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Fungal Phytopathogens Decrease Plant-Insect Interactions

Abstract:

Rusts are pathogens of rapidly growing plant tissue and are of particular concern in agricultural settings due to their reduction of plant fitness. These phytopathogens depend heavily on abiotic features such as humidity, temperature, and UV strength, and with a changing climate, the rust ranges may be altered in future years and new hosts can be infected. Since this organism is an obligate pathogen changes in host ranges could introduce rusts into new communities and lead to novel interactions. Not much research has been conducted to understand the natural communities of which rusts are a part. Previous research has indicated mixed effects of fungal presence on arthropods associated with plants. Decreases in herbivory have been noted as well as increase in pollinator attraction. This study aims to elucidate natural patterns of insect herbivory and pollination, and the effects on plant fitness utilizing infected *Helianthella quinquenervis*. Results indicate significant decreases in herbivory on infected plants, as well as reduced number of flowers produced by infected plants. Pollination rates also decreased with infection as pollinators spent less time and visited fewer flowers on infected plants.

Introduction:

Fungal pathogens can infect a variety of organisms and are becoming increasingly well known for their tenacity and reduction of fitness in host species. A subset of fungal pathogens that infect only plants are known as phytopathogens; they have become the focus of research in order to prevent epidemics that reduce crop yield. Rust fungi are a common type of phytopathogen identified to be part of the order Puccinales. These fungi create pustules and infections on healthy plants; unlike other pathogens, rusts target actively growing tissue, which can create problems for cultivators of plant products. Modern agricultural practices, which reduce species diversity through monoculture, increase the severity of infections and allow for rapid spread of the rust population, especially if the environmental factors in the region are suitable for a particular species of rust (Thrupp, 2000). Lower plant diversity has been shown in

natural systems to be correlated with higher severity of infections but lower rust diversity (Rottstock, Joshi, Kummer, & Fischer, 2014). Rusts and other fungal pathogens exert important selective pressures on plants and are crucial drivers of community dynamics.

Like many organisms rusts are limited by abiotic factors such as moisture, temperature and UV strength, as well as biotic interactions. Rusts are obligate biotrophic parasites and require appropriate hosts in order to reproduce and thus are limited to habitats by host availability and requisite abiotic elements. As environmental factors are altered through global climate change events, these changes can alter the distribution of fungal phytopathogens either through changing the range of host plants or through causing more favorable abiotic factors to persist in areas with already suitable host plants (Helfer, 2014)

As fungal rust ranges are altered understanding how these organisms interact in natural systems is increasingly necessary to predict changes in natural and agricultural communities. Fungal pathogens have been demonstrated to lower plant fitness due to the siphoning of photosynthetic products to fungal growth and a subsequent net energy loss. Reproductive elements of fitness are also decreased through the lowering of seed number and viability as well as general alteration of seed morphology (Salama, Edwards, Heard, & Jeger, 2010). These decreases in host fitness may contribute to decreases in competitive abilities and alter biotic relationships with conspecific and heterospecific plant species. However, recent gains in knowledge indicate that infection via fungal pathogens can structure interactions beyond direct plant-fungi interactions.

Insects are well known as herbivores of different plants and as such have direct, usually negative, impacts on the plant's fitness. When rusts are introduced and a plant-insect-fungi system is established, many new direct and indirect interactions occur. Directly, insects--either herbivores or pollinators--can act as spore dispersers for fungi, enabling the phytopathogens to spread to other plants in the area. Fungal infections can lower available plant material for consumption due to stunting of plant growth, or lowering seed and pollen production; these deleterious effects on plants could negatively affect insects (Salama et al., 2010). Previous research indicates that various rusts can decrease herbivory and oviposition when insects are given a choice between infected and uninfected leaf tissue (Sholes, 1894; Simon & Hilker, 2005). The provided tissue was not contaminated with open lesions thus the fungus indirectly affected insect behavior through the plant. This alteration of behavior without prevalent spores

could be due to altered volatile organic chemical (VOC) emissions which have been documented by Toome et al. (2010). These chemicals could deter insects leading to a decrease in herbivory and pollination or in the case of some rusts an increase in interactions, specifically pollination. For example, the infection of an *Arabis* by a rust can alter the form and emissions of the plant to attract pollinators and aid in the spore dispersal of the fungi (Roy, 1996). Little is known about many natural plant-fungi-insect systems and more research is needed to clarify the interactions within the system and how these systems interact in a larger community.

Fungal effects on plant-insect interactions could be important influences on communities with varied species compositions. Study of these natural communities is needed to elucidate the complicated indirect and direct effects that phytopathogenic fungi, especially rusts, have on hosts and the community as a whole. I plan to study the effects of rusts on a small community by using 1) field observations of the relationship between rust incidence, herbivore damage and plant fitness and 2) observations of pollinator behavior in regards to infected *Helianthella quinquenervis*.

Methods:

Study System:

Helianthella quinquenervis is a long-lived perennial in the family *Asteraceae*. It is found predominantly in the western part of the United States in montane habitats. In the area surrounding the Rocky Mountain Biological Laboratory it is a very common plant that occurs in relatively high densities throughout meadows and even shaded wooded habitats. Basal rosettes are present early in the season and will form tall stalks on which the flower blooms. Both bumblebees and butterflies have been seen pollinating the flowers. Herbivory is common due to insects as well as mammals, including deer. Unpublished observations indicate that certain individuals are repeatedly infected year after year, once they are initially infected.

1. How does the presence of a fungal pathogen affect herbivore behavior?

I located 30 infected plants of each level (specified below) and 30 uninfected *Helianthella quinquenervis* specimens in the meadow behind Bench cabin, Maxfield Meadow and the Weather Station Meadow by Judd Falls (Figure 5). Ten plants in each infection category were found in each location. Study plants were located at least 0.5 m away from another study plant.

Plants of different infection levels were identified with a scale of 1-3 with the first level being minor infection of less than a third of the leaves and the third level being complete infection of all of the leaves of the plant. All plants were tagged with the lab provided GPS unit. Attributes of the level of infection and the spatial block were entered for each plant tagged. I then monitored all plants for infection and percent of each leaf consumed. I visually estimated the percent area consumed of each plant by averaging the percent consumed of each leaf. Data were collected per plant, and each plant was regarded as a unique observation. Plants were identified as separate individuals by feeling for connecting roots and visually determining separate clumps. Ten plants per level of infection per field site constituted the sample size. Therefore, the total uninfected sample size was 30; the total sample size for level one was 30, and 30 again for both level two and three for a total of 120 plants sampled.

2. How does the presence of a fungal pathogen affect plant fitness with herbivore presence?

For the plants used in the field observations of herbivore damage with and without rust outlined above—I also recorded additional measurements of plant fitness. Plant height was recorded for each plant along with the number of leaves per plant and the number of flowers produced per plant. I measured plant height from the base of the plant to the top of the tallest leaves when gently stretched to a vertical position. The number of leaves was counted, a leaf was counted once it was longer than 2 centimeters or consumed by an herbivore. I counted the number of flowers and buds that were well developed or at least partially open on a well developed stalk.

3. How does the presence of a fungal pathogen affect pollinator behavior?

Seven uninfected plants with at least one flower present on each plant were selected in Judd Falls Meadow, and five uninfected plants were monitored in Maxfield. Seven plants of level three infection with flowers were used for comparison data for Judd Falls site, and five were used in Maxfield Meadow. The plants were monitored for open flowers in the fields. The plants were found within 1 meter of each other and observations were conducted per pair. I recorded the amount of time and the number of flowers each pollinator visited between the pair.

4. How does the presence of a fungal pathogen affect plant reproductive fitness with pollination presence?

Tagged plants that had previously been observed for herbivore damage in the Bench Meadow, Maxfield Meadow and the Weather Station Meadow by Judd Falls were allowed to flower normally and once visitations concluded and the flowers faded, I collected the flowers to analyze seed set. I collected measurements including: seed mass, seed length, number of seeds produced and germination rate. The seed mass and seed length were measured with a finely calibrated balance and calipers respectively and seed number per flower was counted manually. I determined the germination rate by isolating five seeds per petri dish and providing the seeds with 12 hour growing days and adequate moisture to allow germination after a cold period to prepare them. I then calculated percent viability based on infection status.

Statistical Analysis:

I used multiple ANOVA analyses to compare means obtained with the observational experiments. I compared the average leaf damage in each infection level and the means of the plant fitness data in each condition to look for an effect of infection level on the dependent variables measured (herbivory damage, plant height, leaf number and flower number). The pollinator observations were also analyzed with ANOVA manipulations to detect differences in the means.

Results:

Herbivory damage decreased when plants were infected with fungal rusts. Percent consumption was significantly different between each infection level and the uninfected plants (Figure 1). The most significant difference was between plants of level three-infection status and the uninfected plants. None of the infected plants significantly differed from the others in regards to mean percent of leaf area consumed (Table 1).

Plant fitness, measured in number of flowers per plant, was significantly decreased by infection of the plant (Figure 2). A similar trend was seen as with percent consumption, the higher levels of infection had more significant differences (Table 2). There was no significant difference between the mean numbers of flowers produced by plants of any infected status.

Pollinator interactions were decreased significantly when a flower was part of an infected plant. Both the average time that the pollinator spent on the flower, and the number of visitations that were made to the infected plant were significantly decreased (Figure 3 & Figure 4). The significance was consistent whether the observations were made per pollinator or averaged into interactions per plant. Figures included display the data for interactions by plant.

Discussion:

Infected plants have different interactions with insect communities compared to uninfected individuals. Herbivore damage was significantly reduced when *Helianthella* was infected with fungal rust. The percent consumed decreased significantly with all levels of infection, compared to the uninfected plants. However, there was not a significant difference between the mean percent consumed of each of the three infected conditions. A decrease in herbivory could be the result of the rust repelling the insect predators or of otherwise changing the suitability of the plant to act as a food source. Previous studies indicate that for certain larvae infected tissues result in a loss of body weight. Since the type of herbivores feeding on individuals were not recorded the effects on the organism population and the herbivore community cannot be known without further experimentation. A reduction in damage supports either the idea of a reduction in the number or a reduction in the diversity of the insect population.

Pollinator visitation also significantly decreased when a flower was a part of an infected plant. The time that the pollinator spent on the flower was significantly less when the plant was infected at level three compared to the uninfected control. Besides the time spent on the flower, the number of visits paid to 'infected' flowers was also significantly decreased compared to the number of visits made to flowers of uninfected plants. *Bombus bifarius* was the only pollinator seen to visit the flowers and although flies were observed to associate with the flowers, they were not marked as pollinators. This small pollinator population may respond to the visual cues of the infected leaves, which are warped and smaller on some of the infected plants. However, olfactory cues are also important for pollinator attraction by rust (Roy & Raguso, 1997). Similarly, altered volatile organic chemicals, which could be important as attractants, could be a factor in the differential behavior of the bees (Toome et al., 2010). A reduction in nectar level or pollen--due to a partitioning of resources elsewhere in infected plants--could have been

associated with the visual and olfactory cues and led to learned avoidance of the poor food source. Behavioral studies and floral assays could be used to determine how important the visual cues of the plant are in this altered behavior. A reduction in pollinator visitation could have negative effects on the plant's reproductive fitness. Thus, fungal rusts can lower plant fitness through deterring pollinators, possibly lowering seed set.

An alteration of the severity of herbivore damage and the loss of pollinators can have impacts at the population and community level of the plants. The population of *H. quinquenervis* would seemingly benefit from a reduction of predation as the plant can focus resources on defense against the rust instead of insects. Although there was no significant difference detected in the number of leaves produced or the height of infected or uninfected plants; the infected plants did have significantly less flowers than the uninfected comparison plants, which we interpreted as the infected plants having a lower fitness. Fewer flowers combined with having less pollinator visitation seems to indicate a lower overall reproductive fitness, despite any protective qualities against herbivory. Since rusts are recurring on a perennial plant and stalks are preformed underground before emergence, the continuous infection could limit the amount of resources available to form stalks and flowers as a result. Rusts have previously been demonstrated to reduce the seed set of flowers (Salama et al., 2010). In order to determine more accurately the fitness costs of infection seed set will be analyzed at the end of the season.

Understanding how the community surrounding *Helianthella quinquenervis* is altered depending on infection level is important to predict changes in population and associated communities. *Helianthella* serves as a food source for insects, which in turn provide important effects on other plants as pollinators or herbivores, and as prey for larger organisms. Assessing the ability of infected plants to act as a food source, both the leaf tissue and the flower resources, is important to further understand the effects of the rust on insect communities. Further experiments and observations could analyze the difference in food quality, including nectar production, tissue with and without lesions, and pollen quantity. In addition to the effects of the rust at the insect community level, changes in plant fitness could impact the population of *H. quinquenervis* and potentially the surrounding plant community. Lowering the seed set of individuals could alter their competitive abilities temporally.

Differences in the *H. quinquenervis* population could create changes in the plant community and the rust can indirectly affect the fitness of surrounding heterospecific plants by potentially reducing the competitive abilities of the infected aspen sunflowers. In order to understand the broader community effected by rusts, competitive abilities can be measured by planting infected and uninfected plants with common neighbors that compete for pollinators. Surveying the pollinator community surrounding *H. quinquenervis* would be necessary to understand the negative impact on pollinators and the spatial scale of this impact. Infected plants could have a reverse magnet effects in which a plant prevents insects from visiting nearby flowers due to the distaste for flowers of infected plants. Further experimentation is needed to determine if the repulsion can effect nearby flowers or if nearby flowers can experience increased visitation since the flowers of infected plants are compromised.

Understanding rusts and their associated communities is important to predict effects of climate change on certain communities. Many factors favoring rust development are predicted to create more habitable ranges for rusts (Helfer, 2014). Studying the natural systems of these already occurring rust-plant associations gives a broader understanding of how communities will fair when rusts are introduced and begin to colonize.

Appendices

Table 1a: Results for ANOVA comparing means of the percent consumed of each plant per treatment or infection level.

DF	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	3	2126	708.8	7.3e-09
Residuals	116	5073	43.7	

Table 1b: Results for Post-Hoc Tukey HSD analysis of the ANOVA comparing percent consumption between infection levels.

Treatment	Diff	Lwr	Upr	P adj	significance
infected2- infected1	-0.800000	-5.251026	3.651026	0.9658073	
infected3- infected1	-2.166667	-6.617693	2.284359	0.5845257	
uninfected- infected1	8.566667	4.115641	13.017693	0.0000113	*
infected3- infected2	-1.366667	-5.817693	3.084359	0.8541136	
uninfected- infected2	9.366667	4.915641	13.817693	0.0000015	*
uninfected- infected3	10.733333	6.282307	15.184359	0.0000000	*

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Table 2a: Results of ANOVA analyzing the differences in mean number of flowers produced by plants of different infection levels.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	3	445	148.39	5.241	0.00199
Residuals	116	3284	28.31		

Table 2b: Results of Post Hoc Tukey HSD analysis of mean number of flowers produced by plants of different infection levels.

Treatment	Diff	Lwr	Upr	P adj	significance
infected2- infected1	-1.200000	-4.781137	2.381137	0.8185625	
infected3- infected1	-2.733333	-6.314471	0.847804	0.1978700	
uninfected- infected1	2.533333	-1.047804	6.114471	0.2582482	
infected3- infected2	-1.533333	-5.114471	2.047804	0.6803831	
uninfected- infected2	3.733333	0.152196	7.314471	0.0375387	*
uninfected- infected3	5.266667	1.685529	8.847804	0.0011652	*

Appendices

Table 3: Results of ANOVA analysis of time spent by pollinators on flowers of infected and uninfected plants.

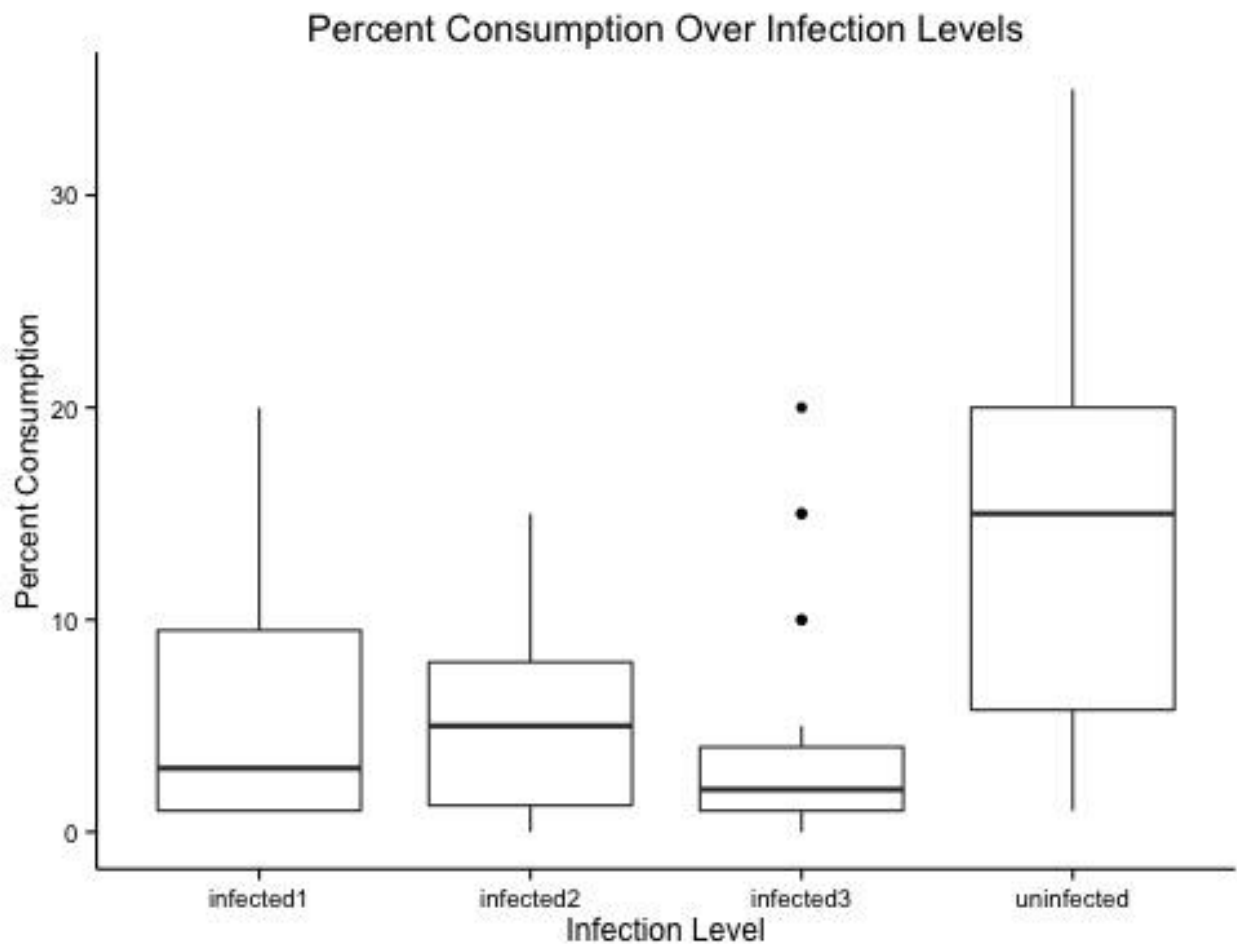
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	1	211	211.03	24.05	6.66e-05
Residuals	22	193.1	8.78		

Table 4: Results of ANOVA analysis of average number of visits by pollinators to flowers on infected and uninfected plants.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	1	51.04	51.04	15.47	0.00071
Residuals	22	72.58	3.3		

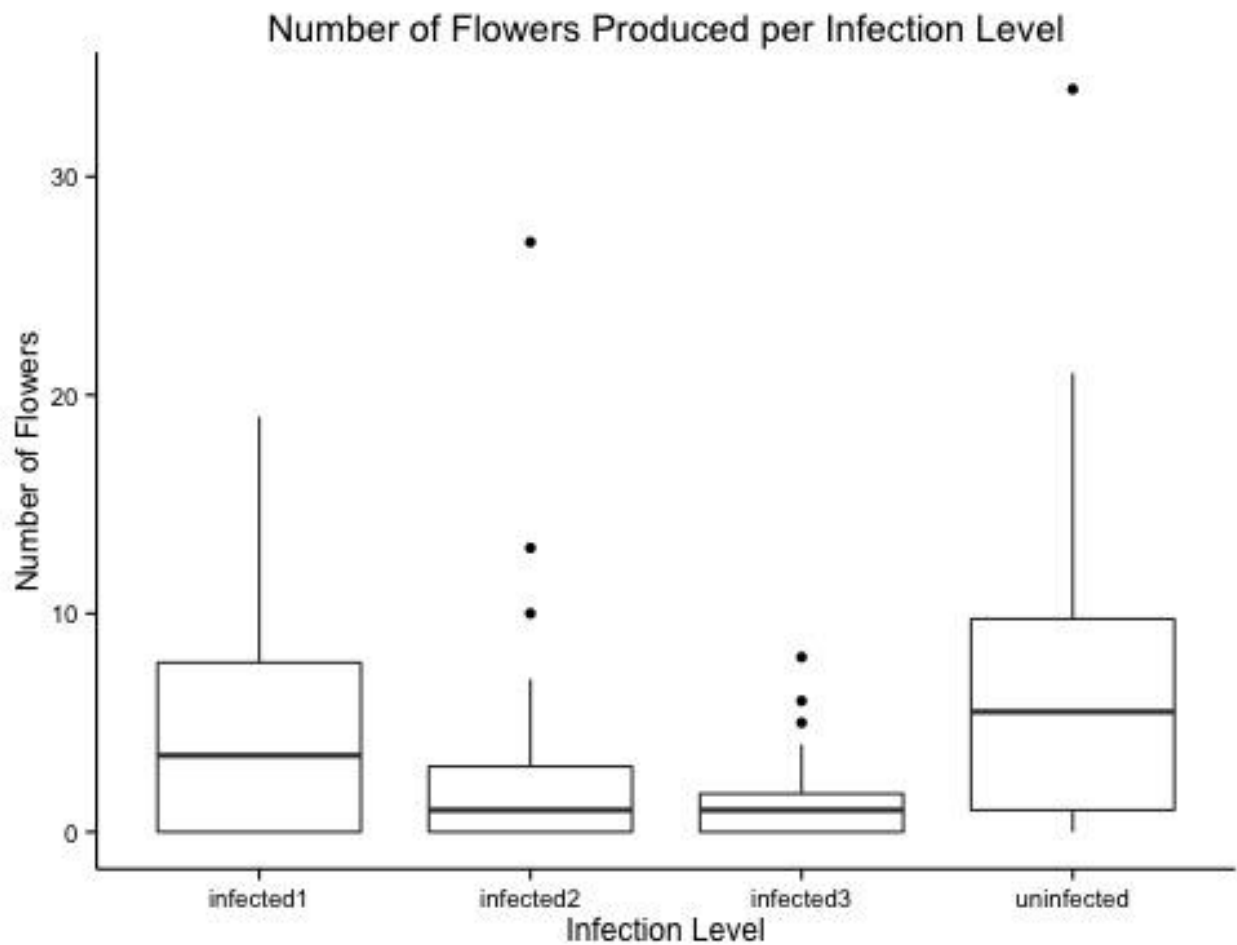
Appendices:

Figure 1: Average percent consumption significantly decreased between infected plants and the uninfected group. Significance of the difference in consumption increased as the level of infection increased, the most significant difference in consumption was between uninfected plants and level three infected plants.



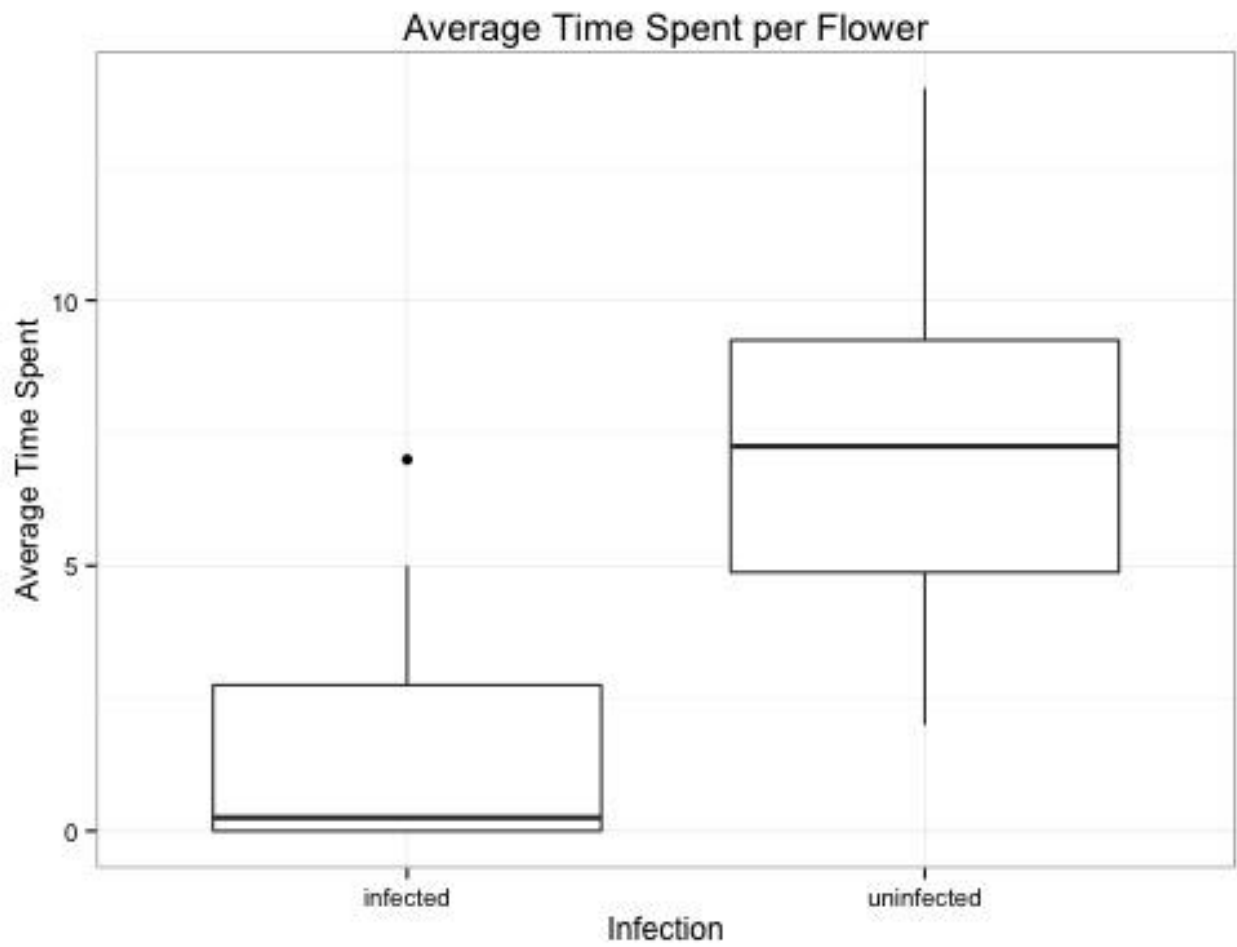
Appendices:

Figure 2: The average number of flowers produced by a plant at each infection level. The number of flowers was significantly decreased by infection of the plant. The most infected plants in level three category had the most significant difference compared to the uninfected plants.



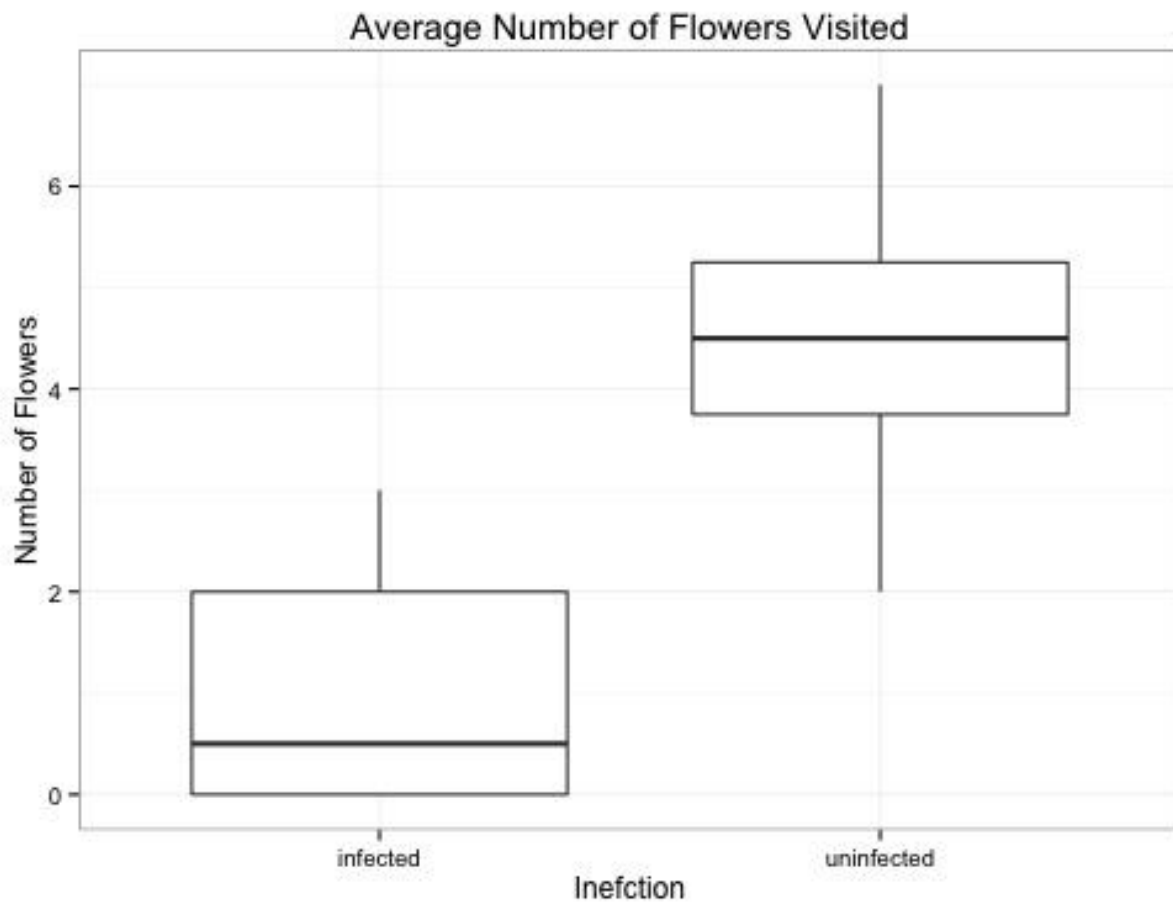
Appendices

Figure 3: The average time that a bee spent on each flower was significantly decreased when the plant was infected. Averages are calculated per plant, not per pollinator.



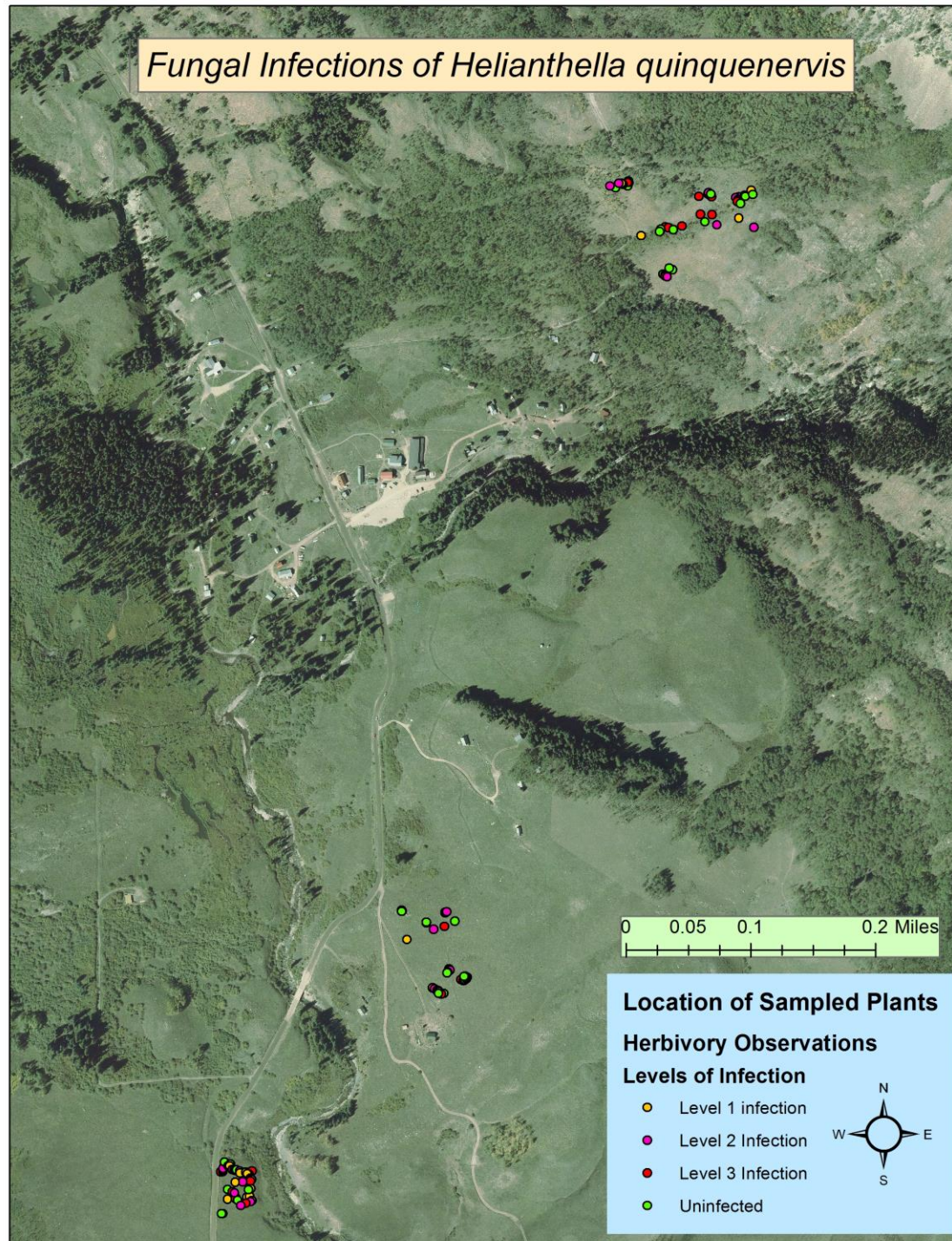
Appendices

Figure 4: The average number of visits made by bees to flowers of level three infected plants was significantly decreased compared to flowers of uninfected plants. Averages are calculated per plant not per pollinator.



Appendices

Figure 5: Map of all tagged plants at three study locations, Judd Falls, Bench Meadow and Maxfield Meadow.



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