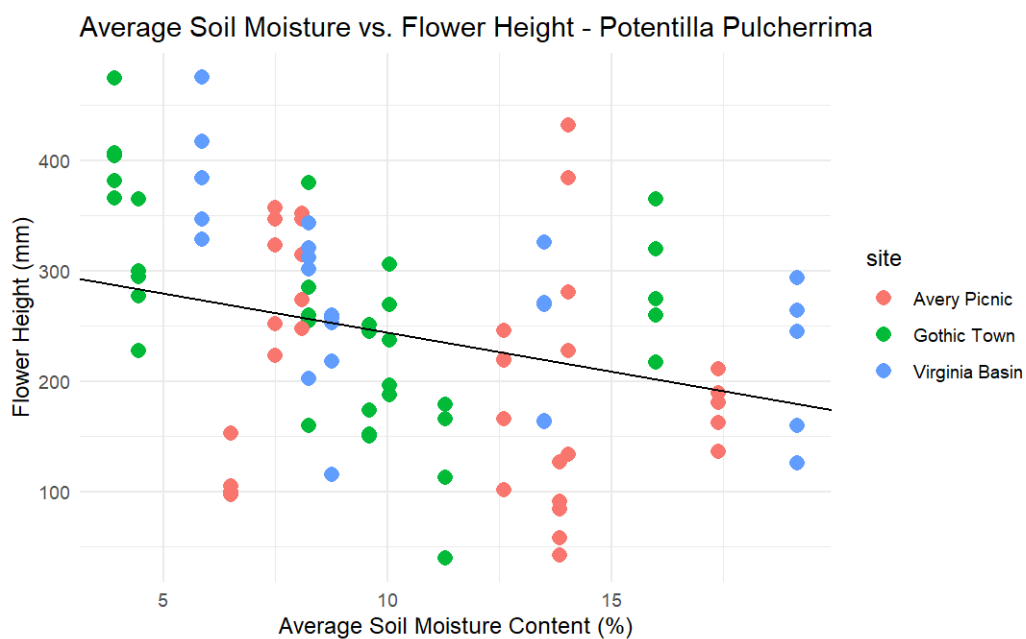


Figure 4.

*Flower height of *Potentilla Pulcherrima* and associated average daily soil moisture content. Analyzed using a mixed effect model.*

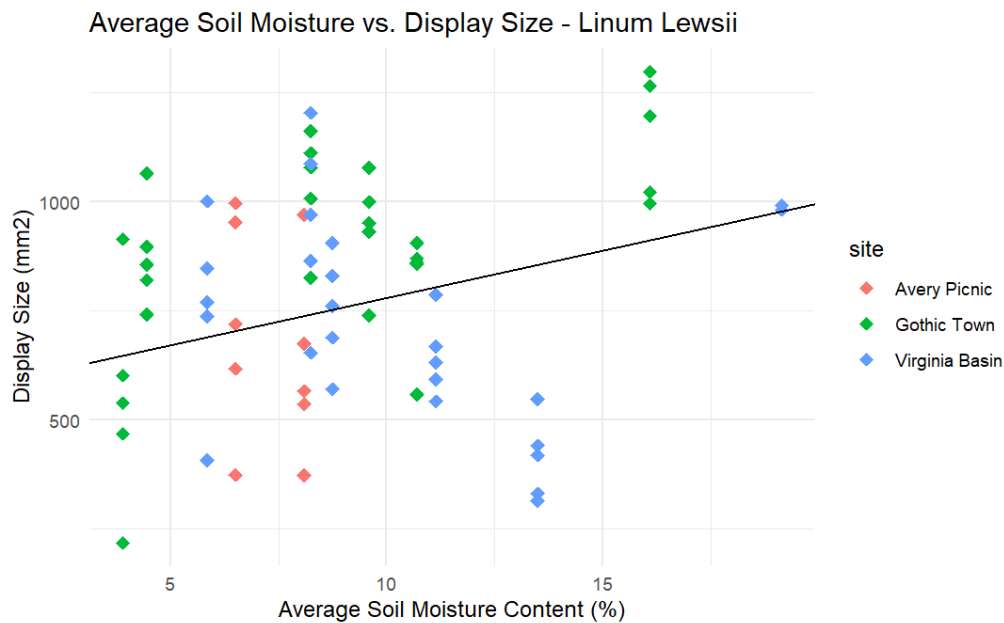


Figure 5.

*Floral display of *Linum Lewisii* and associated average daily soil moisture content. Analyzed using a mixed effect model.*

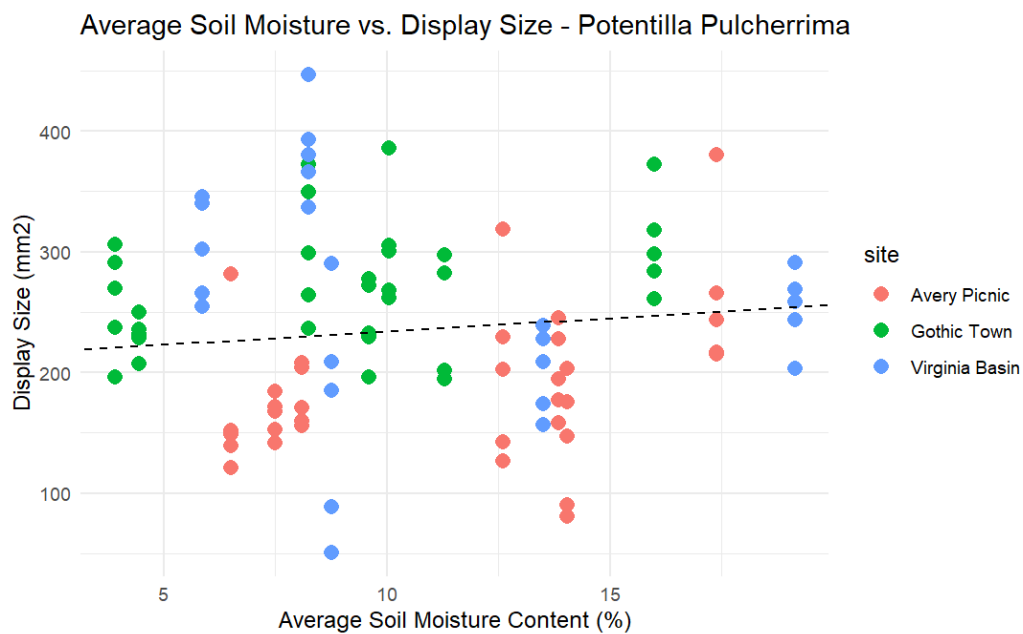


Figure 6.

*Floral Display of *Potentilla Pulcherrima* and associated average daily soil moisture content. Analyzed using a mixed effect model, results are statistically insignificant.*

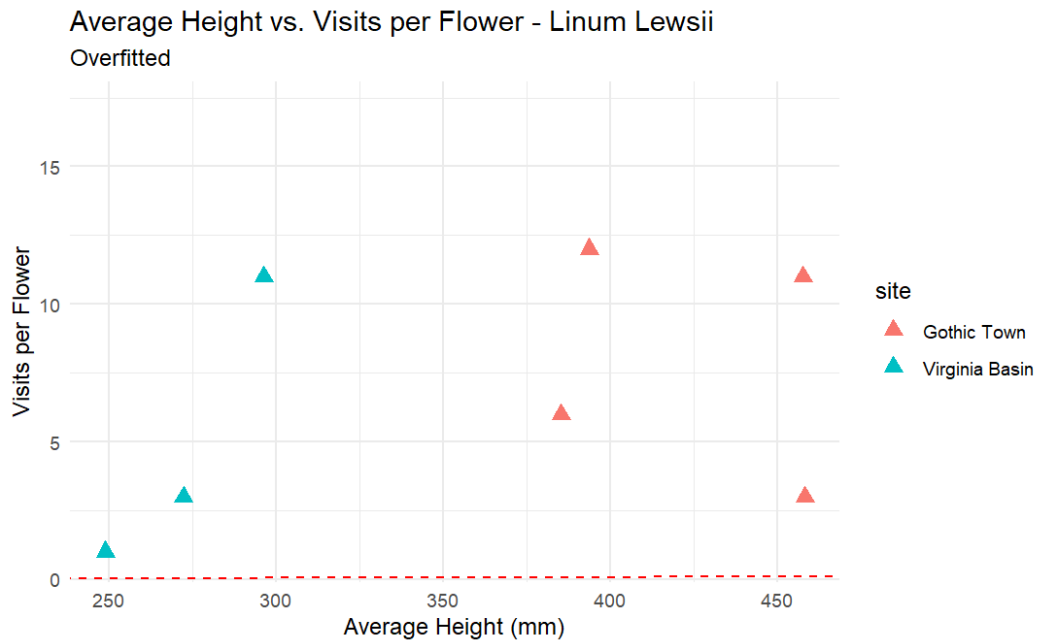


Figure 7.

*Average floral height of *Linum Lewisii* and its pollinator visitation relative to daily flower count. Analyzed using a mixed effect model, data overfitted to the model.*

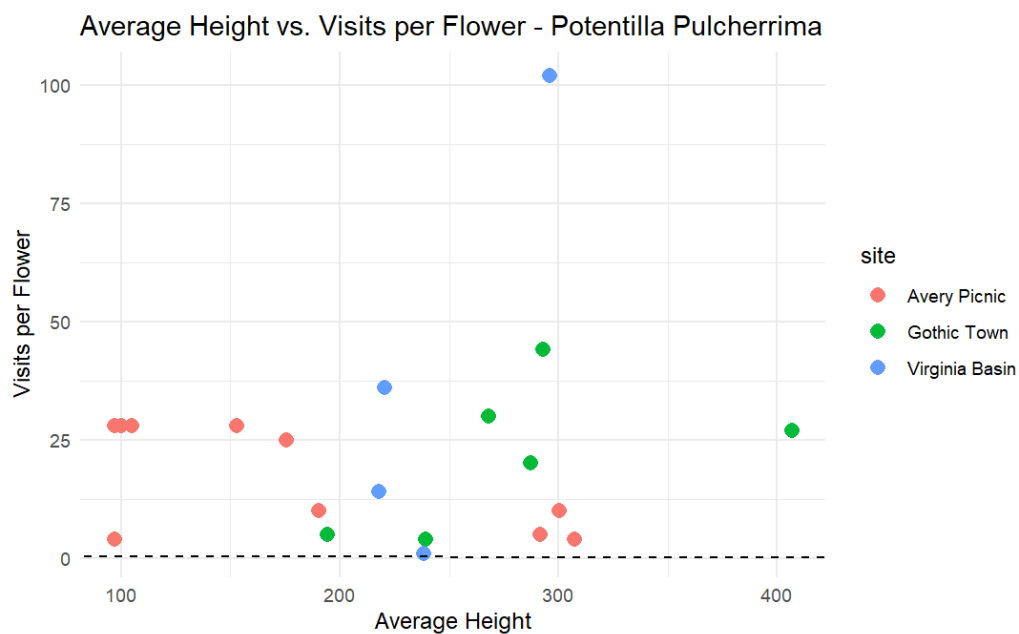


Figure 8.

*Average floral height of *Potentilla Pulcherrima* and its pollinator visitation relative to daily flower count. Analyzed using a mixed effect model, results are statistically insignificant.*

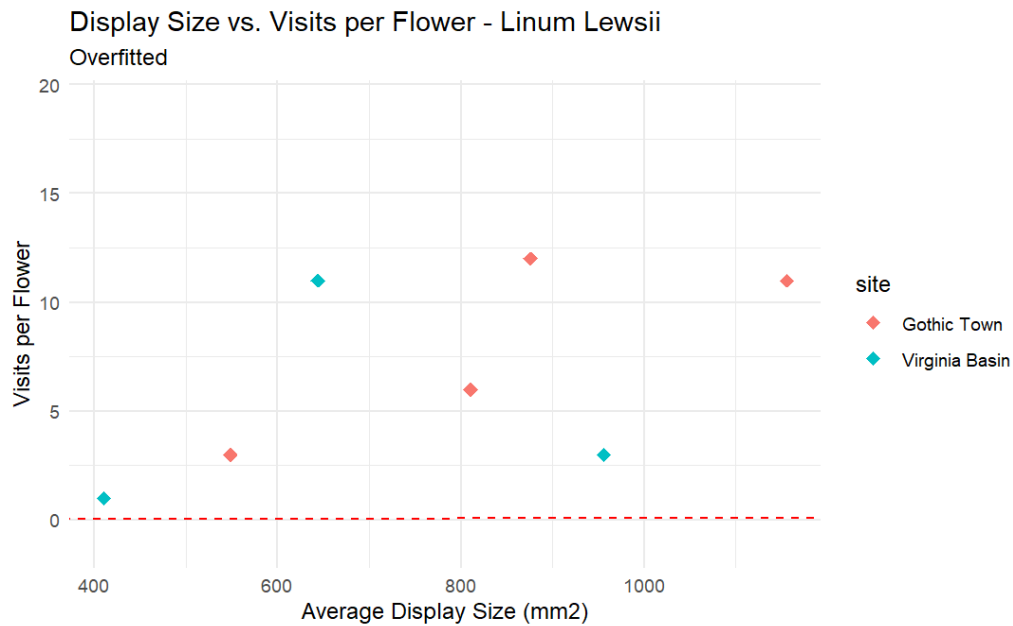


Figure 9.

*Average floral display size of *Linum Lewisii* and its pollinator visitation relative to daily flower count. Analyzed using a mixed effect model, data overfitted to the model.*

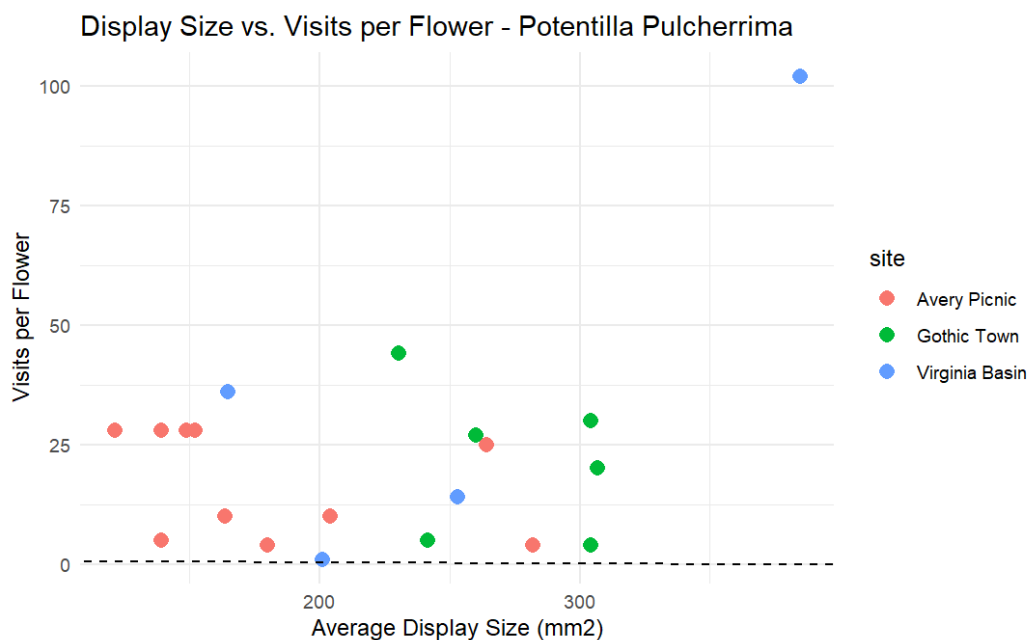


Figure 10.

*Average floral display size of *Potentilla Pulcherrima* and its pollinator visitation relative to daily flower count. Analyzed using a mixed effect model, results are statistically insignificant.*

Discussion

Soil Moisture and Time

Originally, our project set out to streamline data analysis by categorizing years as either drought or non-drought based on categorical measures. However, this approach became untenable as an unusually diminished monsoon season this year blurred the clear boundaries between these categories. As a result, we shifted our focus towards using soil moisture levels as a surrogate for drought conditions. This approach enabled us to continue investigating the impact of water scarcity on floral traits. However, the fluctuation of moisture levels throughout a season created underlying patterns to our data. This complicated identifying correlations between variables in our study.

Additionally, our assessment of seasonal soil moisture trends may have been limited by our utilization of linear analysis. In the rocky mountain region, seasonal moisture patterns frequently showcase non-linear behaviors, owing to the increase of moisture from spring snowmelt early in the season and summer monsoons at the end. These combined effects tend to shape a U-shaped trend rather than a linear one. Unfortunately, due to time limitations and the complexity of this project, we were unable to conduct analyses better suited for these patterns.

Soil Moisture and Flower Height

Our findings indicate a unique trend in *Potentilla Pulcherrima*'s response to soil moisture levels. Contrary to established literature, our study reveals that these plants exhibit increased height at lower soil moisture levels. Examination of moisture trends uncovers a mid-season drought pattern, which holds specific significance for *Potentilla Pulcherrima* due to its peak blooming period coinciding with mid-season drought occurrences. As a perennial, *Potentilla Pulcherrima* undergoes seasonal growth from sprouts each year. Notably, times when the plant would naturally reach its tallest points align with periods of greater aridity. This observation suggests that seasonal growth patterns potentially exerts a more substantial impact on plant height compared to soil moisture, at least within the context of a single growing season. Notably, differences in findings compared to similar studies could stem from their experimental manipulation of moisture levels, which remain consistent throughout the growing season.

Soil Moisture and Floral Display

The relationship between soil moisture levels and flower display is marked by a distinct negative correlation. This trend suggests that the presence of ample water within the soil exerts a positive impact on overall plant health, leading to enhanced flower displays. Conversely, periods of drought have been observed to diminish the display of flowers, a phenomenon attributed to the plant's resource allocation strategy that prioritizes survival over reproductive efforts. Notably, this correlation is particularly pronounced in the case of *Linum Lewisii*, where the only statistically significant connection was found. This species, characterized by its daily production of new flowers, is inherently more susceptible to phenotypic alterations triggered by drought conditions.

Floral Traits and Visitation

Our analysis of visitation data revealed minimal correlation between plant traits and pollinator visitation. This relationship may have been confounded by many different factors. One factor could be attributed to our inclusive categorization of pollinators, ranging from bees to flies and moths, each exhibiting distinct flower preferences and foraging behaviors. While certain pollinators might favor larger and taller flowers, others may not, resulting in attenuated correlations within the dataset. Other confounding factors may have been due to other floral traits which may matter more than height or display such as nectar content or floral density. Additionally, our analysis of visitation data violated assumptions of the model we were using due to low sample sizes and non-normally distributed data.

In conclusion, our research endeavor provides valuable insights into the intricate dynamics between soil moisture, floral characteristics, and pollinator visitation. Nevertheless, these insights are inherently nuanced by the constraints we encountered. Notably, our sample sizes may not have been adequate to detect statistically significant associations. There is also a possibility that other variables could have played a more influential role or introduced confounding effects, thereby impacting the results. However, within these constraints, we emphasize the value of extracting meaningful interpretations from the discreet relationships that have surfaced in our analysis. In contrast to our initial hypothesis, our findings did not support the expectation that heightened soil moisture conditions would yield an increase in both floral display size and height, consequently attracting greater visitation from pollinators. Rather, we found that floral traits had minimal impact on pollinator visitation, and characteristics such as flower height did not consistently exhibit growth in response to increased soil moisture.

In order to achieve a more comprehensive grasp of the ecological dynamics at play, there is ample opportunity for further exploration. This could entail the expansion of data collection efforts, encompassing a broader range of plant species to justify generalized interpretations of results. Additionally, it would be prudent to factor in other pertinent variables that might influence the observed patterns. Integrating random slopes alongside random intercepts within a mixed-effects model would be good. This approach would adequately address the varying degrees of response to fluctuations in soil moisture across distinct sites or years. Additionally, employing methods to manipulate water resources could offer valuable insights. As comparing soil moisture levels while maintaining typical season moisture trends might unveil more pronounced results. Contrasting soil moisture levels while upholding typical seasonal moisture trends may reveal relationships overshadowed by seasonal growth patterns. Another suggestion is a concentrated investigation into specific pollinator species or taxa, this may eliminate variation in flower preferences and foraging behavior.

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