

Effects of soil moisture and temperature on plant palatability and herbivore plant preference.

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RMBL Summer 2021

Independent Research/REU

Abstract

Climate change is predicted to lead to increased water and temperature stress for plants. Reduced soil moisture and higher temperatures have the potential to impact plant growth, development, and survival. Changes in plant physiology due to climate change may also impact insect herbivores. Herbivores influence plant community composition and plant distribution, so changes in herbivore feeding with climate change could alter plant communities. To better understand the potential impacts of climate change on plant-insect interactions, this study investigates the combined and separate effects of soil moisture and increased temperature on leaf palatability and herbivore plant preference. Four treatment types, ambient precipitation + ambient temperature, watered + ambient temperature, ambient precipitation + warmed, and watered + warmed, were established in the field in plots containing five common plants growing near Gothic, Colorado, *Erigeron speciosus*, *Helianthella quinquenervis*, *Potentilla pulcherrima*, *Heliomeris multiflora*, and *Lupinus bakeri*. Plants were warmed with open topped chambers and watered twice a week. Multi-choice feeding tests with the generalist herbivore *Melanoplus borealis* were conducted to assess plant palatability and herbivore preference in different treatments. In single species trials, fewer *Helianthella quinquenervis* leaves were eaten from warmed plots than ambient temperature plots and fewer *Heliomeris multiflora* leaves were eaten from watered plots than ambient rainfall plots. In multiple species trials more *Helianthella quinquenervis* was eaten than *Lupinus bakeri* and *Potentilla pulcherrima* and more *Heliomeris multiflora* was eaten than *Potentilla pulcherrima* in heated only treatments. More *Erigeron speciosus* was eaten than *Lupinus bakeri*, and *Potentilla pulcherrima* and more *Heliomeris multiflora* was eaten than *Potentilla pulcherrima* in watered only treatments. Changes in

herbivore preferences in treatments that reflect possible future climate scenarios suggest that shifts in herbivory could reshape plant communities in the coming decades.

Introduction

With climate change, plants will likely face increasing water and temperature stress. In Colorado, climate change is predicted to result in a decrease in summer rainfall, an increase in drought intensity, and higher temperatures (Frankson et al., 2017). Increased drought is likely to have many impacts on plant growth, development, and survival as water deficits have been shown to reduce photosynthetic rate, growth rate, leaf nitrogen content, and seed mass (Lipiec et al., 2013); (Rosbakh et al., 2017). Higher temperatures may reduce photosynthetic productivity and biomass (Zhang et al., 2010).

Since changes in precipitation and increased temperature are likely to occur simultaneously in future years, it is important to understand the combined effects of these factors to predict how a changing climate may impact plant communities. Complex and varied interactions between water availability and temperature have been seen in past studies. One factor may exacerbate the stress of another. A study of alpine grasslands showed no indication of stress in plants exposed only to high temperatures but showed vegetation browning in plants exposed to heat and drought together (De Boeck et al., 2015). The study suggested that temperature stress increased due to an inability to manage heat through transpiration (De Boeck et al., 2015). Drought and temperature can also have opposite effects, as seen in one study where high temperatures made plants more preferable to herbivores while drought made plants less preferable (Kuczyk et al., 2021).

In addition to directly effecting plants, climate change can result in impacts on plants mediated by herbivores. Herbivores affect plant reproduction, growth, and survival (Maron et al., 2006). Insect herbivores can influence the local distribution of plant species (Fine et al., 2004); (Louda et. al., 1982). Herbivores also influence plant community composition (Olf et al., 1998). Herbivores may create competitive asymmetry between plants, influencing plant competitive interactions (Borgstrom et al., 2016).

Drought and high temperatures may have plant-mediated effects on insect herbivores. Drought and temperature increases have been shown to change plant chemistry, negatively impacting plant quality for herbivores (Kuczyk et al., 2021). Herbivores fed drought stressed plants have been shown to have decreased growth rates (Po-An et al., 2020), and reduced abundance (Kansman et al., 2021). Insects feeding on temperature stressed plants may also have decreased performance (Kuczyk et al., 2021).

The effects of temperature and drought on herbivores could shift plant community composition if herbivores shift their feeding preferences with environmental changes. The ways in which drought and temperature interact to affect plant palatability and herbivore preference are not well-known. A previous study found that plants grown under warmer temperatures were more palatable to herbivores. In contrast, studies have shown that drought reduces the nutritional value of plants to chewing herbivores and decreases their survival (Kuczyk et al., 2021); (Po-An et al., 2020). To better understand the potential impacts of future drought and warming on plant-insect interactions, this study investigates the combined and separate effects of drought and increased temperature on leaf palatability and herbivore plant preference.

Methods

Temperature and soil moisture were manipulated in experimental plots in the field. Four treatments were established; watered and warmed, watered at ambient temperature, ambient rainfall and warmed, and ambient rainfall and ambient temperature. Plots were established in meadows near in Gothic, Colorado (Figure 1). Plots were placed in eight blocks of four plots containing one plot from each treatment. Heated plots were warmed with 0.84 square meter open-top chambers. Plots at ambient temperature were not heated. Watered plots received 10 liters of water per square meter approximately twice a week. Not watered plots experienced ambient rainfall.

Each experimental plot contained one or more individuals of five plant species common in the study site: *Erigeron speciosus*, *Helianthella quinquenervis*, *Potentilla pulcherrima*, *Heliomeris multiflora*, and *Lupinus bakeri*. Before and after the manipulation, herbivore damage was estimated on ten leaves of each of the focal species in each plot to account for possible effects of treatment on herbivory and for the effects of induced resistance to herbivory. Before the manipulation, percent cover of common plant species in the plots, rock, and bare ground was measured.

The heating and water treatments were conducted from June 28th, 2021, to August 6th, 2021. During this period, soil moisture was measured in the plots twice a week. Hobo temperature loggers were deployed in the plots to monitor the effect of the heating treatments.

Multi-choice feeding tests were conducted with the generalist insect herbivore *Melanoplus borealis* to assess plant palatability and herbivore plant species preference under the different treatments. Female *Melanoplus borealis* individuals were collected in the same meadows where the experimental plots were placed. The insects were starved for approximately 12 hours before

the start of feeding trails. Leaves from the focal species were collected from the experimental plots for use in the trials. Three leaves with minimal herbivore damage were collected from a single flowering plant of each species in each plot. At the time of collection, for the collection plant, percent herbivory was estimated and distance from the center of the plot was recorded.

Herbivore feeding trials were conducted in 21.5cm x 21.5cm plastic clamshell boxes. Leaf circles were cut out using a hollow metal punch tool and placed on a damp paper towel. One grasshopper was placed in each box and allowed to feed. Trials were stopped when $\frac{1}{4}$ of the total leaf area in the box was eaten. Boxes were photographed and the leaf area eaten of each leaf circle was calculated in ImageJ.

To test the effect of drought and temperature on leaf palatability, in single-species trials herbivores were given a choice between leaves of a single species experiencing the four different treatments. Three $\frac{3}{8}$ -inch diameter leaf circles were offered per treatment from leaves originating in plots in a single block. One trial was conducted per plant species per block for the five focal species, for a total of forty trials.

To test the effects of treatments on herbivore plant species preference, in multi-species trials herbivores were given a choice between leaves of different species experiencing the same treatment. Six $\frac{1}{4}$ inch diameter leaf circles were offered per plant species from leaves originating from a single plot. One trial was conducted per plot for a total of 32 trials.

Data were analyzed in R version 1.3.1093 (R Core Team 2020). Linear models were used to analyze how treatments influenced soil moisture and temperature. Linear models were used to test whether percent eaten varied by warming or watering for each species in single species trials. An analysis of variance and Tukey HSD test was used to compare herbivory between species in multiple species trials.

Results

The climate manipulations affected soil moisture and temperature in the plots. Watered plots were significantly wetter than plots that were not watered ($t=8.766$, $p<0.001$, Figure 2). Plots that were both watered and heated had higher soil moisture than those that were only watered ($t=7.374$, $p<0.001$, Figure 2). Plots that were only heated had a lower average soil moisture than control plots ($t=7.374$, $p<0.001$ Figure 2). The average air temperature was higher in plots heated with open-topped chambers ($t = 7.031$, $P < 0.001$, Figure 3). There was no effect of watering on temperature.

In single species trials, the amount of *Potentilla pulcherrima*, *Lupinus bakeri*, *Heliomeris multiflora*, and *Erigeron speciosus* eaten was not significantly affected by warming. For *Helianthella quinquenervis*, fewer leaves from warmed plots were eaten than leaves from ambient temperature plots ($t = -2.24$, $P = 0.03$, Figure 4). Watering did not affect the amount of *Helianthella quinquenervis*, *Heliomeris multiflora*, *Lupinus bakeri*, or *Potentilla pulcherrima* eaten. Less *Heliomeris multiflora* from watered plots was eaten than *Heliomeris multiflora* from ambient rainfall plots ($t= -2.49$, $P = 0.02$, Figure 4).

In multiple species trials, there was no significant difference in the amount of each plant species eaten in trials with leaves originating from control plots and heated + watered plots. In trials with leaves originating from heated only plots, significantly more *Helianthella quinquenervis* was eaten than *Lupinus bakeri* (ANOVA, $P = 0.011$, Figure 5), and *Potentilla pulcherrima* (ANOVA, $P < 0.001$, Figure 5). Significantly more *Heliomeris multiflora* was also eaten than *Potentilla pulcherrima* (ANOVA, $P = 0.02$, Figure 5). In trials with leaves originating from watered only plots, significantly more *Erigeron speciosus* was eaten than *Lupinus bakeri* (P

= 0.01, Figure 4), and *Potentilla pulcherrima* (ANOVA, $P = 0.001$, Figure 5). Significantly more *Helioeris multiflora* was eaten than *Potentilla pulcherrima* (ANOVA, $P = 0.03$, Figure 5).

Discussion

Temperatures are predicted to increase in Colorado in future decades. In the single species trials, the responses of plant species to increased temperature varied. For *Erigeron speciosus*, *Potentilla pulcherrima*, *Helioeris multiflora*, and *Lupinus bakeri*, heating had no effect on amount eaten, suggesting that herbivory on these plants will not be affected by warming temperatures. However, heating resulted in less consumption of warmed *Helianthella quinquenervis*. Heat has been shown to affect plant chemistry (Kuczyk et al., 2021). Changes in plant chemistry induced by heat may have reduced palatability to herbivores of *Helianthella quinquenervis* leaves. In some studies, herbivores that feed on plants grown at higher temperatures have demonstrated decreased performance and survival, indicating that heat may reduce plant nutritional quality (Kuczyk et al., 2021); (Po-An et al., 2020). If heat reduced the nutritional value of *Helianthella quinquenervis* leaves, this may have made the leaves less attractive to *Melanoplus borealis*. Another possible reason for decreased herbivory on heated *Helianthella quinquenervis* leaves is increased herbivory defense. Plants grown at higher temperatures have been shown to have increased glucosinolate concentrations, making them more defended against herbivores (Kuczyk et al., 2021). *Helianthella quinquenervis*, though unlikely to use glucosinolates in defense, may have seen comparable changes in its defensive chemistry. If heated *Helianthella quinquenervis* plants manufacture more defensive chemicals, their consumption by insect herbivores may decrease in future climate scenarios in the presence

of less defended alternatives. In addition, these findings suggest that herbivores feeding on *Helianthella quinquenervis* may be especially vulnerable to plant mediated effects of climate change.

Predictions for future precipitation trends in Colorado present uncertainty, with either an increase or a decrease in precipitation possible. In this study, watering resulted in higher soil moisture, suggesting that future precipitation increases may heighten soil water availability for plants. In single species trials, watering did not affect herbivory of leaves of *Helianthella quinquenervis*, *Erigeron speciosus*, *Potentilla pulcherrima*, or *Lupinus bakeri*. At the beginning of summer 2021, much of Colorado, including the study site, was in a drought. Past studies have demonstrated lower growth rates and lower leaf nitrogen content in plants exposed to drought conditions (Rosbakh et al., 2017). Drought has also been seen to increase plant palatability to herbivores (Hamann et al., 2021). However, a possible explanation for our finding of no effect of water on herbivory of *Helianthella quinquenervis*, *Erigeron speciosus*, *Potentilla pulcherrima*, and *Lupinus bakeri* is that, despite the early summer conditions, plants in the ambient rainfall treatment did not experience water stress. During the field manipulation, in July 2021, 0.62 inches more of rain than average fell in Gunnison County, Colorado (NOAA). This rain eased drought conditions. Thus, the watering treatment may have provided water exceeding that which was required for plant growth and function and may have had little impact on *Helianthella quinquenervis*, *Erigeron speciosus*, *Potentilla pulcherrima*, and *Lupinus bakeri* plants. It is also possible that, in these four species, water stress did occur but did not induce changes in the plant that affect herbivory. If so, if precipitation decreases in the future, herbivore-plant interactions in these species are likely to remain stable barring other changes in pressures on herbivores.

While watering did not affect the amount of *Helianthella quinquenervis*, *Heliomeris multiflora*, *Lupinus bakeri* or *Potentilla pulcherrima* eaten, more *Heliomeris multiflora* from ambient precipitation plots was eaten than from watered plots. Past studies have suggested that herbivores feeding on plants exposed to drought had increased performance, possibly indicating increased plant nutritional value of the plants (Kuczyk et al., 2021). It has also been shown that drought stress often leads to increased foliar herbivory (Hamann et al., 2021). Plants in ambient precipitation plots may have experienced more water stress than plants in watered plots. Despite frequent rainfall during the summer, the study site experienced the effects of a low snowpack and drought conditions (NOAA). If water stress occurred, ambient precipitation *Heliomeris multiflora* may have been unable to maintain strong herbivory defenses. In addition, it is possible that excess water provided watered *Heliomeris multiflora* with an opportunity to increase investment in herbivory defense. Past research supports this idea, as excess watering has been shown to allow increased productivity even when the water is more than is normally required (Post et al., 2021). Thus, *Heliomeris multiflora* leaves without water addition may have been more palatable to herbivores thanks to a reduction in defenses compared to watered leaves. Since an effect of water on herbivory was seen only in *Heliomeris multiflora*, if precipitation increases in future years, *Heliomeris multiflora* could experience a decrease in herbivory compared to other plants, and herbivores that rely on *Heliomeris multiflora* could be affected by changes in the plant.

In multiple species trials, I examined the effects of watering on herbivore preference between species. Under control conditions, all plant species were eaten the same amount. However, when watered at ambient temperature, the herbivore expressed a preference for *Erigeron speciosus* over *Helianthella quinquenervis*, *Potentilla pulcherrima*, and *Lupinus bakeri*.

A preference was also expressed for *Heliomeris multiflora* over *Potentilla pulcherrima*. These results suggest that herbivory patterns on these five common species may shift if rainfall increases in the future. Increased herbivory on *Erigeron speciosus* and *Heliomeris multiflora* are possible with increased rainfall. As herbivory has the potential to affect plant abundance (Maron et al., 2006), a decrease in *Erigeron speciosus* and *Heliomeris multiflora* abundance is possible in wetter conditions. Herbivory also has the potential to affect plant distribution (Fine et al., 2004); (Louda et. al., 1982). With increased herbivory, the distribution of *Erigeron speciosus* and *Heliomeris multiflora* could shift, altering plant community composition.

Heating alone also influenced herbivore preferences. When plants were heated only, the herbivores expressed a preference for *Helianthella quinquenervis* over *Lupinus bakeri* and *Potentilla pulcherrima*. Herbivores also showed a preference for *Heliomeris multiflora* over *Potentilla pulcherrima*. These results suggest that under future climate warming, *Helianthella quinquenervis* and *Heliomeris multiflora* may experience increased herbivory. Herbivores have important effects on plant survival (Maron et al., 2006), and thus influence the plant species distribution (Fine et al., 2004); (Louda et. al., 1982), and plant community composition (Olff et al., 1998). Through new preferences, herbivory could shift abundance, distribution, and plant community composition in alpine meadows.

Lupinus bakeri and *Potentilla pulcherrima* were the least preferred plants in both watered only and heated only treatments, while in the control condition treatment, the herbivore expressed no preference. This change in preference for *Lupinus bakeri* and *Potentilla pulcherrima* suggests that these plants may be more defended against herbivores under heated only conditions and watered only conditions or may be less nutritionally valuable. If an herbivore relies heavily on one of these plants, a decline in survival of this herbivore is possible

if the plant is less palatable and the herbivore is unable to shift consumption to another species. A decline in an insect herbivore could have wide ranging effects on the plant community. The loss of an herbivore competitor may benefit other herbivore species, leading to increases in their abundance. This has been seen in past studies where drought increased the survival of some herbivores due to reduced abundance of competing herbivores (Po-An et al., 2020). Loss of an herbivore specialist could also lead to increasing abundance of the plant it feeds on. Herbivory has been shown to create competitive asymmetry between plants, impacting competitive interactions between plants, and reshaping plant communities (Borgstrom et al., 2016). However, resilience of herbivores to the effects of heating or water availability on plants is also possible. Studies have indicated that in some cases herbivores can use host plants of variable quality without a decrease in performance (Po-An et al., 2020).

The results of multi-species trials suggest that the distribution of herbivory pressure depends on the exact configuration of future climate conditions. Warming is predicted in Colorado, suggesting increased herbivory on *Helianthella quinquenervis* and *Heliomeris multiflora* and decreased herbivory on *Lupinus bakeri* and *Potentilla pulcherrima*. However, our study shows that increased precipitation may have a mitigating effect on these changes. Despite the effects of watering alone and heating alone on herbivore preference, in treatments with simultaneous watering and heating herbivores showed no preference between plant species, mirroring control conditions. Thus, it seems that water availability may mitigate the effect of heating on these plants. Past studies have seen this buffering effect. A study found that heating in conjunction with drought resulted in vegetation browning (De Boeck et al., 2015). However, heating had no impact on productivity when water was also available. It is likely that water stress

made it harder for plants to endure high temperatures since transpiration, which reduces heat stress, was reduced (De Boeck et al., 2015).

Our results suggest that herbivore plant preferences may shift with climate change. In future climate conditions, changes in herbivore preferences may reduce the abundance of some plants while increasing the abundance of others, likely causing many cascading effects on alpine ecosystems. However, if increased precipitation occurs in tandem with increased temperatures, changes in herbivore preference are less likely. In a scenario possible in many locations across the world, a combination of drought and heating could lead to impacts on plant-herbivore interactions that have the potential to reshape plant communities.

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Figure 1. Map of the field site. Plots containing the four treatments are placed in blocks of four. Treatments are watered at ambient temperature (NA), not watered and heated (NH), watered at ambient temperature (WA) and watered and heated (WH).

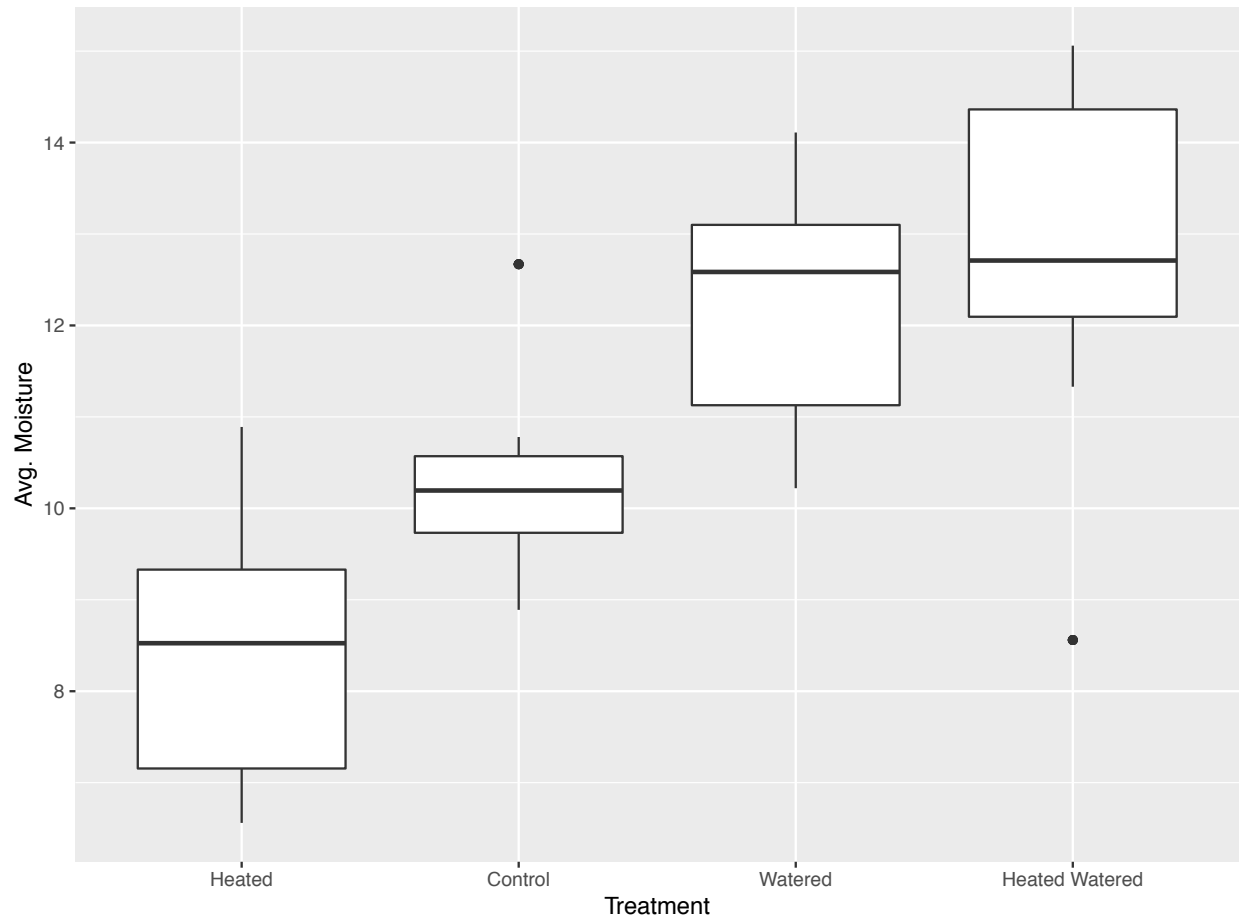


Figure 2. Average soil moisture in plots by treatment. Soil moisture was measured twice a week between 7/13/21 and 8/2/21 in experimental plots.

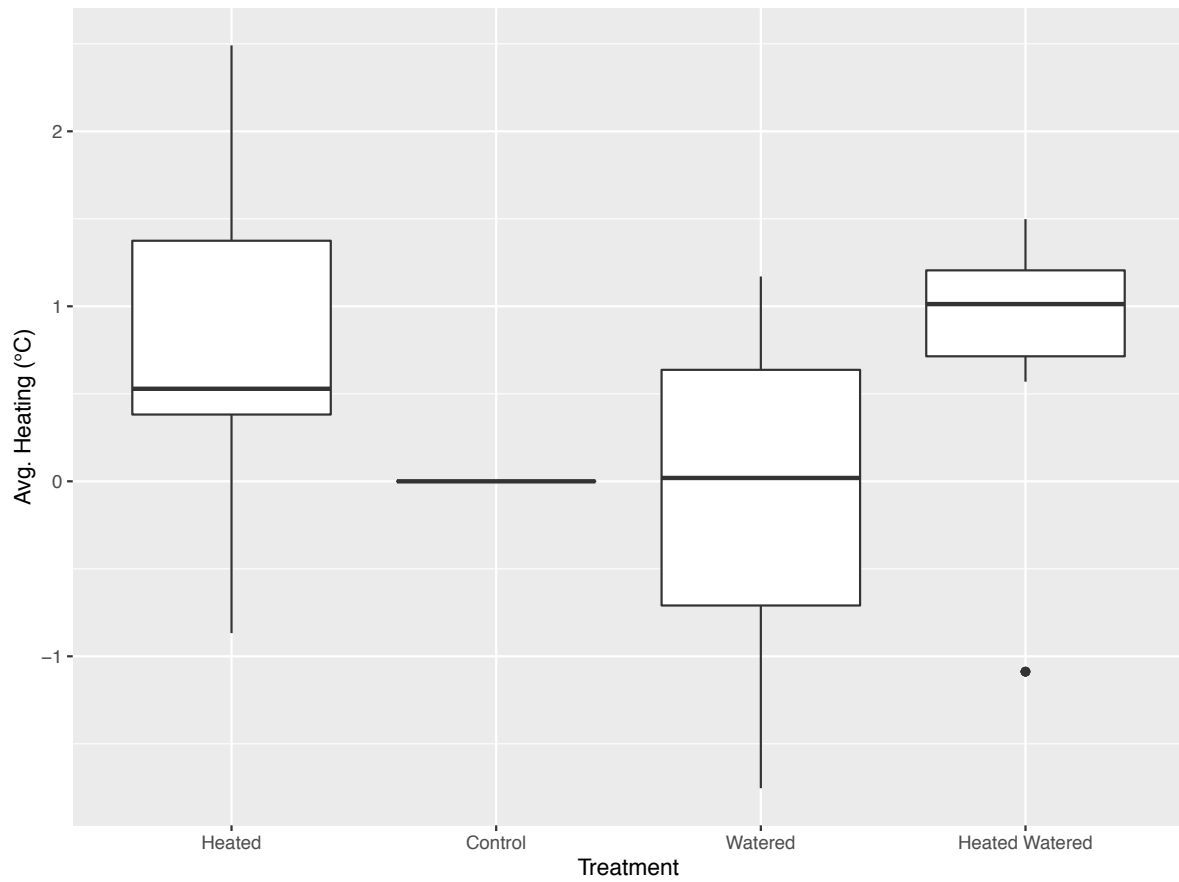


Figure 3. Average difference by treatment in temperature from the temperature of the control plot in the same block. Temperature was monitored in plots with hobo data loggers.

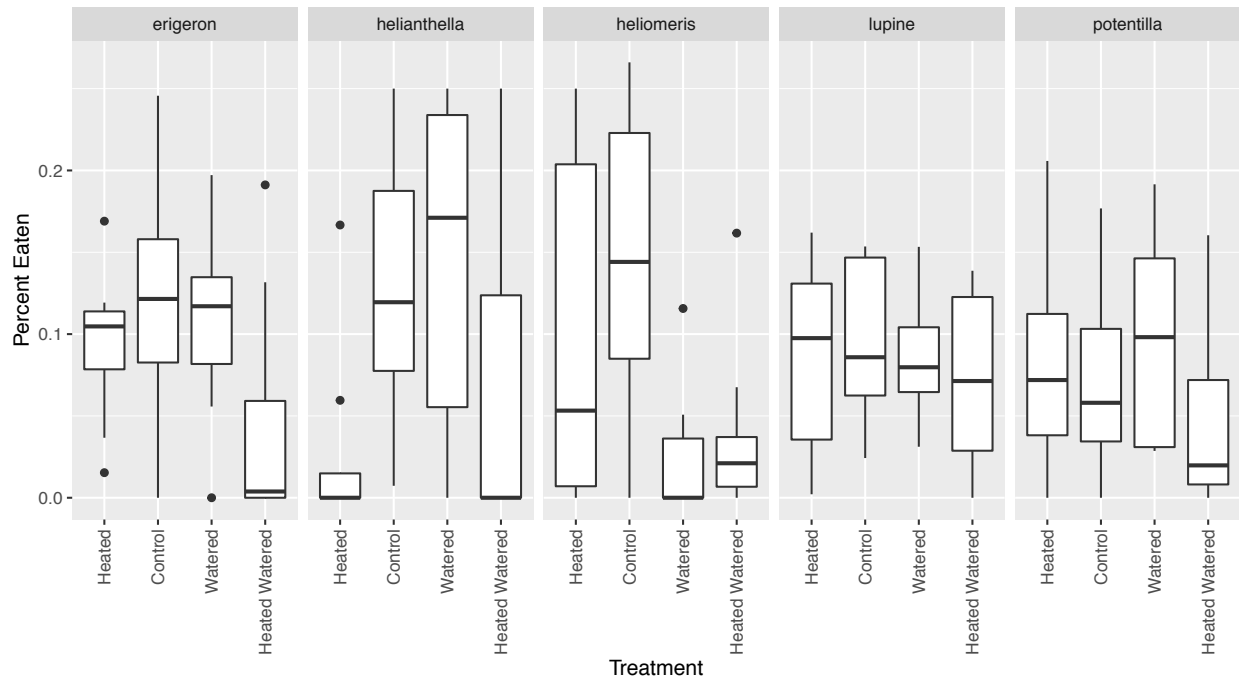


Figure 4. Percent of total leaf area in a trial consumed of leaves of the same species exposed to different treatments. Each panel shows data from boxes containing only leaves from one species of plant.

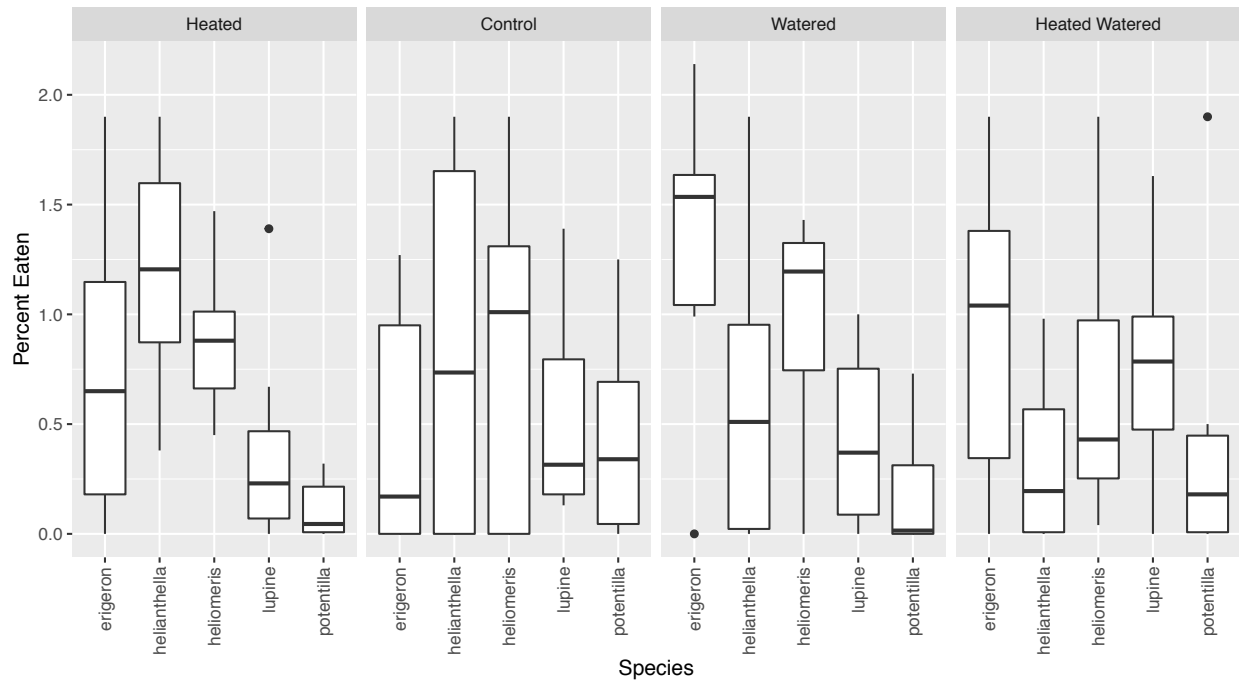


Figure 5. Percent of total leaf area in a trial consumed of leaves of different plant species under the same treatment conditions. Each panel shows data from boxes containing leaves of different species experiencing the same treatment.