

**Effects of Insect Damage to *Helianthella*
quinquenervis (Asteraceae) Inflorescences on
Pollinator Visitation**

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Abstract

Plant reproductive success is often influenced by not only direct effects of flower-eating herbivores (florivores) consuming floral tissues, but also the indirect effects of pollinator visitation. Previous literature suggests that florivore damage can negatively influence pollinator visitation, consequently having both direct and indirect effects on plant reproductive fitness. Studies have also shown that conspecific and heterospecific floral density can increase or decrease pollinator visitation. Here I determine the effects of inflorescence damage on pollinator visitation rate of a common perennial native to the Rocky Mountains, the aspen sunflower (*Helianthella quinquenervis*, Asteraceae). Damage on both the ray florets and disk florets of the sunflowers were found to decrease visitation of their two most common pollinators, bumble bees (*Bombus spp.*) and Muscid flies (Muscidae). Visitation rate was also observed for co-flowering heterospecifics of *Helianthella* within 1x1 meter observation plots to determine if damaged sunflower inflorescences influenced the visitation of blooming neighbors. However, data was insufficient to observe any effects. With *Helianthella* experiencing phenological shifts due to climate change—blooming earlier in early snowmelt years—floral abundance has declined due to frost susceptibility (Inouye 2008). Given that florivore damage also directly and indirectly affects plant fitness, it may be possible that *Helianthella* will experience an accelerated decline in abundance due to and increase in florivore competition and a decrease in pollinator visitation.

Introduction

Plant communities often have dense floral displays composed of many simultaneously flowering plant species. Floral displays are vital for approximately 87% of flowering plants (Ollerton et al. 2011) as they attract pollinators that mediate sexual reproduction and help maintain plant populations. However, the resources provided by flowers are not only attractive to mutualists, but also to antagonists. Flower-eating herbivores (florivores) are attracted to and damage flowers. It is well known that damage caused by florivores can reduce reproduction directly, by consuming floral tissue, but also indirectly, by reducing pollinator visitation to plants with florivore-damaged flowers (Cardel & Koptur 2010; Carper, Adler & Irwin 2016; McCall 2008; Söber, Moora & Teder 2010). This latter effect on pollinator visitation may also extend to co-flowering plant neighbors. Plant species often share pollinators with other plants in communities, and the presence of co-flowering neighbors can increase or

decrease a focal plant's pollination (Mitchell et al. 2009; Bruninga-Socolar, unpublished). An unexplored question is how damage by florivores can affect not only the damaged plant's pollination, but also the pollination to co-flowering neighbors that share pollinators.

The objective of this study is to determine if flower damage from florivores influences patterns of pollinator visitation. Tephritid flies (Tephritidae) are known to oviposit in inflorescence buds of the sunflower family (Asteraceae), where their larvae feed on the developing seeds. These flies not only hinder seeds from maturing, but discolor the inflorescence in the process. If pollinators find these discolored inflorescences visually unappealing, the inflorescence may receive fewer pollinator visits therefore produce fewer seeds and have lower reproductive success. If these inflorescences receive fewer pollinator visits, it is possible that the surrounding floral neighbors will receive fewer visits as well. The indirect effects of inflorescence damage affecting the surrounding community is an example of an ecological

phenomenon described as “associational effects” (Underwood, Inouye & Hambäck, 2014). These effects, in turn, may ultimately change floral composition in a community; as some rare or sensitive flowers may experience lower abundances or even local extinction as a response to an increase in florivores.

Aspen sunflower (*Helianthella quinquenervis*) is a ubiquitous perennial in the Rocky Mountains, spanning from northern Mexico to Northern Montana (USDA, NRCS 2016). *Helianthella* is considered a dominant species in the East River Valley, home of the Rocky Mountain Biological Laboratory (RMBL), typically flowering from mid to late July with average densities as much as 8 flowers per square meter in some years (Inouye 2008). However, since seasonal growth is initially dependent on the date of snowmelt, *Helianthella* has experienced phenological shifts due to climate change causing earlier snowmelt (Inouye 2008). Subsequently, inflorescence abundance has decreased in recent years, as buds develop sooner in the season but are often killed by frost (Inouye 2008). *Helianthella* is also known to be a target of florivory from tephritid flies among other insect pests (Inouye & Taylor 1979). With floral abundances decreasing, it is possible that *Helianthella* inflorescences could experience greater florivory in response to increased fly-specialist competition for floral resources. If pollinators are deterred by damaged flowers, pollinator services to *Helianthella* may decline, thus contributing further to the effect of climate change on the reproductive success of montane flowers.

In this study, I investigated the effects of *Helianthella quinquenervis* inflorescence damage on pollinator visitation. Inflorescences with greater damage from insects were expected to receive significantly fewer pollinator visits. A study by Prathyusha Pamidi in 2014 at the RMBL found that damaged *Erigeron speciosus* inflorescences received significantly fewer pollinator visits than undamaged inflorescences (unpublished). Neighboring flowers were also expected to receive fewer pollinator visits due to “associational susceptibility” (described by Underwood, Inouye & Hambäck, 2014) of damaged *Helianthella* reducing the pollinator attractiveness to floral neighborhoods.

Methods

In the summer of 2016, I spent four weeks conducting an observational study at the RMBL in Gothic, Colorado. In this study, I selectively placed 1 x 1 meter quadrats over aspen sunflowers (*Helianthella*

quinquenervis) representing a range of flower damage at five different sites: two next to Gothic Road near the East River and three next to Gothic Road near Bellevue Mountain (Figure 1). In each of these plots, I categorically estimated damage from insects on each inflorescence as a function of discoloration on the disk of each flower within the plots. I also estimated damage to ray florets. To measure pollinator visitation, I recorded the number of pollinator visits and pollinator type to each inflorescence in each plot a various times between 1000 and 1800 h during 30 min observation periods between July 13 and August 13, 2016. I also noted floral abundances of heterospecifics in each plot and their respective pollinator visits. Additionally, I noted weather conditions, floral density, inflorescence height (from ground), inflorescence diameter, and fungal rust presence were as potential effects on visitation.

Statistics

Because the number of pollinators to each plot is a discrete non-negative number, the data were not particularly well suited for analysis by simple linear models with Gaussian (normal) residuals. Instead I used generalized linear models, which allowed me to model the number of pollinator visits with a Poisson distribution. To account for lack of independence among flowers within the same plot, I included the plot identity as a random factor in generalized linear mixed models (GLMMs). Since pollinators on *Helianthella* were predominantly bumble bees (*Bombus spp.*) and Muscid flies (Muscidae). I ran separate analyses on each visitor group to determine if visitation behavior was influenced differently. Inflorescence height and diameter were not recorded for the first nine observations so I ran two GLMM analyses including and excluding inflorescence height and diameter data. To determine if there was an effect of *Helianthella* density on pollinator visitation, I used linear regression with averaged data across observation periods including weather conditions (wind, cloud cover, rain) and floral characteristics data (inflorescence height and diameter) as covariates to better explain variation in the data. All analyses were run in R 3.2.4 (R Core Team, 2016).

Results

Since damage to the ray and disk florets had the same effect on pollinator visitation, I combined them into one variable for simplification. Flower damage significantly decreased both bumble bee and fly visitation in all GLMM analyses (Figure 2a,b,c,d; $P < 0.01$). Fly visitation significantly decreased as time progressed throughout the day (Figure 2c,d; $P < 0.05$). Bumble bees visited flowers with a greater flower diameter more than those of smaller diameters (Figure 2b, $P < 0.001$), whereas flies visited flowers with greater diameters relative to the average diameter of flowers within the plot (Figure 2d, $P < 0.001$). Relative flower height only marginally influenced fly visitation (Figure 2c, $P = 0.0647$). *Helianthella* at lower sites received fewer bumble bee visits when compared to those at higher sites (Figure 2a, $P < 0.05$). Location, however, did not significantly influence bumble bee visitation when including flower diameter as a variable (Figure 2b, $P > 0.05$). With flower diameter included as a variable, bumble bees visits significantly increased as the season progressed (Figure 2b, $P < 0.001$).

Using regression analyses on summarized data, bumble bee visits significantly increased as *Helianthella* density increased using flower damage, observation time, and location as covariates (Figure 3b, $P < 0.001$, $R^2 = 0.785$). Plots of the other covariates used are included in Figure 3 to display trends in the relationships. Cloud cover, wind, presence of fungal rust on the vegetation, and number of flowering neighbor species were found to not significantly influence pollinator visitation in any of the analyses ($P > 0.05$).

Discussion

Consistent with previous findings in other plant-pollinator systems (Cardel & Koptur 2010; Carper, Adler & Irwin 2016; McCall 2008; Söber, Moora & Teder 2010), insect damage on *Helianthella* inflorescences significantly decreased pollinator visitation. With *Helianthella* floral abundances declining (Inouye 2008), the effect of insect damage may reduce reproductive success both directly (florivory) and indirectly (fewer pollinator visits), potentially reducing seed production and recruitment into the population. This may ultimately contribute to declining population sizes.

Literature Cited

Previous studies have shown that conspecific and heterospecific flower density can either increase or decrease pollinator visitation to a focal species (e.g., Mitchell et al. 2009; Bruninga-Socolar, unpublished). Considering only 27 pollinators were observed on neighbor flowers among all 30 plots, these data were insufficient to determine if *Helianthella* inflorescence damage influences visitation rate to heterospecific neighbors. However, it may be possible that the presence of *Helianthella* inflorescences reduced pollinator visitation to neighbors, potentially in favor of *Helianthella*. To determine this, visitation on heterospecifics with a flowering period that overlaps that of *Helianthella* could be observed. If visitation of heterospecifics decreased while *Helianthella* was flowering, it could be reasoned that *Helianthella* increases associational susceptibility of neighbors.

Interestingly, bumble bees were significantly influenced by the absolute diameter of *Helianthella* inflorescences, whereas flies were influenced by the relative diameter of the inflorescences compared to the average of the plot they were observed in. This could be a result of their differences in foraging behavior. In my observations, flies spent more time moving to nearby inflorescences. Conversely, bees would travel further distances, perhaps in search of larger flowers with greater pollen and/or nectar rewards. Studies have shown that both bees and flies visit larger flowers more frequently (Galen & Newport 1987, Conner & Rush 1996), but little is known about how bumble bees and flies differ in their foraging response to flower characteristics.

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{ Map removed because it made the file too big for the RMBL publications database. Contact gis@rmbll.org for GPS data. – J.Reithel. Sept, 2016 }

Figure 1. Site map of all 5 observation locations. Reference points were included for identification. All 5 sites occurred in Gothic Valley, north of Crested Butte, CO.

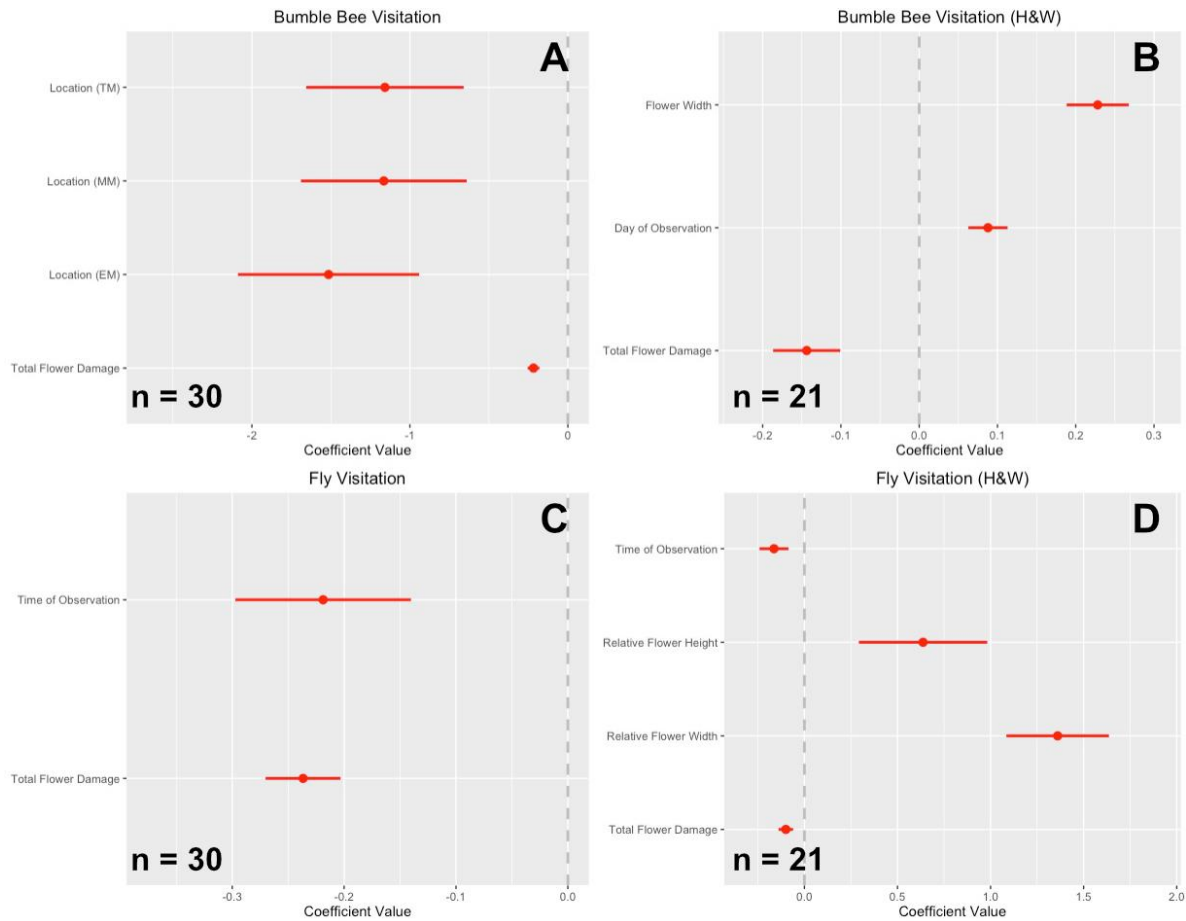


Figure 2. GLMM analyses of pollinators visitation expressed as coefficient plots. Panel A and B represent bumble bee visitation in response to various variables. Panel C and D represent fly visitation. Panel B and D are figures where flower height and diameter were included in the analyses. All values present are significant with the exception of “Relative Flower Height” in plot D which is marginally significant ($p = 0.0647$). Bars represent standard error. Separate plots were made including flower height and flower diameter due to lack of data for nine of the 30 observations.

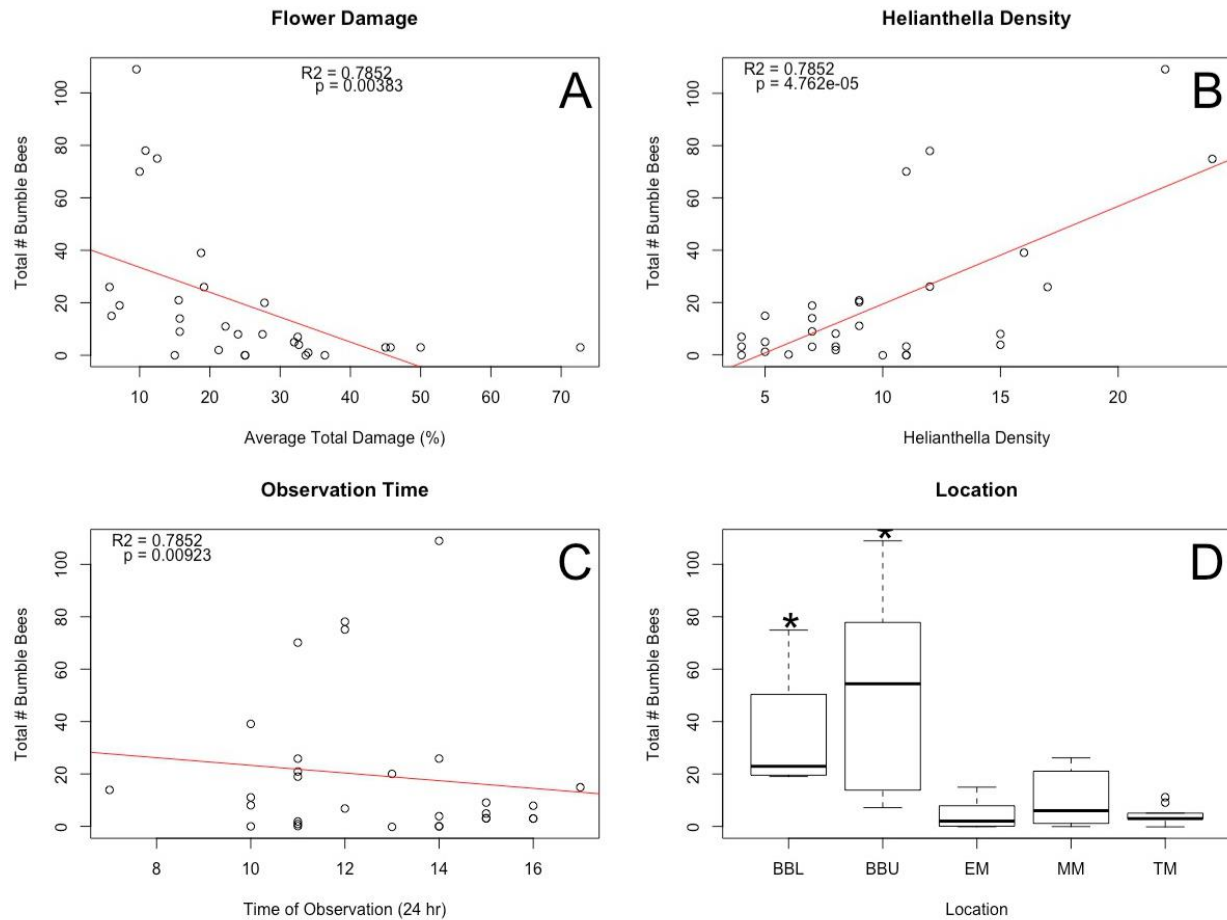


Figure 3. Regression analyses of bumble bee visitation using averaged data for each plot. Each panel represents bumble bee visitation response to various variables. Flower damage, *Helianthella* density, observation time and location were used as covariates. Each covariate significantly influenced visitation with the exception of location. Locations that were significantly different from each other are indicated with an asterisk (*).

