

Effects of frost and neighborhood on Tephritid fly herbivory of *Erigeron speciosus*

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Abstract

With earlier snowmelt, the impacts of a late frost in the Rocky Mountains have been more consequential for the production of flowers in species such as *Erigeron speciosus*. Several species of Tephritid flies (Diptera: Tephritidae) are pre-dispersal seed herbivores of *E. speciosus*. The neighborhood around an individual plant has been shown to play an important role in plant fitness/life history of an individual. In addition, plant neighborhoods have been found to have an impact on Tephritid fly behavior. This study examined the direct and indirect impacts of frost and neighborhood on Tephritid fly herbivory of *Erigeron speciosus*. In addition, it investigated how frost affects adult Tephritid fly abundance. This study was conducted by establishing plots at sites with different levels of frost. Data were analyzed in RStudio using ANOVA, linear models and a structural equation model. Results suggest that frost and neighborhood have a significant impact on *Erigeron* herbivory. In addition, there were significantly more Tephritids in areas with low frost than in areas with high frost. These results indicate that climate change is having many direct and indirect impacts on biological systems and both such impacts must be examined to more holistically understand the effects of climate change.

Keywords: *Erigeron speciosus*, frost, herbivory, neighborhood effects, Tephritid flies

Introduction

Climate change is impacting ecosystems across the world, but alpine and high elevation ecosystems are often considered some of the most vulnerable to the effects of climate change (Tsering et al. 2010; Kohler & Maselli 2009; Scott 2006). One major impact of climate change has been disruptions of plant/pollinator and plant/herbivore networks as environmental cues are shifting (Potts et al. 2010; Coley 2010). This is causing discrepancies in phenology and timing of emergence as different species respond differently to climate change. However, these studies

often only look at the direct impacts of climate change, while ignoring the complexities of indirect impacts on biological systems. In addition, climate change has caused an increase in the number of unpredictable and severe weather events, increasing the amount of disturbance in ecosystems and making them more vulnerable to decline.

In Gothic, CO, approximately 2895 masl., situated in the East River Valley of the Rocky Mountains, spring snow melt has been occurring earlier and is now taking place in Mid-April despite historic trends of snowmelt occurring in June. As a result of the earlier snowmelt date, plants are beginning to grow and form buds earlier in the season. As a result, when frosts occur in May or early June, they are occurring at a time when buds are particularly vulnerable, often killing the buds. This results in years with significantly fewer flowers produced (CaraDonna & Bain). *Erigeron speciosus* has been found to have buds that are particularly vulnerable to late frosts (Inouye 2008). Despite the overall decrease in the flowering, microclimate plays an important role in determining the severity of frost in particular meadows or on individual plants (Inouye 2008; Pardee et al. 2018; Inouye 2000). Adjacent meadows may have very different levels of frost, as a result of aspect, slope or various other factors. In 2020, frost events occurred on the nights of June 9th and June 10th. On both nights the temperature dipped below freezing and caused many of the developing buds to die.

In addition to frost affecting plants, it may also affect the herbivores and pollinators that rely on those plants. Tephritid flies (Diptera: Tephritidae), are pre-dispersal seed herbivores of *Erigeron*. Tephritids rely on *Erigeron* to oviposit in the buds and then the larvae feed on the seeds as they mature. Globally, they have large economic impacts on agricultural crops by eating and destroying a year's harvest. Many tephritid flies that are causing the most devastating economic and agricultural damage are invasive and have been difficult and costly to attempt to

contain or remove (Papadopoulos 2014).

Plant neighborhoods are the plant species adjacent to an individual. Neighborhoods have been found to play an important role in the fitness/life history of an individual (Underwood et al. 2014; Stoll & Prati 2001; Bartkowska & Johnston 2014; Hegland 2014). Neighborhood density and frequency of certain species also plays an important role in herbivory, through both resource concentration and resource dilution (Kim & Underwood 2015). In addition, Tephritid fly behavior has been found to be influenced by the neighborhood of species surrounding *Erigeron* such as *Helianthella quinquenervis* (Zuniga 2017, unpublished). Tephritid fly behavior in the Rocky Mountains is most likely to be affected by other Asteraceae species, as they are the most common host plant in the area. In Goth such as *Heliomeris multiflora* and *H. quinquenervis* are the most common neighboring species.

While the direct effects frost on flowers has been well studied, much less is known about the indirect impact of frost or neighborhood on herbivory. This study aims to examine the indirect effect of frost damage on *Erigeron* herbivory by exploring the following:

1. What is the effect of frost on Tephritid fly herbivory of *Erigeron*?
2. What is the effect of *Erigeron* neighborhoods on fly herbivory?
3. What is the effect of different levels of frost (i.e in adjacent meadows) on Tephritid fly abundance?

Methods

Study site

This study took place in montane meadows near Rocky Mountain Biological Laboratory (RMBL). RMBL is located approximately 2895 masl. in the old mining town of Gothic,

Colorado. Gothic is located in the East River Valley at approximately 38.9585°N and 106.9877°W. RMBL consist of high altitude wet and dry meadows, aspen forest and conifer forest ecosystems. These high altitudes meadows have an extremely short growing season, beginning when the snow melts between April and June to the time of the first snowfall in September or October. Due to the short growing season and severe climate the majority of species in this area are long lived perennials.

Study organisms

Erigeron speciosus, or Aspen daisy, is a native species to the central and southern Rocky Mountains in the Asteraceae family. *Erigeron* is a long-lived perennial herb found across the western United States and Canada, although it is most common in the Great Basin and Intermountain region. *Erigeron* is found at elevations between 600 – 3420 masl. *Erigeron* is tolerant of a wide variety of soil types and can be found in wet and dry meadows, big sagebrush communities, quaking aspen forests and conifer forests. *Erigeron* flowers in late June or early July, depending on the year and the microhabitat. *Erigeron* produces many purple/white flowers on a single plant (Gucker & Shaw 2018). Additionally, *Erigeron* was used by Navajo peoples medicinally as a contraceptive and to treat menstrual pain in addition to other abdominal pains (Shemluck 1980; Vestal 1952; Moerman 2003; Gucker & Shaw 2018).

Tephritid flies are a family of fruit flies, known as true fruit flies, that are found across the world in sub-tropical and temperate regions. In the Rocky Mountains. Tephritid flies are common pre-dispersal seed herbivores of various Asteraceae species, including *Erigeron*, *Helianthella* and *Heliomeris*. Tephritids overwinter as adults and oviposit in the buds as soon as they emerge in the spring. The larvae hatch and eat the seeds, leaving a distinctive brown spot in the center of *Erigeron* disc flowers. Tephritids are found at several different elevations surrounding RMBL based on previous surveys (Kearns 1992).

Data collection

Five sites were established in montane meadows surrounding the Gothic townsite (Figure 1). Site 2 is located on a west facing slope. Sites 1 and 3 are located on an east facing slopes. Site 4 is located on a south facing slope and site 5 is located in a flat meadow. All sites are at elevations of 2900 masl $\pm$ 25m. Sites were selected based on observed differences in the amount of frost damaged buds on *Erigeron*. At each site, data were collected from 24 one-meter squared plots. Of the 24 plots, 15 were placed randomly along a 15m transect in the middle of each site. These plots were established in order to collect landscape level data about each site. Among the nine remaining plots, three were placed at low, medium and high densities of *Erigeron* respectively. These plots were established in order to ensure plots with a variety of *Erigeron* density were observed at each site.

In each plot, I measured the percent cover of *Erigeron*, as well as the percent cover of *Heliomeris* and *Helianthella* when present within a plot. Tephritid flies, rely on both *Heliomeris* and *Helianthella* in addition to *Erigeron* as a host plant. In addition, fly behavior has been found to be influenced by the presence of neighboring Asteraceae species (Zuniga 2017, unpublished). I counted the total number of viable flowers and buds on in addition to the number of buds with frost damage. These same data were collected for *Helianthella* and *Heliomeris* when present in a plot.

At approximately the time of peak flowering, the total number of flower and the number of flowers with fly damage were counted in each plot in order to gain an understanding of the severity of Tephritid fly herbivory at each site. To examine the effect of *Erigeron* frost severity on adult fly density at each site, an insect net was used to do sweeps at each site. Three transects

were walked at each site. Each transect consisted of 30 steps in a line, where the insect net was swept across the ground with each step.

Additionally, GPS coordinates and elevation data were obtained at the beginning and end of each transect, at the center of the nine specific density plots at each site and at the beginning of each fly sweep.

Data analysis

Once all data were collected, they were organized in excel and uploaded in to RStudio for further analysis. Once in RStudio, data were analyzed using ANOVA tests, Tukey tests, linear models and structural equation models. Data were analyzed both as one variable as well as in a multivariate manner, taking into account interactions between different variables. Scatter plots were created for integer data and were analyzed using linear models. An alpha value of 0.05 was used to determine significance. A structural equation model (SEM) was created to better understand the system as a whole and how different parts interact with and affect each other both directly and indirectly.

Proportions were created to analyze and compare frost and herbivory data. Proportion of frost (or herbivory per plot) = the total number of buds with frost (or herbivory damage) / (the number of flowers + the number of buds + the number of frost damaged buds per plot). The number of flowers, buds and frost damaged buds were added together and used as a proxy for number of stems. The total number of stems was very strongly positively correlated with percent cover (Figure 2). As a result, percent cover and the total number of stems were used interchangeably to examine *Erigeron* abundance. The same proportions were created to analyze and compare the amount of frost damage for *Heliomeris* and *Helinathella*. In addition, among the five sites where data were collected, Site 1, 3 and 4 did not have significantly different

proportions of frost from each other, nor did Site 2 and 5 from each other. However, the level of frost was different between these two groups (Figure 3). Site 1, 3 and 4 had a low level of frost and Sites 2 and 5 had a high level of frost. Sites 2 and 5 are located on the west side of County Road 317. Sites 1, 3 and 4 are located on the east side of County Road 317.

All spatial data were obtained using Gaia GPS app for iPhone and uploaded in to QGIS3.6 for analysis and to create a map of site location and plot types. ArcMap satellite imagery was used for the base map.

Results

In order to compare the amount of frost and herbivory on *Erigeron* at each site, the raw data were transformed into proportions. The results show that in plots with higher proportion of frost, there are lower proportions of herbivory (Figure 4). The slope of this line was negative and the equation of the line of best fit = $-0.11049x + -0.13202$ and the p-value = $6e-5$, indicating a statistically significant negative linear relationship between the proportion of herbivory as a function of the proportion of frost.

In order to examine the effect of common neighboring Asteraceae species on herbivory the percent cover of *Heliomeris* or *Helianthella* were compared to the proportion of *Erigeron* herbivory. Figure 5 shows the proportion of *Erigeron* herbivory as a function of the percent cover of *Heliomeris* per plot. This figure shows a positive linear relationship that indicates in plots with a higher percent cover of *Heliomeris*, there is also a higher proportion of herbivory on *Erigeron*. The equation of the line of best fit = $0.011048 + 0.074754x$, and the p-value = 0.000208 , indicating a statistically significant linear relationship between the proportion of herbivory on *Erigeron* and the percent cover of *Heliomeris*. Figure 6 shows the proportion of herbivory on *Erigeron* as a function of the percent cover of *Helianthella*. This figure shows a negative linear relationship which indicates that in plots with a lower percent cover of

Helianthella, there is a greater proportion of buds with herbivory on *Erigeron*. The equation of the line of best fit = $0.003924x + 0.106228$, and the p-value = 0.000773.

The number of adult flies caught with a sweep net was compared between sites with a low proportion of frost damage on *Erigeron* and sites with a high proportion with frost damage (Figure 7). The results show a significant difference in the number of flies caught at sites with low or high frost damage, $p = 0.0463$. Sites with lower frost damage had a greater number of adult Tephritid flies than did sites with high frost damage.

In order to examine how different variables may jointly affect fly herbivory on *Erigeron*, an SEM diagram was created (Figure 8). This diagram looks at many possible factors that may have a direct or indirect effect on herbivory of *Erigeron*, including the proportion of frost on *Erigeron*, the floral abundances of *Erigeron*, *Heliomeris* and *Helianthella*, and the frequency of *Erigeron*. The portion of frost and the floral abundance of *Heliomeris* were both found to have a significant impact on herbivory, p-value = 0.00 and p-value = 0.040, respectively. The frequency of *Erigeron* was found to be affected by *Erigeron* and *Heliomeris* floral abundance, p-value = 0.000 for both. However, *Erigeron* frequency has no significant effect on herbivory, p-value = 0.934. The floral abundance of *Helianthella*, has no significant impact on either *Erigeron* frequency or herbivory, with p-value = 0.313 and p-value = 0.091, respectively.

Discussion

As a result of climate change and earlier snowmelt in Gothic, CO there have been an increase in the frequency of frost events that are occurring at the same time as bud formation in *Erigeron*. As a result, many buds are aborted and killed by the frost. This study shows that not

only does frost directly affect the flowering potential of *Erigeron*, but it also indirectly affects herbivores, such as the Tephritid flies that rely on *Erigeron*. This result make sense logically, as one would expect that in an areas with less frost damage and more viable buds, it would be more attractive to flies looking to oviposit.

The composition of an individual's neighborhood, or the surrounding species in an area can play an important role in fitness and reproduction of an individual. Tephritid flies rely on *Erigeron* among other aster species, including *Helianthella* and *Heliomeris*, which were the next most abundant Asteraceae species in the meadows where sampling took place. Both *Heliomeris* and *Helianthella* had a significant effect on *Erigeron* herbivory, however, the effect was different for each species. For *Heliomeris*, as the percent cover increased, the amount of herbivory on *Erigeron* also increased. *Heliomeris* is not vulnerable to frost events as the buds are produced late enough in the season that they are unharmed. Thus, in areas where there is a higher percent cover of *Heliomeris*, a large number of flowers are also produced, which may be attracted to the Tephritids. There may also be a positive linear correlation between *Erigeron* herbivory and *Heliomeris* percent cover, as same fly species can use both as a host plant. However, for *Helianthella* an increase in percent cover was correlated with a decrease in the amount of herbivory on *Erigeron*. This is likely related to frost, *Helianthella* buds are particularly vulnerable the frost and very few flowers were produced this year. In plots with a high percent cover of *Helianthella*, there was likely also a lot of frosted killed buds. Additionally, different species of Tephritid flies rely on *Helianthella* and *Erigeron*. These results support work done by Zuniga 2017, showing fly behavior to be affected by neighboring Asteraceae species.

The results from this study also show that sites with lower levels of frost have a greater number of flies than sites with a higher level of frost. These data indicate that flies may be using landscape level cues related to the amount of frost in a meadow to decide where to spend time and oviposit as adults. Frost is highly variable by microhabitat; adjacent meadows may have very different degrees of frost damage on buds, especially depending on the direction of the slope. Thus, flies may not have to travel long distances to find meadows with less frost damage. Previous studies on a different species of Tephritid flies have found that the flies use visual cues to differentiate between various landscapes (Roitberg & Mangel 1997). It is possible that the flies in Gothic are also responding to microhabitat cues visually to choose meadows with less frost.

Tephritid fruit flies are a major agricultural and/or invasive pest in many parts of the world. Like in the Rocky Mountains, much of the Northern Hemisphere is also seeing the climate trend toward earlier spring snowmelt and warmer spring temperatures. While this decreases the overall number of frosts, it increases the chances of a late spring frost damaging early buds and vegetation (Scheifinger et al. 2003 & Cooter & LeDuc 1995). A decrease in frosts may cause an increase in the number of Tephritid flies in such areas as there is also a decrease in the number of aborted buds. In addition, studies have projected that there will be an increase in the abundance of Tephritid flies and changes in their distribution. In general, they are projected to move towards the poles and eastward into more temperate regions. Tephritids are projected to move into these areas due to a decrease in the number of frosts, an increase in the growing season and warmer nights (Hill et al. 2016). Hill et al. 2016 supports the results from this study that show there is a connection between frost and Tephritid fly abundance, and that less frost events may result in an increase in flies.

This study emphasized the importance of understanding both the indirect and direct impacts of climate change on biological systems. Few studies have taken place that examine the indirect effects of climate change. In order to gain a more holistic understanding of climate change such studies need to take place on other biological systems. In addition, these studies will help improve conservation and management plans to promote resilient ecosystems by better understanding how different systems are impacted both directly and indirectly.

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Figures

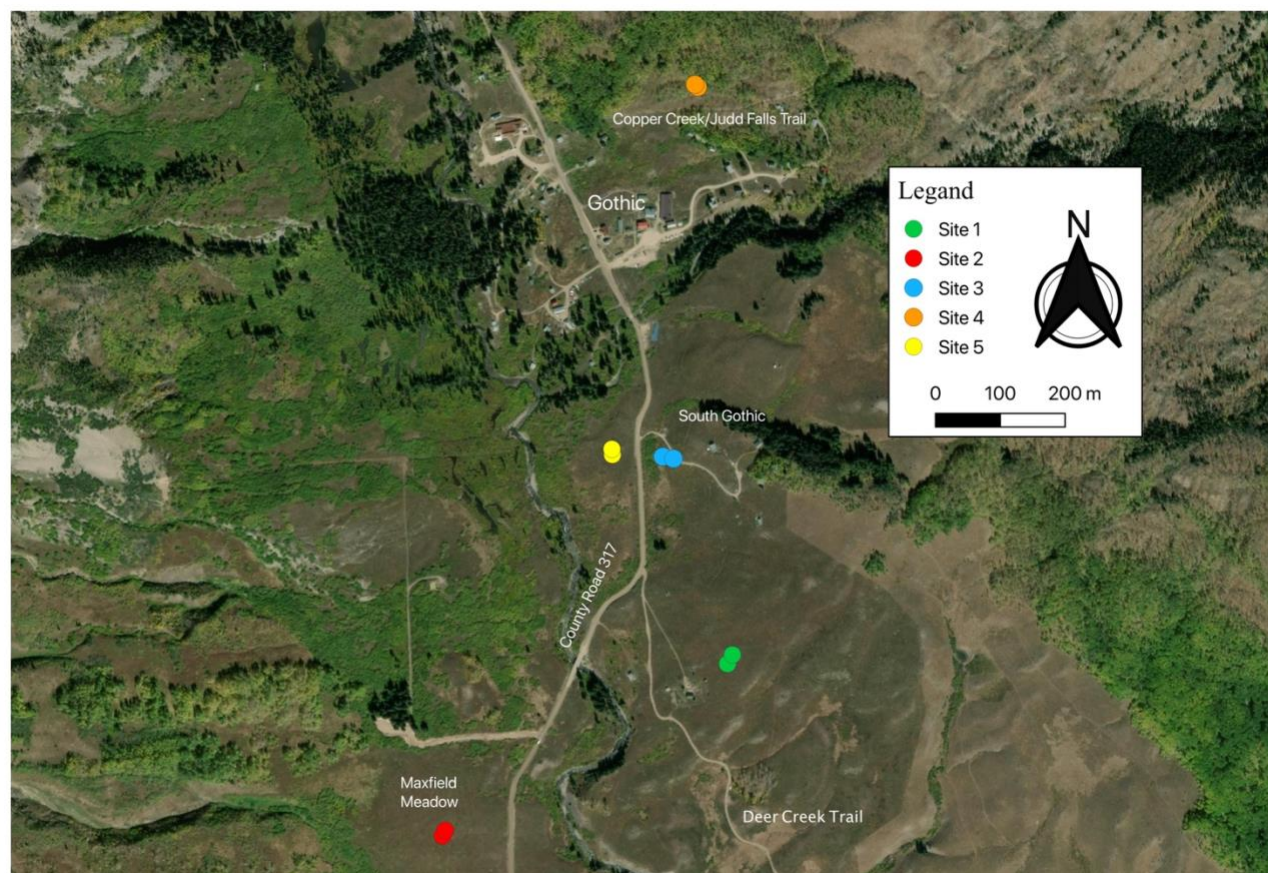


Figure 1: Map of study site. Each dot represents a different site. The two dots at each site represent the beginning and end of the transect at each site. Sites 2 and 5 had high levels of frost and Sites 1, 3 and 4 had low levels of frost.

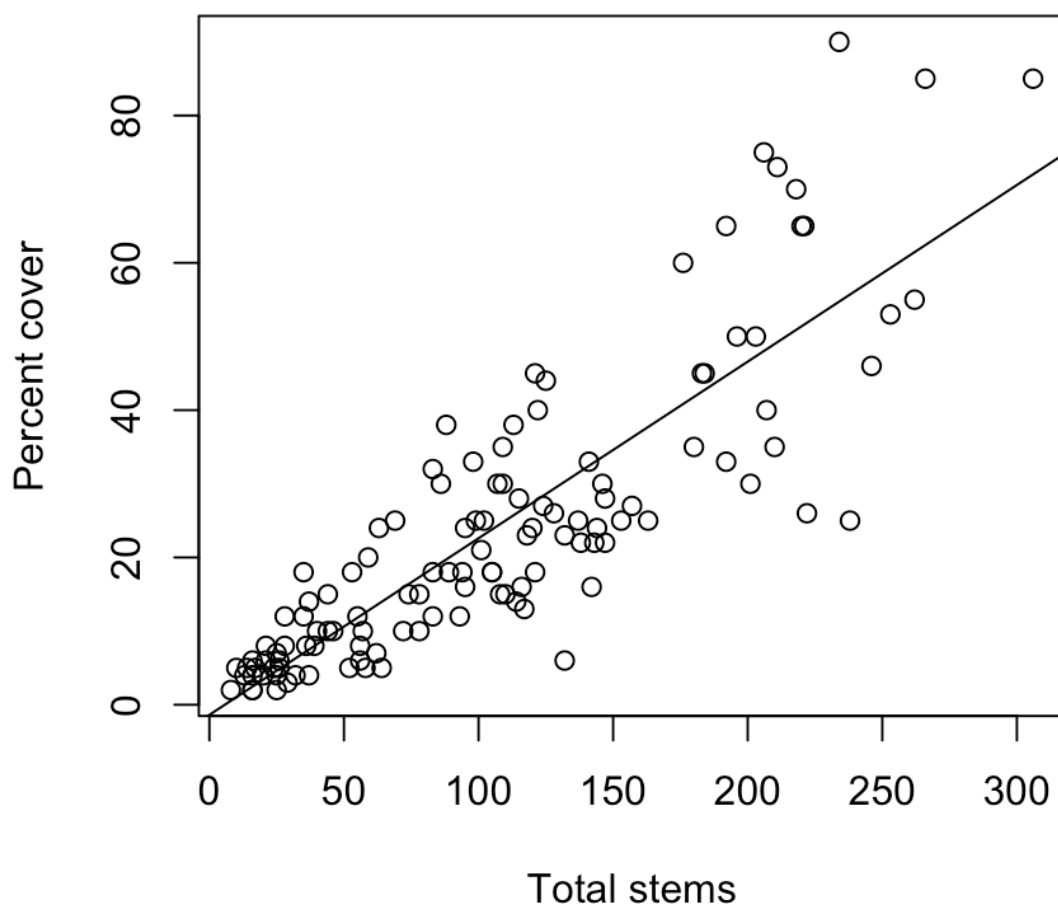


Figure 2: The percent cover of *Erigeron* as a function of the number of stems in each plot. The number of stems = the total number of flowers + buds + frost aborted buds. Data shows a significant positive linear relationship. $p\text{-value} = <2e-16$. $r^2 = 0.723$. $n = 120$ plots.

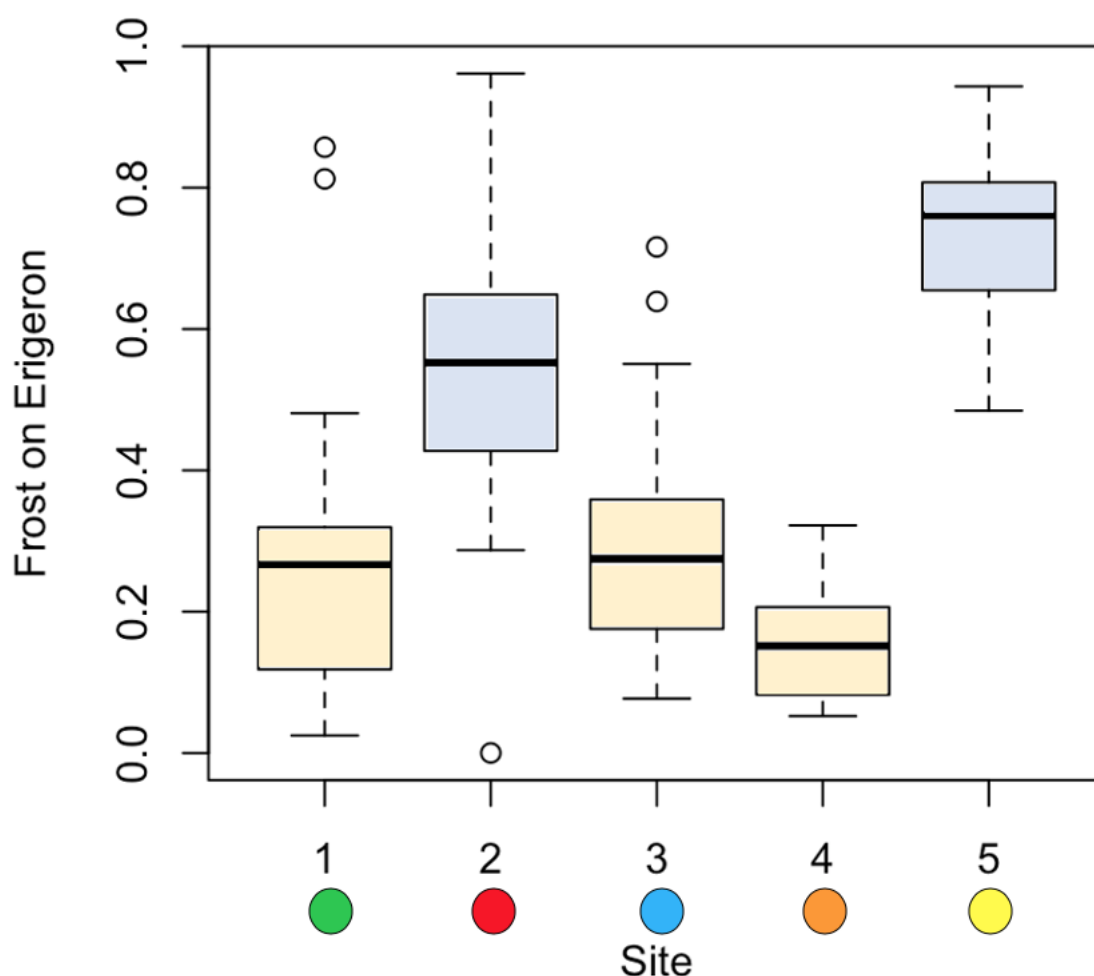


Figure 3: The proportion of frost on *Erigeron* at each site. Sites 1, 3 and 4 (low frost, yellow) different significantly from Sites 2 and 5 (high frost, blue). The colored dot under corresponds with color of each site on the map (Figure 1). The mean $\pm$ standard deviation is 0.276 ± 0.204 , 0.544 ± 0.202 , 0.305 ± 0.166 , 0.162 ± 0.088 , and 0.730 ± 0.117 for sites 1, 2, 3, 4 and 5 respectively. . p-value = $< 2e-16$. n = 24 plots per site.

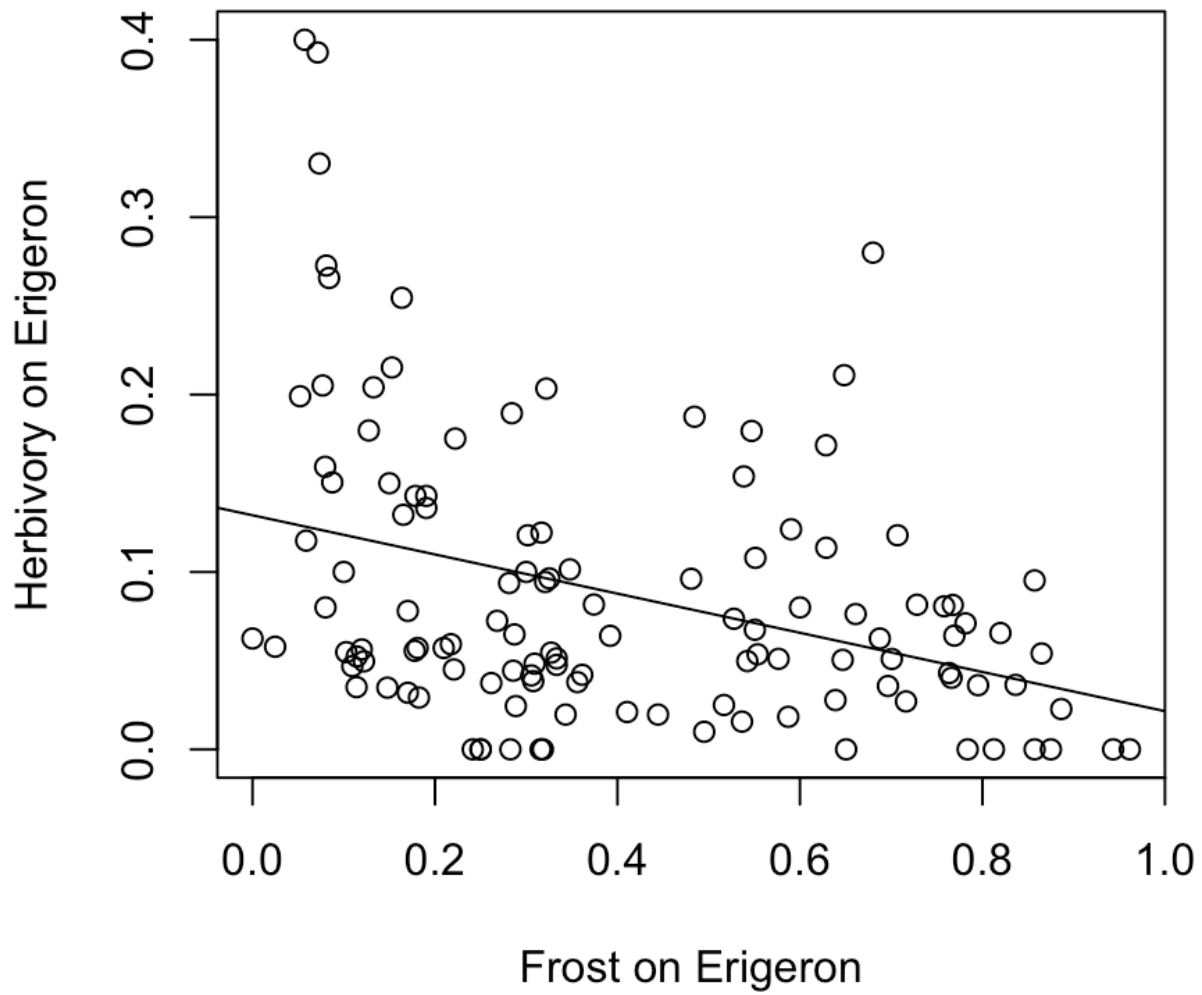


Figure 4: The proportion of fly herbivory on *Erigeron* as a function of the portions of frost at in each plot. Data show a significant negative linear relationship. $p\text{-value} = 6e-5$. $n=120$ plots.

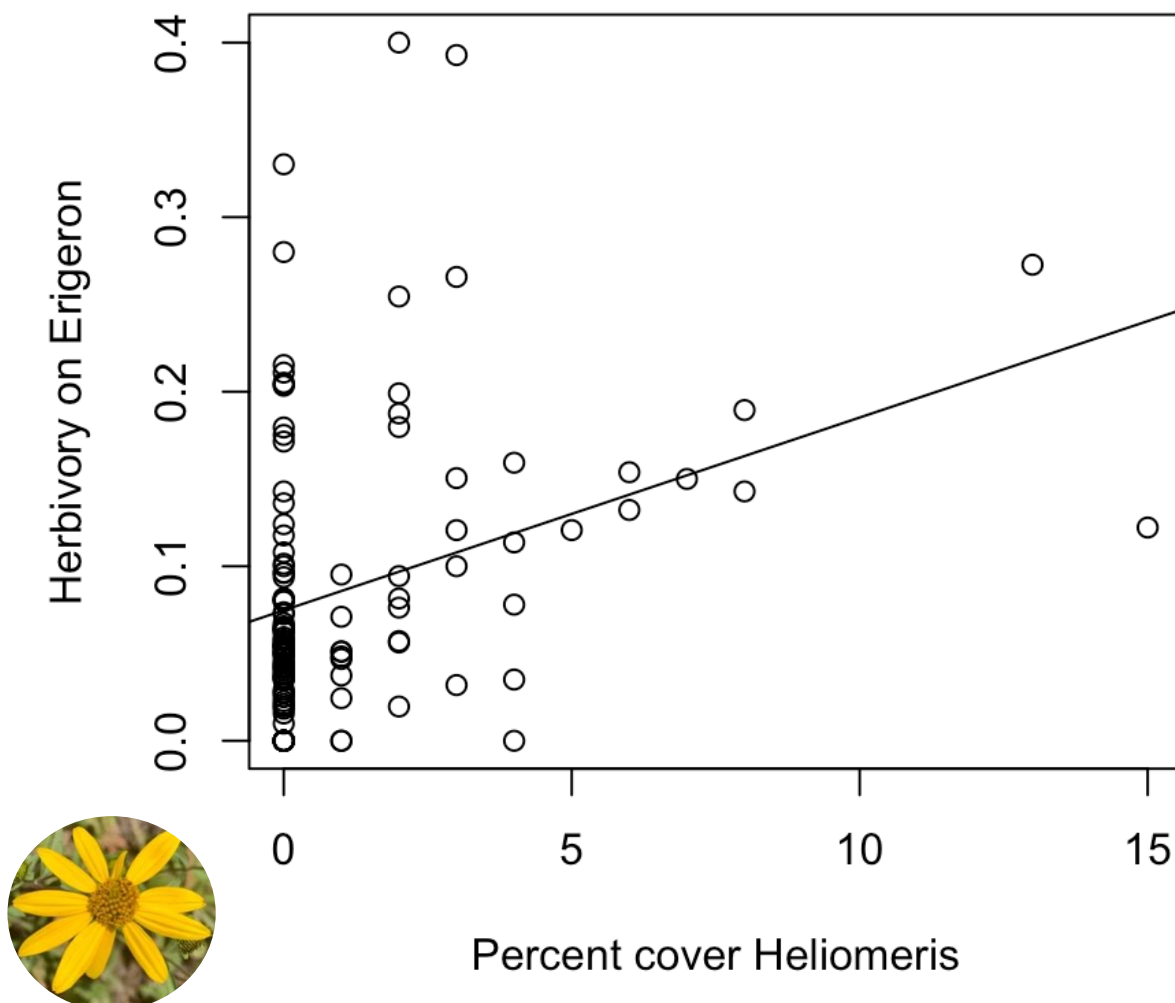


Figure 5: The proportion of *Erigeron* buds with herbivory as a function of the percent cover of *Heliomeris* in each plot. Zero values indicate no *Heliomeris* present in the plot. Data shows a significant positive linear relationship. $p\text{-value} = 0.000208$. $n = 120$ plots.

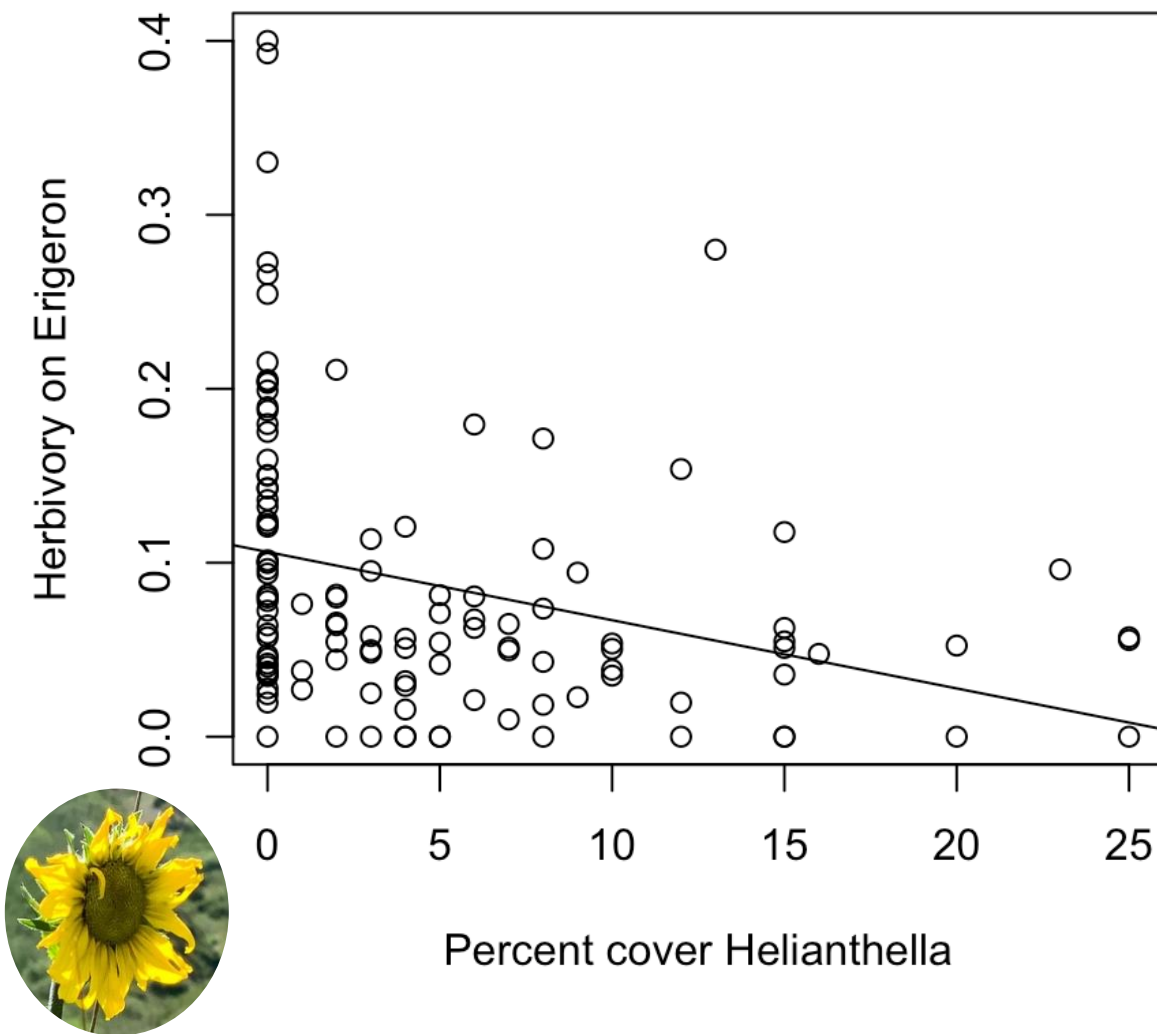


Figure 6: The proportion of *Erigeron* buds with herbivory as a function of the percent cover of *Helianthella* in each plot. Zero values indicate no *Helianthella* present in the plot. Data shows a significant negative linear relationship. $p = 0.000773$. $n = 120$ plots.

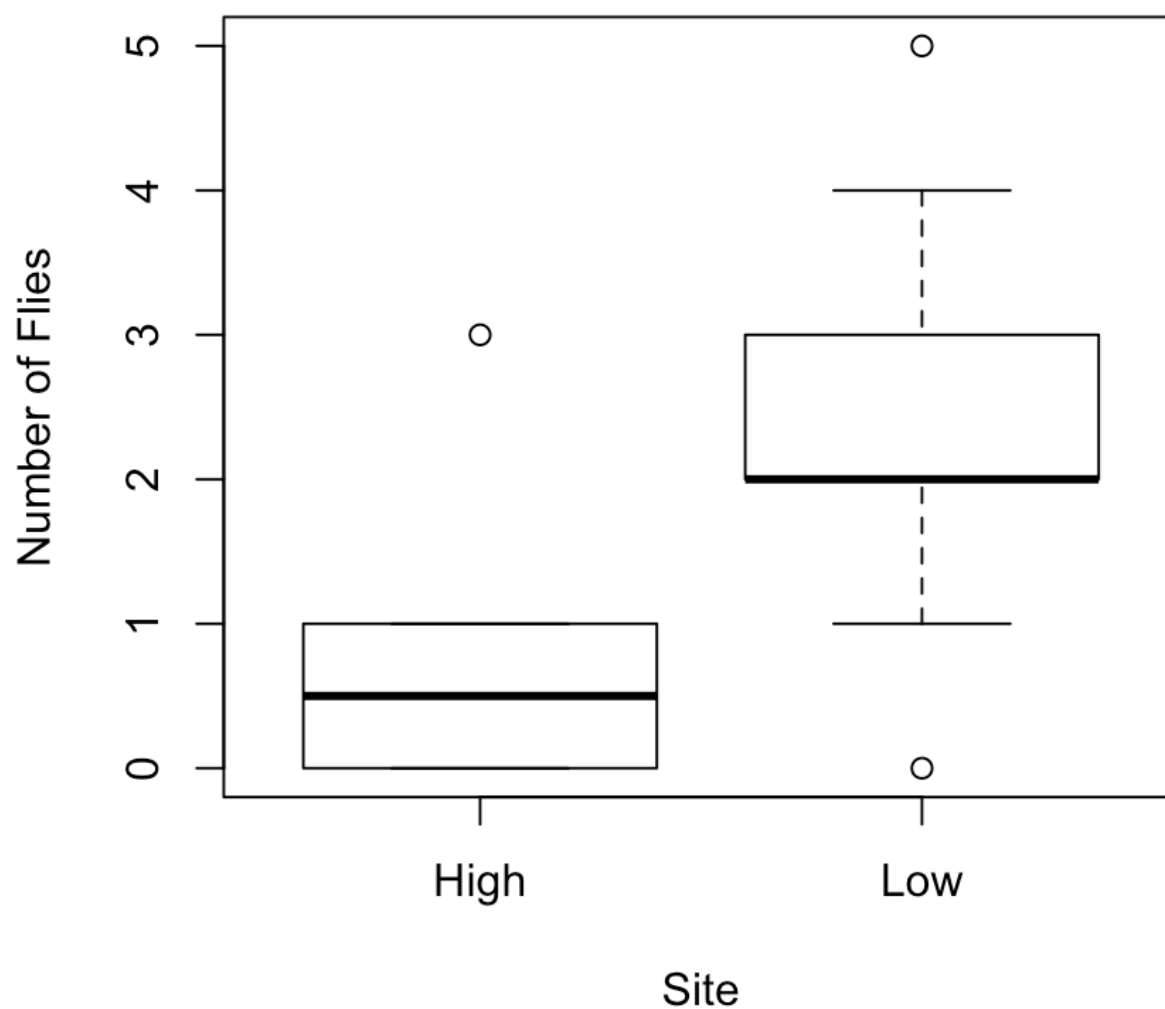


Figure 7: The number of Tephritid flies caught in a sweep net at each site as a function of site. Sites were aggregated together into either low frost density (Sites 1, 3, 4) or high frost density (Sites 2 and 5). Sites with low frost densities had a significantly greater number of flies. $p=0.0463$. In sites with low frost density the mean $\pm$ standard deviation = 2.3 ± 1.5 . In sites with high frost density the mean $\pm$ standard deviation = 0.83 ± 1.17 . $n = 3$ sweep nets per site.

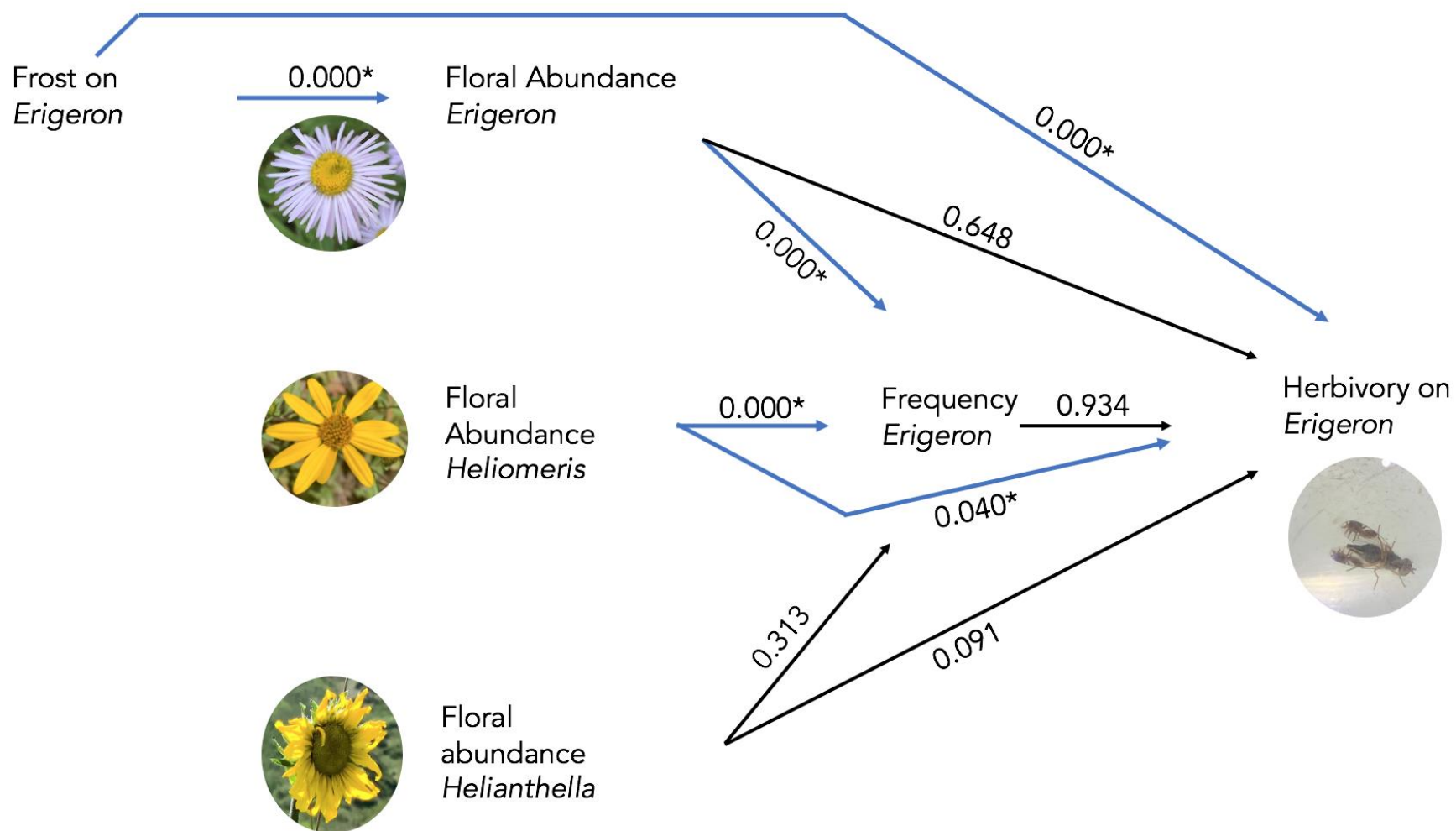


Figure 8: Structural equation model and path diagram showing all possible direct and indirect factors effecting herbivory of *Erigeron*. P-values are shown above each arrow. * indicates significant p-values. Significant relationships are shown with blue arrows.