

RMBL Research Proposal

Effects of high temperature and early snowmelt on floral morphology and volatile organic compounds in *Ipomopsis aggregata*

Student: David Hopp

Mentor: Diane Campbell

Summer 2021

Abstract

Climate change is causing warmer temperatures and earlier snowmelt throughout the mountains of Western North America. This study was designed to investigate how these two factors of temperature and snowmelt date influence floral traits, using *Ipomopsis aggregata* as a model species. While phenological shifts caused by temperature and snowmelt date have been studied in depth, impacts on physical traits such as floral morphology and emitted volatile organic compounds (VOCs) are less well known. In this research, both floral morphology and VOC identity and quantity were measured over one summer to determine if manipulated temperature and date of snowmelt have any effect on these characteristics. *I. aggregata* individuals treated with higher temperature developed longer corollas and produced more nectar and sugar. Volatile emissions were not strongly impacted by either higher temperature or early snowmelt.

Introduction

With the ever-present threat of climate change, understanding how plants will respond to this rapid change is critical if the natural world is to be protected. Increased average global temperatures is one of the most obvious symptoms of climate change (Pachauri et al., 2014), and along with these increased temperatures and reduced winter precipitation comes an earlier initial date of snowmelt every year in the Rocky Mountains of Colorado (Wadgymar et al., 2018). Earlier date of snowmelt is known to impact the phenology of plants in montane environments, usually pushing forward the date of flowering for many species of wildflower. This early date of flowering often corresponds to fewer flowers in the middle of summer, a challenge for pollinator species who historically have relied on flower nectar as a food source all summer long (Aldridge et al., 2011). Understanding how floral traits are influenced by climate change could give researchers a better view of how pollinator attraction is influenced by climate change. Researching this attraction is key to predicting impact on fitness for both pollinator species who rely on nectar production as a food source and for plants that rely on pollination for seed production and therefore successful reproduction.

As sessile organisms, it is important for plant species to find ways to interact with their environment without the use of movement. One evolved method of interaction comes from chemical communication through the emission of volatile organic compounds (VOCs) detectable to animals by olfaction. VOCs are compounds characterized by their high vapor pressures and low molecular weights. These chemical signals are synthesized in various processes of plant metabolism and are released from all parts of the plant. These volatiles often are multi-purpose

and can function in both attraction of pollinator species as well as a defense mechanism, deterring antagonistic species such as seed predators from laying eggs on the plant. Common classes of VOCs include terpenoids, phenylpropanoids/benzenoids, fatty acid derivatives, and amino acid derivatives (Farré-Armengol et al., 2013). For some pollinator species, such as honeybees and bumblebees, the process of learning and retaining odors of flowers is quicker than learning visual cues such as color (Farré-Armengol et al., 2013). A common example of a VOC is α -pinene, a monoterpene ($C_{10}H_{16}$) that humans often associate with the smell of pine trees. Previous studies done on the VOCs of *Ipomopsis aggregata* (Polemoniaceae) have shown that α -pinene is one of the primary floral VOCs emitted (Campbell et al., 2019). Interestingly, the quantity of VOCs emitted by *I. aggregata* can depend on abiotic conditions such as water availability (Campbell et al., 2019). Abiotic dependency of volatile emission has been documented in other plant species as well, although such studies are still rare, and none have investigated the impact of snowpack or snowmelt date. One study done on subarctic plants in Sweden determined that the quantity of VOCs released by whole plants increased with temperature (Valolahti et al., 2015) and it is possible that flowers of *I. aggregata* will show this same temperature dependence. The quantity of these volatiles may influence the attraction of pollinators as well as the deterrence of the seed predator *Delia* sp. (Diptera: Anthomyiidae), whose larvae consume the developing offspring of *I. aggregata* (Campbell et al., 2002).

I. aggregata depends on hummingbird pollination, primarily from the residential *Selasphorus platycercys* and the mid-summer visitor *S. rufus* in the Elk Mountains of Colorado, and it seems that long corollas, color intensity, and high nectar production are three important factors in hummingbird flower choice, as flowers with these traits have been found to be preferentially visited by hummingbirds (Campbell et al., 1997). Wide corollas and wide sepals are also associated with oviposition by the seed predator *Delia* (Campbell et al. 2002). Physical characteristics such as these are also thought to be dependent on abiotic conditions in many plant species (Gallagher & Campbell, 2017, Kuppler et al. 2021). Nectar production in some plants, including *I. aggregata*, is heavily dependent on water availability (Mueller et al., 2020), and therefore it is possible that the date of snowmelt and temperature will have an influence on the ability of *I. aggregata* to express the characteristics that attract hummingbird pollinators.

In this study, the goal was to find how early snowmelt and high temperature influence nectar production, corolla length and width, color intensity of the flower, VOC output, as well as oviposition by seed predators. Increased temperature can influence physiochemical properties of the VOCs themselves such as volatility, solubility, and diffusivity. Generally, increased temperature increases volatility (increased vapor pressure) and diffusivity, therefore reducing the resistance in the emission pathway of the VOCs. Second, the increased temperature can affect plant processes such as the biosynthesis or release of the volatiles. Increased temperature has been shown to increase the activity of enzymes involved in the biosynthesis of VOCs, therefore the cause of the increase in VOC emission is likely a combination of physiochemical changes as well as physiological (Farré-Armengol et al., 2014). It is likely the *Delia* fly seed predators will preferentially target larger flowers, potentially meaning that healthier flowers likely to make

more seeds will be targeted (Brody, 1992; Juenger et al., 2005). Some questions that were considered are: (1) what is the effect of early snowmelt and higher than average temperatures on floral traits? (2) How would *Delia* flies respond to higher temperatures and early snowmelt; will there be a change in *Delia* fly oviposition choice? I hypothesized that there would be an effect of temperature and snow melt on all traits being measured. I predicted that nectar production would decrease from both increased temperature and early snowmelt due to low moisture associated with both factors. The manipulated conditions would also lead to smaller flowers, possibly due to reduced soil moisture content (Kuppler et al., 2021). The color of the flowers was predicted to be less vibrant. I predicted that the quantity of the VOCs emitted will increase overall, especially of terpene compounds. I also predicted that *Delia* flies would prefer to visit plants not being treated with higher temperature and early snowmelt.

Methods

Study System

The field site is in Maxfield Meadow and is about 1 km south of Rocky Mountain Biological Laboratory (RMBL) in Gothic, CO at the coordinates 38.9495°N, 106.9908°W (figure 1). In this area, there are six plots of which three were covered late in the winter with a black shade cloth effectively melting the snow in those plots 1 to 2 weeks earlier than the other three control plots. These snowmelt manipulations have been carried out for the past four springs. In each of these plots, there are two subplots that contain various amounts of *I. aggregata*. In each subplot approximately four individuals were studied: half of the individuals functioned as a control and were left exposed to the open air. The other half of the individuals received ambient temperature manipulation using open top chambers (OTCs). These OTCs are made of translucent plastic panels and they function as miniature greenhouses heating the air to a higher average temperature than the outside ambient temperature.

59 individuals were split between the various treatments: a total of 30 plants were contained within OTCs while the other 29 plants functioned as controls. Of the 30 plants within OTCs, 14 were in plots treated with early snowmelt and 16 were in plots of normal snowmelt. Of the 29 plants controlled for temperature, 16 received early snowmelt treatment and 13 were in plots of normal snowmelt (table 1).

To ensure test subjects received higher than average temperature, HOBO temperature loggers were placed inside of each OTC as well as in control areas, and temperature was measured throughout the summer. To test if the OTCs or the date of snowmelt impacted soil moisture, volumetric water content of the soil was periodically sampled in each of the subplots as well as next to each plant sampled for volatiles at time of sampling.

Trait Measurements

A total of 59 individuals were tested weekly for the following traits: corolla length, corolla width, and number of *Delia* fly eggs under sepals. In addition, 48-hr nectar production, nectar concentration, and corolla reflectance were measured twice over the summer and identity and quantity of VOCs were measured four times throughout the summer.

Corolla length and width were measured using calipers; measurements of corolla length were from the opening of the corolla down to the base of the calyx and corolla width was measured across the opening of the corolla. These traits were measured on about 3 flowers per plant throughout the summer, then averaged for the plant prior to analysis.

Nectar production and concentration were captured using 32mm long, 5 μ L microcapillary tubes and measured using a refractometer that allows determination of the sugar concentration in the nectar. Measurement of nectar volume was achieved by first measuring mm of the 5 μ L microcapillary tubes filled up by the nectar produced. This number was then multiplied by 5/32 (volume of each microcapillary tube divided by length of tubes) to convert measurements into volume of nectar produced. To get a true measurement of 48-hr nectar production, access to the desired flowers was blocked with a bent piece of drinking straw for pollinators who would otherwise feed from the flower. In addition to nectar production and concentration, 48-hour sugar production was also calculated by multiplying nectar production and nectar concentration.

Color spectra were measured using a reflectance spectrometer measuring the reflectance at each wavelength between 400 to 700 nm. Flowers of *I. aggregata* are typically red to humans and do not reflect in the ultraviolet (Meléndez-Ackerman, 1997). Each flower analyzed was given a “redness” value calculated by subtracting green reflectance from red reflectance and dividing that value by total reflectance. Green reflectance was defined as the reflectance of wavelengths 476-550 nm. Red reflectance was defined as the reflectance of wavelengths 626-700 nm. Total reflectance was defined as the reflectance of wavelengths 401-750 nm.

$$\text{Redness} = \frac{R_{626-700} - G_{476-550}}{T_{401-750}}$$

VOCs were collected using dynamic head space methods as described in Campbell et al., 2019. Microcapillary tubes filled with Tenax TA (a large polymer commonly used to capture volatile compounds) capped with glass wool were used as scent traps to capture the VOCs from *I. aggregata* flowers. Individual flowers were bagged using modified plastic Reynolds oven bags for 30 minutes to allow the build-up and equilibration of emitted volatiles. Scent traps were connected to Supelco PAS-500 pumps and fitted through small holes cut in the bags during the 30 minutes equilibration period. After the flower was bagged for 30 minutes, the PAS-500 pumps fitted with scent traps were started and run for 15 minutes. Scent traps were handled only with gloved hands and pieces of aluminum foil as an effort to reduce the contaminants captured by the scent traps from the sampler’s hands. Only floral volatiles were of concern in this study, and therefore only single flowers were bagged in each sample to ensure we are testing floral volatiles rather than volatiles from other structural sections of the plant. Ambient air was sampled for volatiles on days and at locations where floral volatiles were being sampled to

ensure that all VOCs come from our species of interest. For analysis, we used only the floral compounds identified from *I. aggregata* by Campbell et al. (2019). Floral compounds found to be dependent on drought by Campbell et al. (2019) (α -pinene, caryophyllene, α -farnesene, β -ocimene, and benzyl alcohol) were analyzed individually to see if similar dependency might be seen with high temperature and early snowmelt. 1,3-octadiene is a compound that is dependent on drought as found by Campbell et al. (2019), however, this compound was not detected in any samples taken in our data collection and therefore is not included in data analysis. Volatile samples were analyzed using thermal desorption GC-MS. At RMBL, where this study took place, there is a Markes Ultra autoloading system connected to a Markes Unity-xr that loads volatiles into a Shimadzu GC-MS QP2020. The temperature ramp program and column were the same as in Campbell et al. (2019). Peak areas were converted to ng emitted per hour per flower using calibration from known standards. Standards were matched to sampled volatiles based on class of compound.

Seed predator fly eggs are found underneath the sepals of the *I. aggregata* flowers. Weekly, every sepal on every individual was peeled back and all eggs were counted.

Data Analysis

Question 1 about the impacts of the treatments on floral traits was addressed using a mixed model ANOVA. The model includes the fixed factors of snowmelt (early vs normal), temperature (OTC vs control), and the snowmelt x temperature interaction. It also included random effects of plot and subplot, and was implemented using the function ‘lmer’ in R (Bates et al., 2014). For length and width of corollas, values were first averaged when there were multiple measurements taken per plant. Since volatiles include many compounds, we used total volatile emissions as the response variable. In addition, we used a canonical analysis of principal coordinates (CAP) to determine the combination of all volatiles that best separates the four treatments (Oksanen et al., 2020). This same CAP analysis was also conducted comparing individuals who received both treatments to individuals who received neither treatment. Question 2 about the impact of high temperature and early snowmelt on *Delia* fly oviposition was addressed using a generalized mixed model ANOVA. This model also includes the fixed factors of snowmelt (early vs normal), temperature (OTC vs control), and the snowmelt x temperature interaction. It also included random effects of plot and subplot, and was implemented using the function ‘glmmTMB’ in R (Brooks et al., 2017).

Results

The OTCs did have an impact on the temperature, depending on the time of day. The average increase in daily temperature was 6.9 ± 1.9 °C (mean $\pm$ SD). The temperature at night was unaffected by the OTCs (figure 2, figure 3).

In addition to higher temperature, the OTCs dried out the soil by 1.0 – 4.5% (VWC) depending on the week measurements were taken (figure 4).

Corollas were longer in plants that received higher temperature treatments ($p=0.0221$ for temperature treatment in mixed model ANOVA). Corolla length did not depend significantly on snowmelt ($p=0.7484$ for early snow treatment in ANOVA) and the effect of temperature did not vary with different snowmelt treatments ($p=0.5651$ for the interaction of these two factors). The average corolla length for individuals who were unmanipulated in both factors (control temperature, normal snowmelt) was 25.3 ± 0.638 mm (mean $\pm$ SE). Average corolla length for individuals subjected to both temperature and snowmelt treatment (OTC, early snowmelt) was 26.8 ± 0.510 mm, 5.93% longer than unmanipulated individuals (figure 5).

Corolla width was not found to be significantly different between individuals treated with higher temperature or early snowmelt and the effect of temperature did not vary with different snowmelt treatments (all p values >0.05 ; figure 6).

48-hour nectar production was significantly higher in plants that received higher temperature treatment ($p=0.0104$ for temperature treatment in ANOVA). Nectar production was not significantly different in individuals treated with early snowmelt ($p=0.3961$ for early snowmelt treatment in ANOVA), and the effect of temperature did not vary with different snowmelt treatments ($p=0.7609$). The average volume of nectar produced in 48 hours by unmanipulated individuals was 3.59 ± 0.619 μ L. Individuals who received both treatments (OTC, early snowmelt) had an average nectar production of 5.78 ± 1.03 μ L, 61.45% higher than individuals who received no treatment (figure 7).

Nectar concentration was not found to be significantly different between individuals treated with higher temperature or early snowmelt and the effect of temperature did not vary with different snowmelt treatments (all p values >0.05 ; figure 8).

48-hour sugar production was significantly higher in individuals treated with higher temperatures ($p=0.0376$). There was no significant impact on sugar production from early snowmelt treatment ($p=0.4552$) and the effect of temperature did not vary with different snowmelt treatments ($p=0.1608$). The average 48-hour sugar production of unmanipulated individuals was 1.18 ± 0.139 mg. The average 48-hour sugar production of individuals who received both treatments (OTC, early snowmelt) was 1.63 ± 0.236 mg, 38.13% higher than individuals who received no treatments (figure 9).

Flower color was not found to be significantly different between individuals treated with higher temperature or early snowmelt and the effect of temperature did not vary with different snowmelt treatments (all p values >0.05 ; figure 10, figure 11).

Based on the multivariate CAP analysis, overall volatile organic compound emissions were not significantly impacted by either treatment of early snowmelt or higher temperature (p values >0.05). (figure 13. In addition, the CAP analysis done comparing only individuals who received

both treatments with individuals who received neither treatment showed no difference in volatile emissions ($p>0.05$).

The sum of total volatiles emitted was not significantly impacted by higher temperature or early snowmelt and the effect of temperature did not vary with different snowmelt treatments (all p values >0.05 ; figure 13, figure 14).

Of the individual floral volatile compounds assessed (α -pinene, α -farnesene, β -ocimene, benzyl alcohol, and caryophyllene) none of them were significantly impacted by the temperature or the early snowmelt treatments. However, α -farnesene was only found in samples taken from plants treated with high temperature (figure 15).

The average number of eggs per flower was significantly lower on plants treated with higher temperature compared to untreated individuals ($p=0.002$). There was no effect seen from early snowmelt, and the effect of temperature did not vary with different snowmelt treatments (figure 16).

Discussion

Corolla Length

An experimentally imposed increase in average temperature caused *Ipomopsis aggregata* to develop longer corolla tubes, a quality correlated with higher visitation rates from hummingbird pollinators (Campbell et al., 1991). This finding is consistent with research done on a related species, *Ipomopsis longiflora*, that found corolla length to be highly impacted by temperature. This particular study treated *I. longiflora* with three different temperatures (20°C, 30°C, and 35°C) and found that of the individuals tested, those that were treated with the 30°C treatment produced the longest corollas (Villarreal & Freeman, 1990). It was predicted that corollas of individuals in control areas would have longer corollas than those within the OTCs because the higher temperature would dry the soil, and dryer soil is associated with smaller flowers (Armando G. Villarreal & C. Edward Freeman, 1990; Campbell & Wendlandt, 2013; Kuppler et al., 2021). Though the OTCs did have dryer soil within, the positive effect of temperature seemed to eclipse the effect of the lower soil water. Likely, if the soil moisture within the OTCs had been equal to soil moisture outside the difference in corolla lengths between the temperature treated individuals and the control would have been even larger. The reason for longer corolla development in higher temperatures is unknown.

Nectar Production

Individuals treated with higher-than-average temperature had higher volumes of nectar production than individuals not treated for temperature. Though it was hypothesized that nectar production would decrease because of higher temperature, this finding is consistent with other findings that show *Ipomopsis aggregata* and other flower species have an increase in nectar production with higher temperatures. A study done by John M. Pleasants (1983) found that the

weather had a significant impact on nectar production of *I. aggregata* and that on overcast days, nectar production was significantly lower than on sunny days. Pleasants suggests that this difference in nectar production is likely the result of either temperature difference, or difference in sunlight exposure. From the results of our study, we can conclude that temperature alone is a big enough factor to generate the difference in nectar production observed by Pleasants because individuals inside OTCs and outside received the same levels of sunlight, or possibly individuals outside OTCs received more. James H. Brown & Astrid Kodric-Brown (1979) also found that nectar production of *I. aggregata* was dependent on the time of day with peak nectar production happening near the middle of the day in the time period 1500-1700, generally considered one of the hottest parts of the day. The reason for this temperature dependency is not known. However, as poikilotherms the internal temperature of plants is completely reliant on environmental temperatures, which means biochemical pathways, such as the generation of sucrose and other nectar components, are happening at ambient temperature. Rates of chemical reactions are generally faster with higher temperatures, and biochemical reactions are no exception to this. However, for biochemical processes to have higher rates because of high temperature, the temperature must not exceed a level at which enzymes are permanently deactivated (denatured) (Daniel et al., 2008). The temperatures *I. aggregata* individuals were subjected to in this experiment therefore must not have been high enough to denature enzymes required for nectar production.

Finding that higher temperatures increase nectar production is surprising for multiple reasons. First, the link between temperature and photosynthesis. Like other C3 plants, *I. aggregata* is limited by the activity of the enzyme Rubisco which fixes CO₂ or O₂ to RuBP in the first step of the Calvin Cycle. In model C3 organisms (tobacco and cotton), Rubisco activation began to decrease above 30°C, and net photosynthetic rates began to decrease above 28°C (Crafts-Brandner & Salvucci, 2000). We know that temperatures inside OTCs consistently got above 30°C, especially from the time-period of 1000-1600 and that photosynthetic rate in *I. aggregata* ceases to rise with temperature by 25°C and levels off, if not declines, above that point (Wu & Campbell, 2006). It seems counterintuitive that nectar production, a process that requires sugar, would increase at temperatures when photosynthetic rate is potentially less efficient. In addition, the increase in nectar production with temperature may affect feeding by the hummingbird pollinators *Selasphorus platycercys* and *S. rufus*. The dependency on temperature that *I. aggregata* shows for nectar production means that nighttime has the lowest rate of nectar production (James H. Brown & Astrid Kodric-Brown, 1979). Cold periods, such as mornings, are the most important feeding time for hummingbirds, because necessary thermoregulation drives up the cost of metabolism (Suarez & Gass, 2002). Since *I. aggregata* has very low rates of nectar production at night, it is reasonable to assume that cold mornings, the most critical feeding time of *I. aggregata*'s main pollinators, would be the time when *I. aggregata* has the lowest relative amount of nectar stored in its corollas.

Volatile Compound Emission

Overall VOC emissions were not impacted by high temperature treatment or early snowmelt.

Delia Fly Oviposition

Fly egg oviposition was significantly lower on individuals within OTCs. It was thought that the *Delia* flies would have similar preferences as pollinators such as long corollas, however the individuals who had longer corollas in the OTCs had fewer eggs. Apparency of *I. aggregata* plays a big role in oviposition choice for *Delia* flies (Juenger et al., 2005). It is possible that individuals inside OTCs were obscured from the view of seed predator flies and therefore the easily visible individuals outside of the OTCs were preferentially targeted. It is also a possibility that the flies themselves did not like the higher temperature inside the OTCs and therefore preferred not to enter them. Another potential impact of the results found could be a matter of sampling bias. The OTCs made working with the plants inside more difficult than working with plants outside, especially looking for eggs. Looking for eggs required leaning over and deep into the OTCs, therefore it is possible that the difficulty of looking for eggs in the OTCs caused us to find fewer.

Conclusion

The results of this study show that *Ipomopsis aggregata* floral traits may be positively impacted by the higher temperature caused by climate change, though higher temperature is not the only impact climate change will have and other factors, such as soil water availability, may impact floral traits differently. Higher temperature exposure may correlate with higher visitation rates from hummingbird pollinators, and therefore increasing pollination. In addition, higher temperatures may decrease seed predation from *Delia* flies. The combination of these two impacts would likely result in increased fitness of *I. aggregata*. Future studies could test to see if pollinator visitation rates are impacted by these experimental conditions, and potentially seed predator oviposition could be tested in higher temperature treatments that don't obscure the view of *I. aggregata*.

References

Aldridge, G., Inouye, D. W., Forrest, J. R. K., Barr, W. A., & Miller-Rushing, A. J. (2011).

Emergence of a mid-season period of low floral resources in a montane meadow ecosystem associated with climate change. *Journal of Ecology*, 99(4), 905–913.

<https://doi.org/10.1111/j.1365-2745.2011.01826.x>

- Armando G. Villarreal & C. Edward Freeman. (1990). Effects of Temperature and Water Stress on Some Floral Nectar Characteristics in *Ipomopsis longiflora* (Polemoniaceae) under Controlled Conditions. *Botanical Gazette*, 151(1), 5–9. JSTOR Journals.
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2014). Fitting Linear Mixed-Effects Models using lme4. *ArXiv:1406.5823 [Stat]*. <http://arxiv.org/abs/1406.5823>
- Brody, A. K. (1992). Oviposition choices by a pre-dispersal seed predator (*Hylemya* sp.). *Oecologia*, 91(1), 56–62. <https://doi.org/10.1007/BF00317241>
- Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Machler, M., & Bolker, B. M. (2017). GlmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, 9(2), 400. <https://doi.org/10.3929/ethz-b-000240890>
- Campbell, D. R., Crawford, M., Brody, A. K., & Forbis, T. A. (2002). Resistance to pre-dispersal seed predators in a natural hybrid zone. *Oecologia*, 131(3), 436–443. <https://doi.org/10.1007/s00442-002-0900-1>
- Campbell, D. R., Sosenski, P., & Raguso, R. A. (2019). Phenotypic plasticity of floral volatiles in response to increasing drought stress. *Annals of Botany*, 123(4), 601–610. <https://doi.org/10.1093/aob/mcy193>
- Campbell, D. R., & Wendlandt, C. (2013). Altered precipitation affects plant hybrids differently than their parental species. *American Journal of Botany*, 100(7), 1322–1331. <https://doi.org/10.3732/ajb.1200473>
- Campbell Diane R., Waser Nickolas M., Price Mary V., Lynch Elizabeth A., & Mitchell Randall J. (1991). Components of Phenotypic Selection: Pollen Export and Flower Corolla Width

- in *Ipomopsis aggregata*. *Evolution*, 45(6), 1458–1467. JSTOR Journals.
<https://doi.org/10.2307/2409892>
- Crafts-Brandner, S. J., & Salvucci, M. E. (2000). Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO₂. *Proceedings of the National Academy of Sciences*, 97(24), 13430. <https://doi.org/10.1073/pnas.230451497>
- Daniel, R. M., Danson, M. J., Eisenthal, R., Lee, C. K., & Peterson, M. E. (2008). The effect of temperature on enzyme activity: New insights and their implications. *Extremophiles*, 12(1), 51–59. <https://doi.org/10.1007/s00792-007-0089-7>
- Diane R. Campbell, Nickolas M. Waser, & Elvia J. Melendez-Ackerman. (1997). Analyzing Pollinator-Mediated Selection in a Plant Hybrid Zone: Hummingbird Visitation Patterns on Three Spatial Scales. *The American Naturalist*, 149(2), 295–315. JSTOR Journals.
- Farré-Armengol, G., Filella, I., Llusà, J., Niinemets, Ü., & Peñuelas, J. (2014). Changes in floral bouquets from compound-specific responses to increasing temperatures. *Global Change Biology*, 20(12), 3660–3669. <https://doi.org/10.1111/gcb.12628>
- Farré-Armengol, G., Filella, I., Llusia, J., & Peñuelas, J. (2013). Floral volatile organic compounds: Between attraction and deterrence of visitors under global change. *Perspectives in Plant Ecology, Evolution and Systematics*, 15(1), 56–67.
<https://doi.org/10.1016/j.ppees.2012.12.002>
- Gallagher, M. K., & Campbell, D. R. (2017). Shifts in water availability mediate plant–pollinator interactions. *New Phytologist*, 215(2), 792–802. <https://doi.org/10.1111/nph.14602>
- James H. Brown & Astrid Kodric-Brown. (1979). Convergence, Competition, and Mimicry in a Temperate Community of Hummingbird-Pollinated Flowers. *Ecology*, 60(5), 1022–1035. JSTOR Journals. <https://doi.org/10.2307/1936870>

- Juenger, T., Morton, T. C., Miller, R. E., & Bergelson, J. (2005). Scarlet gilia resistance to insect herbivory: The effects of early season browsing, plant apparency, and phytochemistry on patterns of seed fly attack. *Evolutionary Ecology*, 19(1), 79. Springer Nature Journals.
<https://doi.org/10.1007/s10682-004-8090-4>
- Kuppler, J., Wieland, J., Junker, R. R., & Ayasse, M. (2021). Drought-induced reduction in flower size and abundance correlates with reduced flower visits by bumble bees. *AoB PLANTS*, 13(1). <https://doi.org/10.1093/aobpla/plab001>
- Meléndez-Ackerman, E. J. (1997). Patterns of color and nectar variation across an Ipomopsis (Polemoniaceae) hybrid zone. *American Journal of Botany*, 84(1), 41–47.
<https://doi.org/10.2307/2445881>
- Mueller, A. L., Berger, C. A., Schittenhelm, S., Stever-Schoo, B., & Dauber, J. (2020). Water availability affects nectar sugar production and insect visitation of the cup plant *Silphium perfoliatum* L. (Asteraceae). *Journal of Agronomy and Crop Science*, 206(5), 529–537.
<https://doi.org/10.1111/jac.12406>
- Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., & Wagner, H. (2020). *vegan: Community Ecology Package* (2.5-7) [Computer software].
<https://CRAN.R-project.org/package=vegan>
- Pachauri, R. K., Allen, M. R., Barros, V. R., Broome, J., Cramer, W., Christ, R., Church, J. A., Clarke, L., Dahe, Q., Dasgupta, P., Dubash, N. K., Edenhofer, O., Elgizouli, I., Field, C. B., Forster, P., Friedlingstein, P., Fuglestad, J., Gomez-Echeverri, L., Hallegatte, S., ... van Ypersele, J.-P. (2014). *Climate change 2014: Synthesis report. Contribution of working groups i, ii and iii to the fifth assessment report of the intergovernmental panel*

on climate change (R. K. Pachauri & L. Meyer, Eds.). IPCC.

<https://epic.awi.de/id/eprint/37530/>

Suarez, R. K., & Gass, C. L. (2002). Hummingbird foraging and the relation between bioenergetics and behaviour. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 133(2), 335–343. [https://doi.org/10.1016/S1095-6433\(02\)00165-4](https://doi.org/10.1016/S1095-6433(02)00165-4)

Valolahti, H., Kivimäenpää, M., Faubert, P., Michelsen, A., & Rinnan, R. (2015). Climate change-induced vegetation change as a driver of increased subarctic biogenic volatile organic compound emissions. *Global Change Biology*, 21(9), 3478–3488. <https://doi.org/10.1111/gcb.12953>

Wadgyamar, S. M., Ogilvie, J. E., Inouye, D. W., Weis, A. E., & Anderson, J. T. (2018). Phenological responses to multiple environmental drivers under climate change: Insights from a long-term observational study and a manipulative field experiment. *New Phytologist*, 218(2), 517–529. <https://doi.org/10.1111/nph.15029>

Wu, C. A., & Campbell, D. R. (2006). Environmental stressors differentially affect leaf ecophysiological responses in two *Ipomopsis* species and their hybrids. *Oecologia*, 148(2), 202–212. OmniFile Full Text Select (H.W. Wilson).

Tables and Figures

Temp	Snow	n
Control	Early	16
Control	Normal	13
OTC	Early	14
OTC	Normal	16

Table 1: quantity of individuals receiving each treatment option.

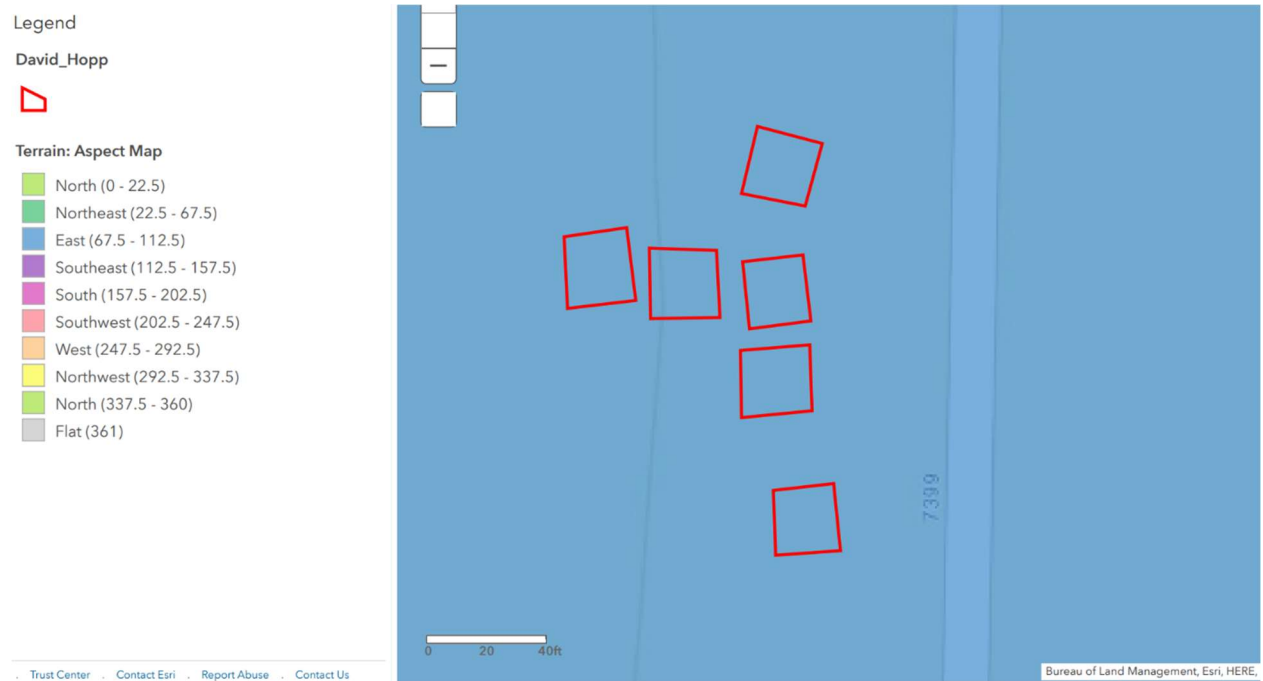


Figure 1. Map of Maxfield meadow 2021 field site along county road 317 ~1km south of Gothic, CO. Blue color shows Eastern aspect of terrain (direction of slope face). Coordinates are 38.9495°N, 106.9908°W.

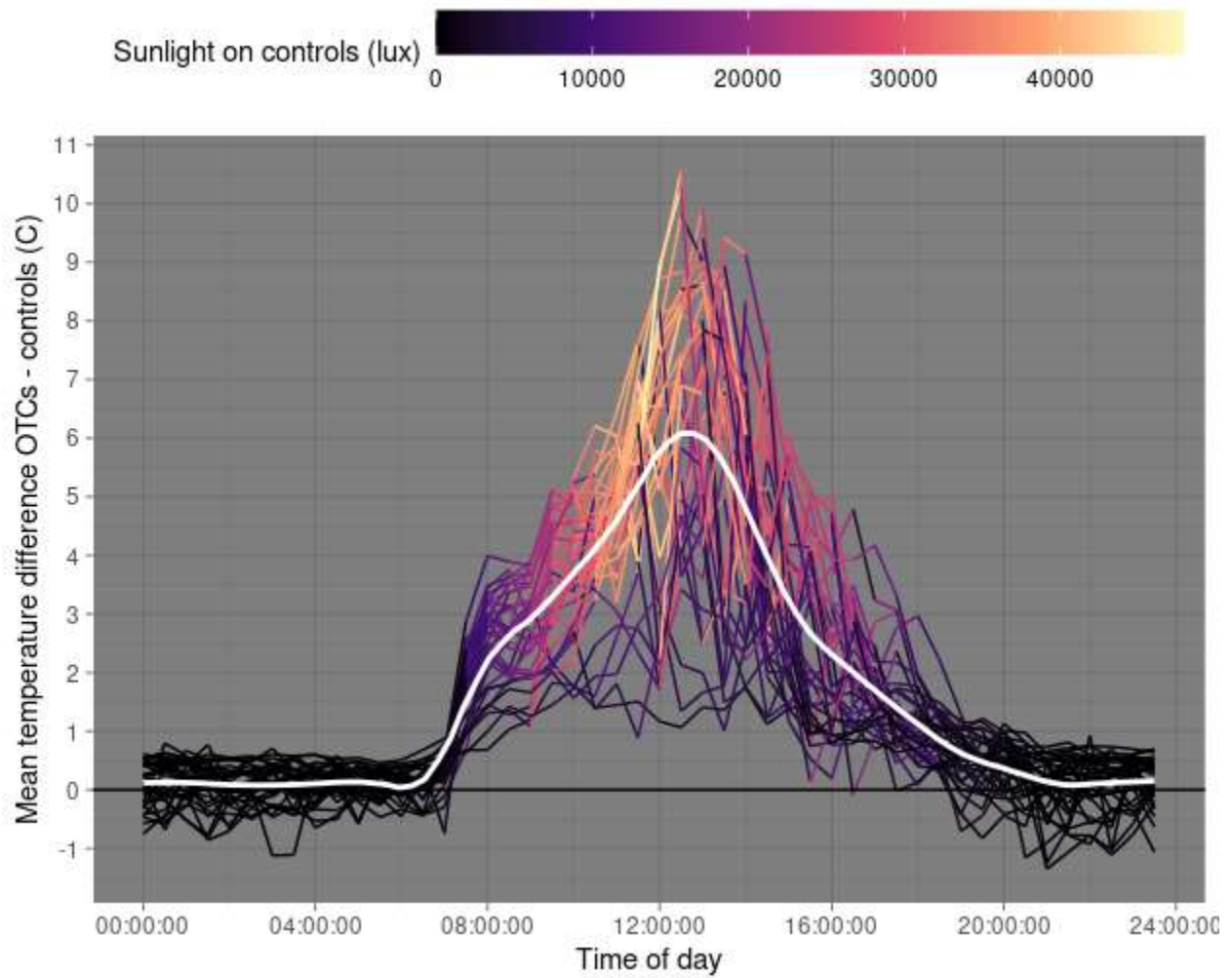


Figure 2. Mean temperature difference between OTCs and outside temperature based on time of day. Color of lines indicates amount of sunlight hitting HOBO loggers outside of OTCs.

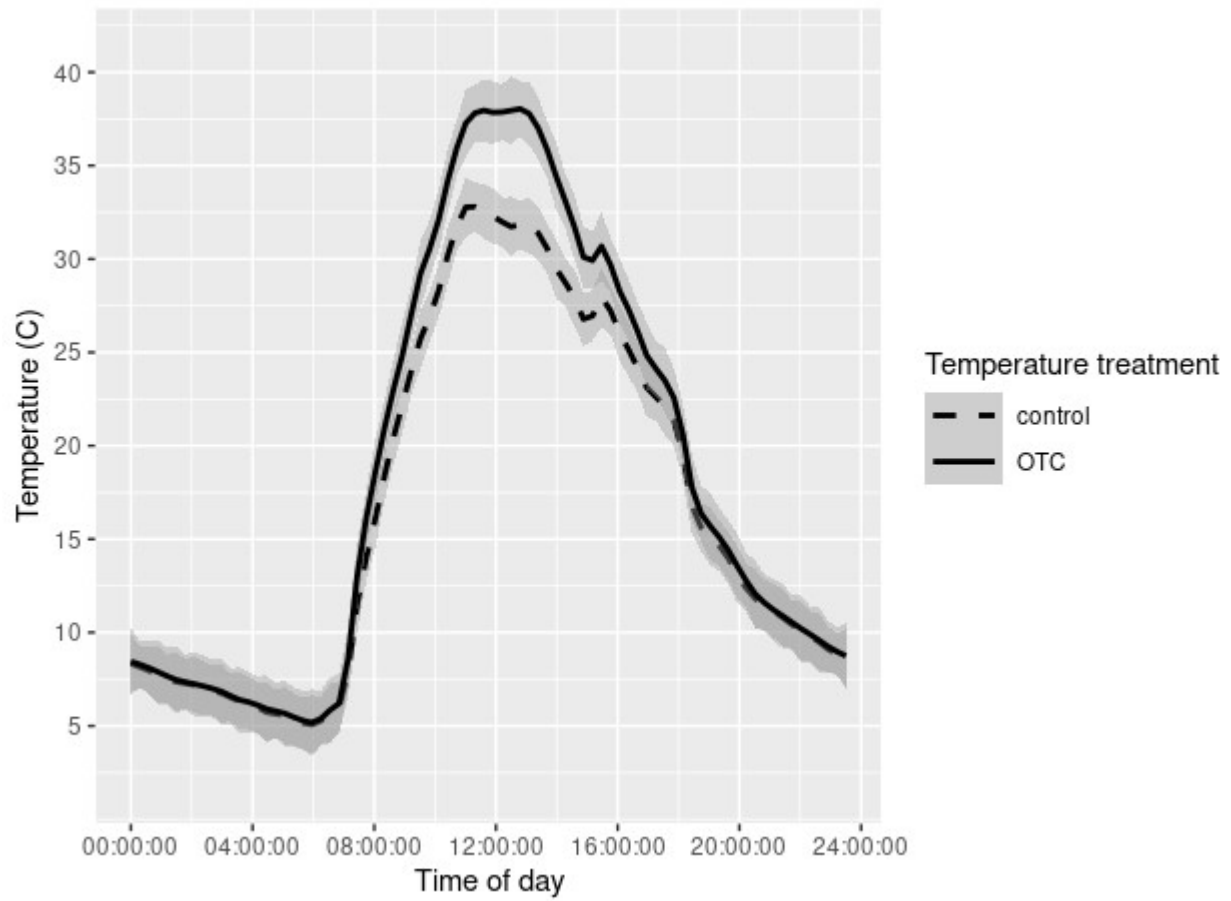


Figure 3. Average temperature (°C) inside and outside OTCs at different times of the day. Gray clouds around lines show 95% confidence interval.

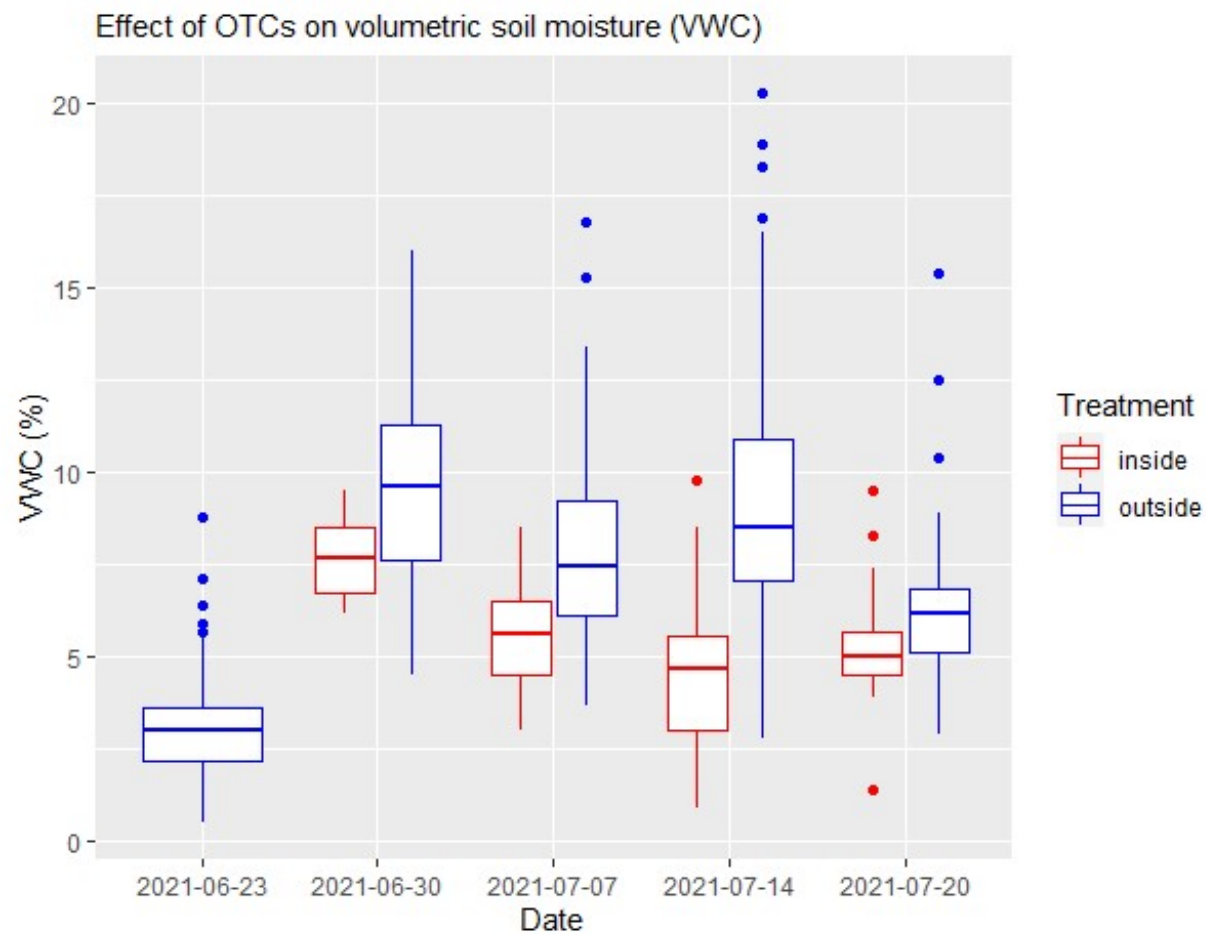


Figure 4. The effect of OTCs on volumetric water content (VWC) of soil (%). OTCs were set up on 2021-06-25. Middle line of boxes depicts medians. Each half of the boxes contain 25% of data spread. Whiskers on either side of box depict min and max points of dataset. Any points beyond whiskers depict possible outliers.

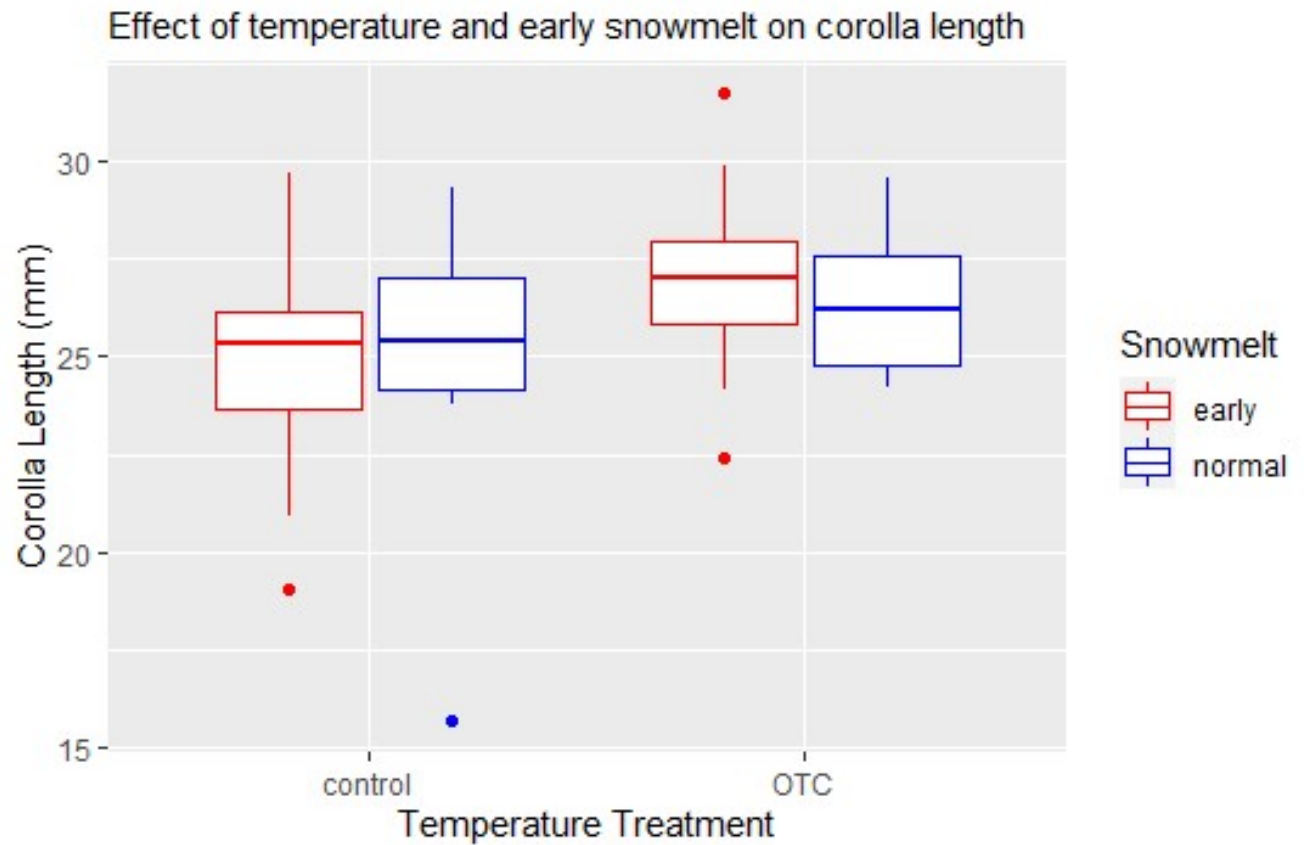


Figure 5. Effect of higher temperature and early snowmelt on corolla length (mm). Middle line of box depicts median. Each half of the box contains 25% of data spread. Whiskers on either side of box depict min and max points of dataset. Any points beyond whiskers depict possible outliers.

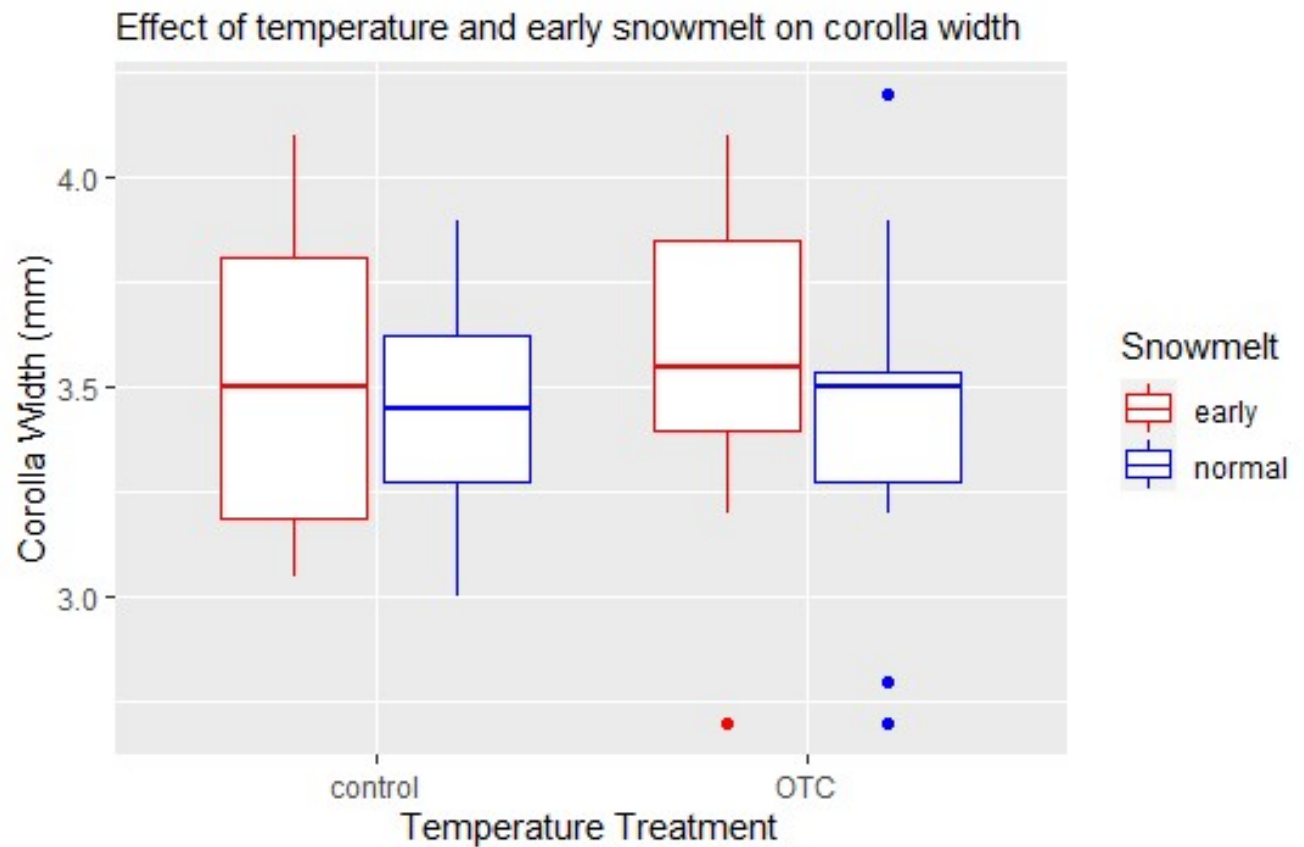


Figure 6. Effect of higher temperature and early snowmelt on corolla width (mm). Middle line of box depicts median. Each half of the box contains 25% of data spread. Whiskers on either side of box depict min and max points of dataset. Any points beyond whiskers depict possible outliers.

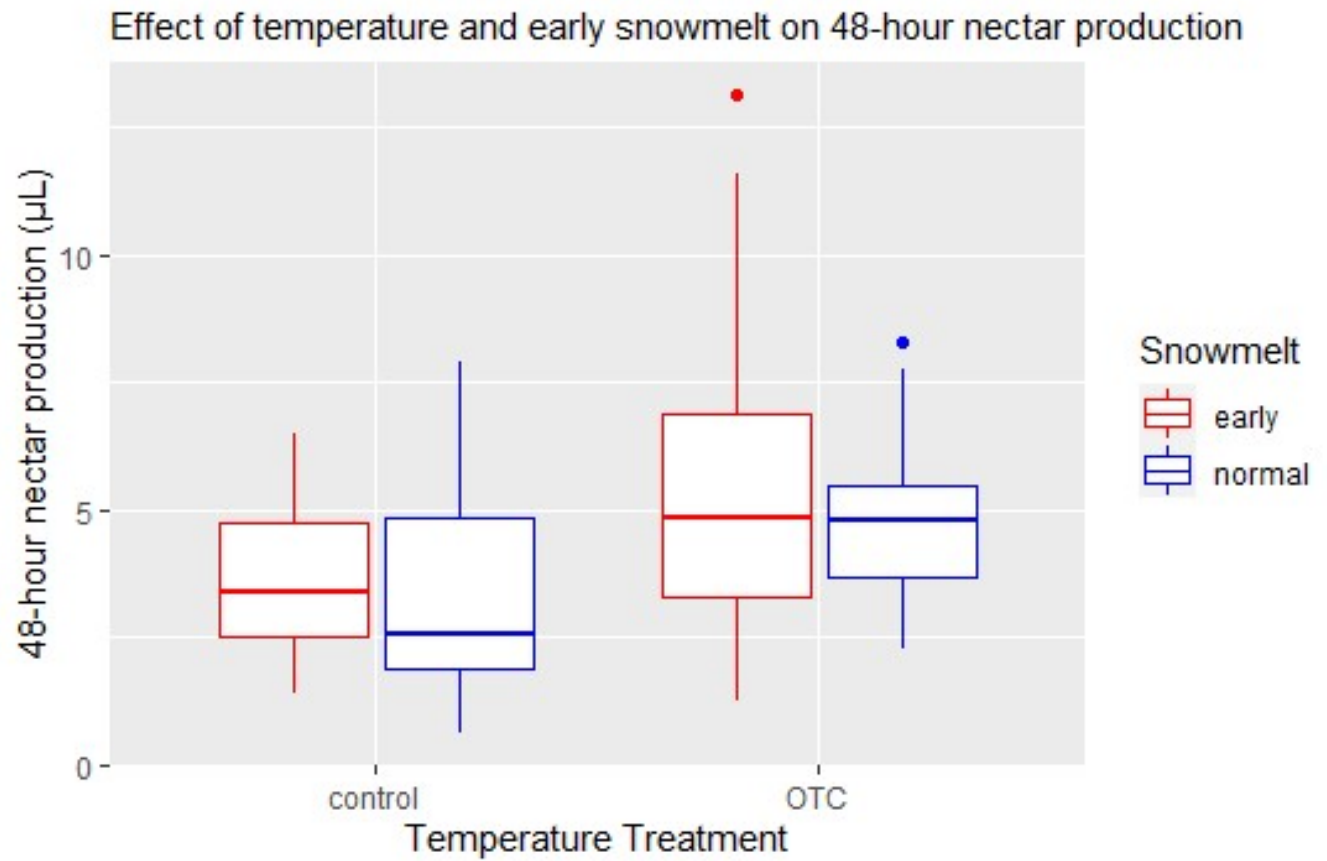


Figure 7. Effect of higher temperature and early snowmelt on 48-hour nectar production (μL). Middle line of box depicts median. Each half of the box contains 25% of data spread. Whiskers on either side of box depict min and max points of dataset. Any points beyond whiskers depict possible outliers.

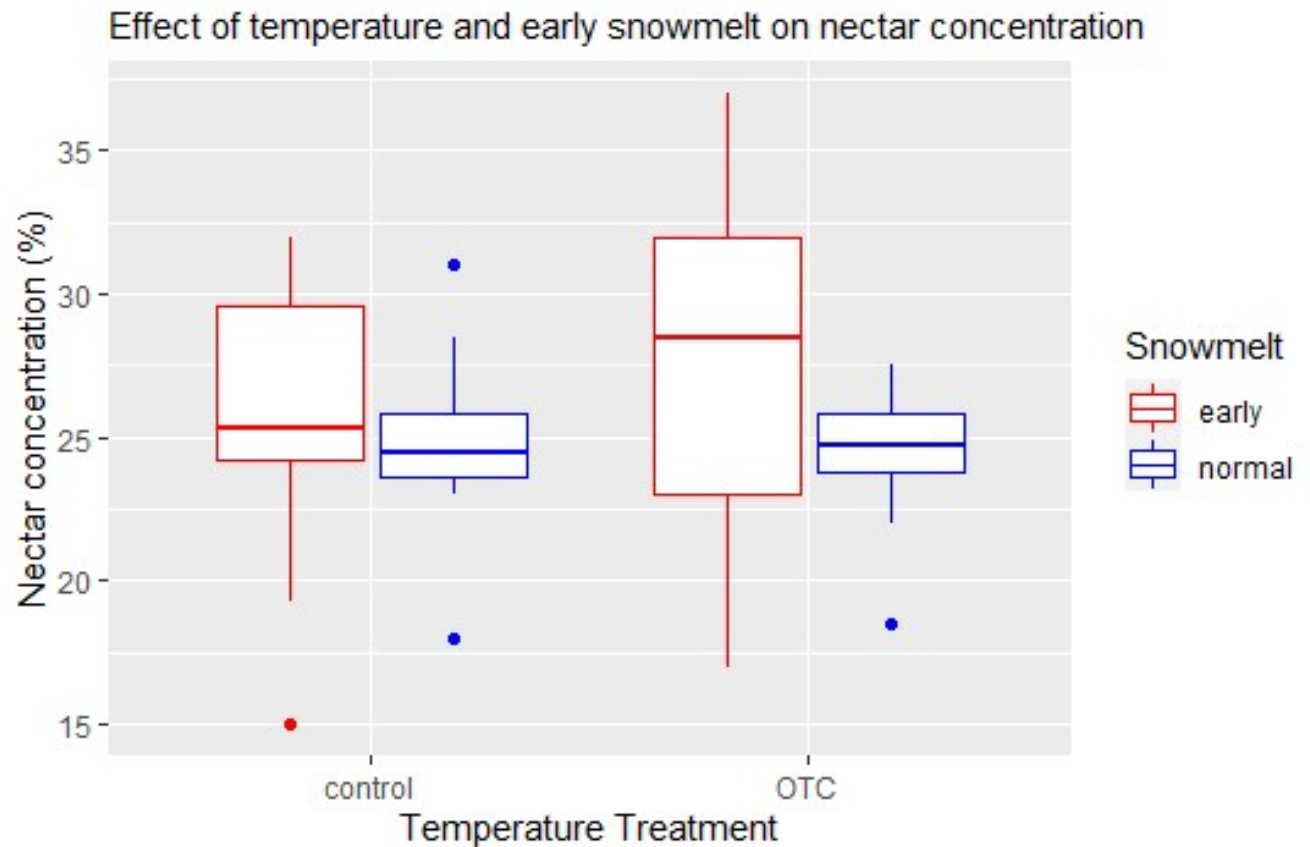


Figure 8. Effect of higher temperature and early snowmelt on nectar sugar concentration (%). Middle line of box depicts median. Each half of the box contains 25% of data spread. Whiskers on either side of box depict min and max points of dataset. Any points beyond whiskers depict possible outliers.

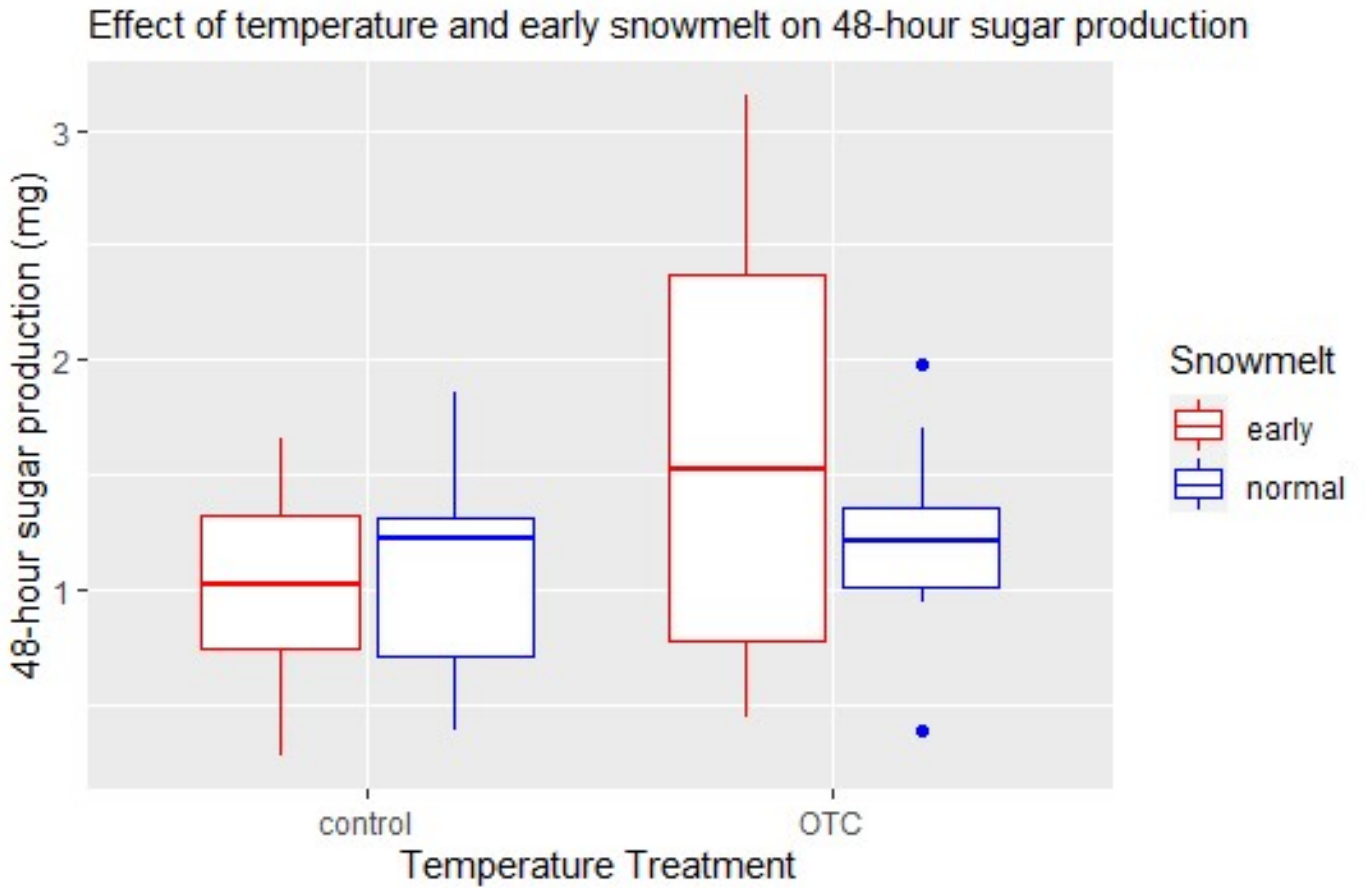


Figure 9. Effect of higher temperature and early snowmelt on 48-hour sugar production (mg). Middle line of box depicts median. Each half of the box contains 25% of data spread. Whiskers on either side of box depict min and max points of dataset. Any points beyond whiskers depict possible outliers.

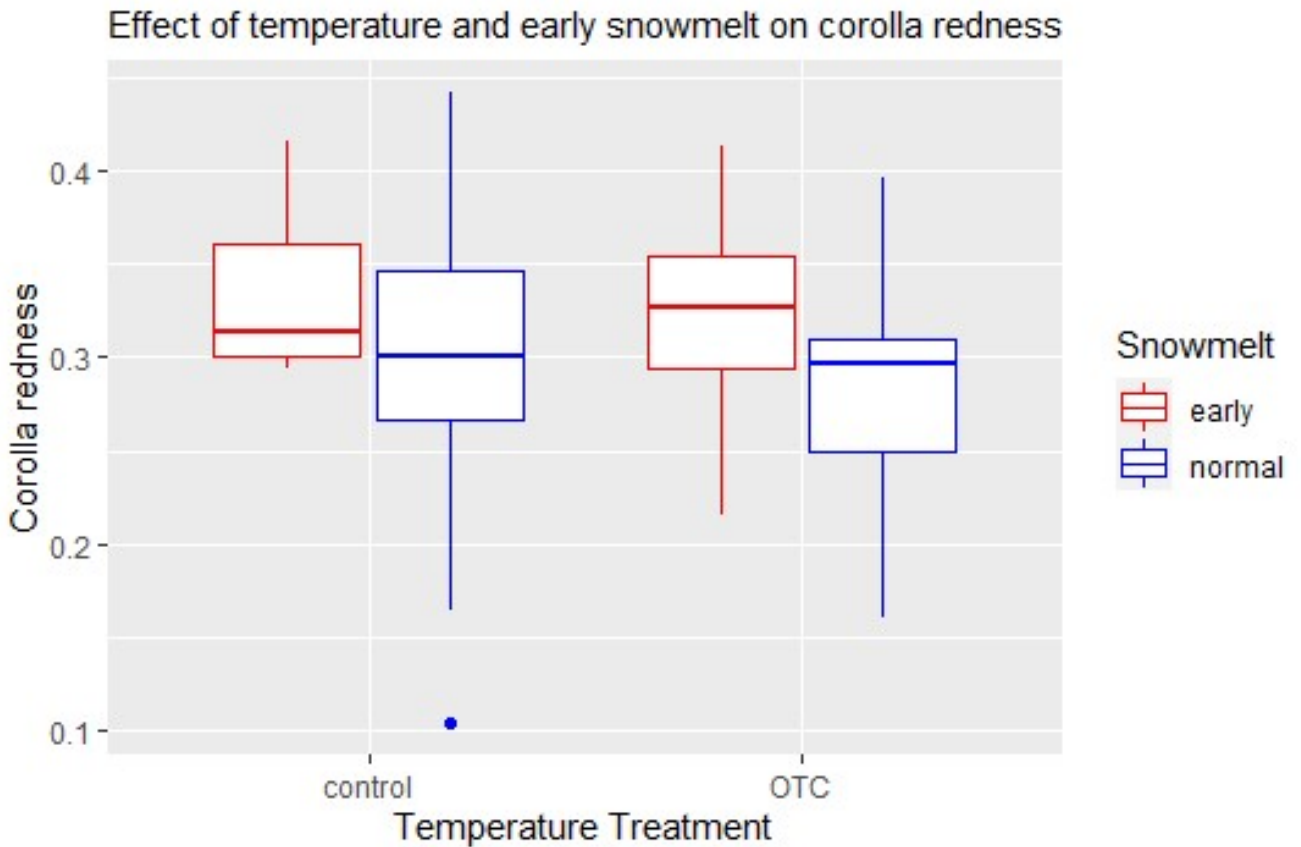


Figure 10. Effect of higher temperature and early snowmelt on flower color. Middle line of box depicts median. Each half of the box contains 25% of data spread. Whiskers on either side of box depict min and max points of dataset. Any points beyond whiskers depict possible outliers.

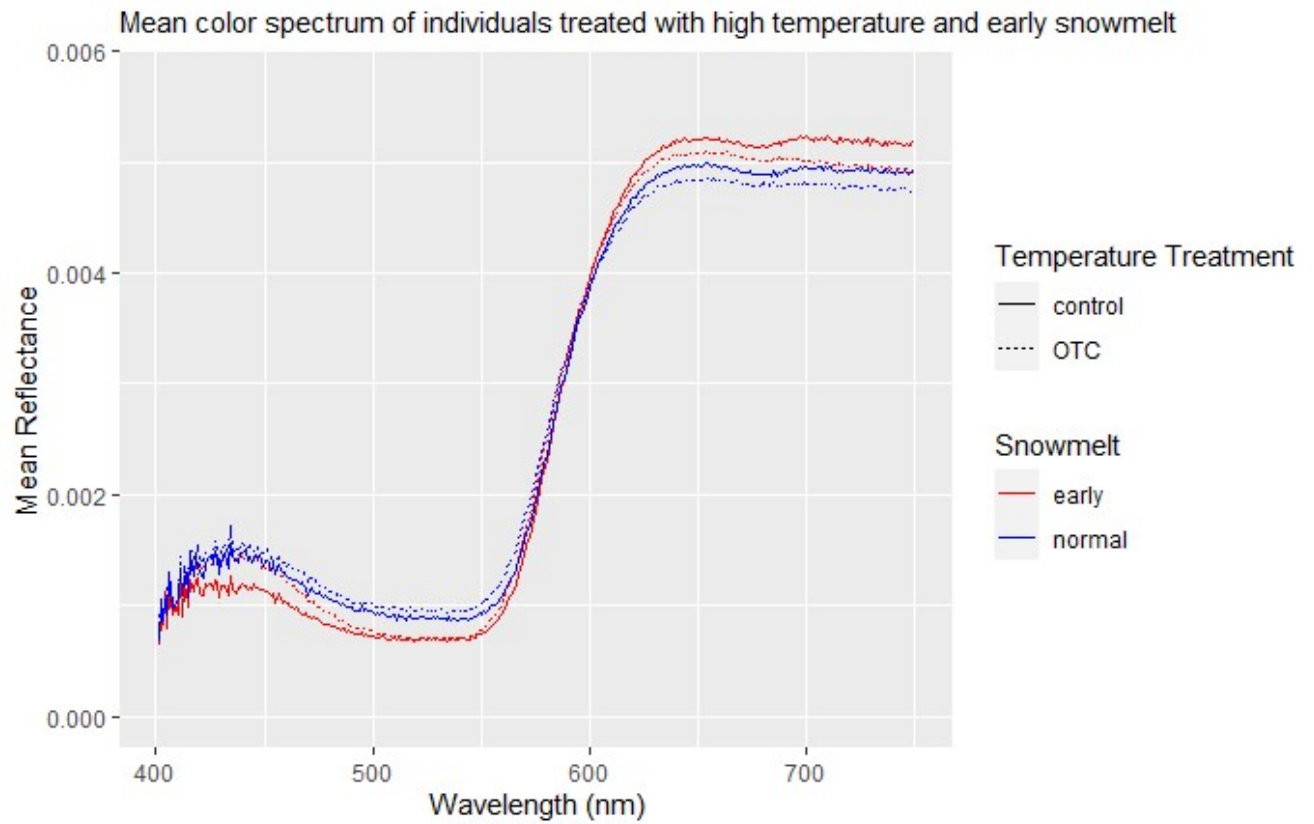


Figure 11. Effect of higher temperature and early snowmelt on corolla color reflectance. Each line is an average of the relative reflectance for each of the four treatment combination options.

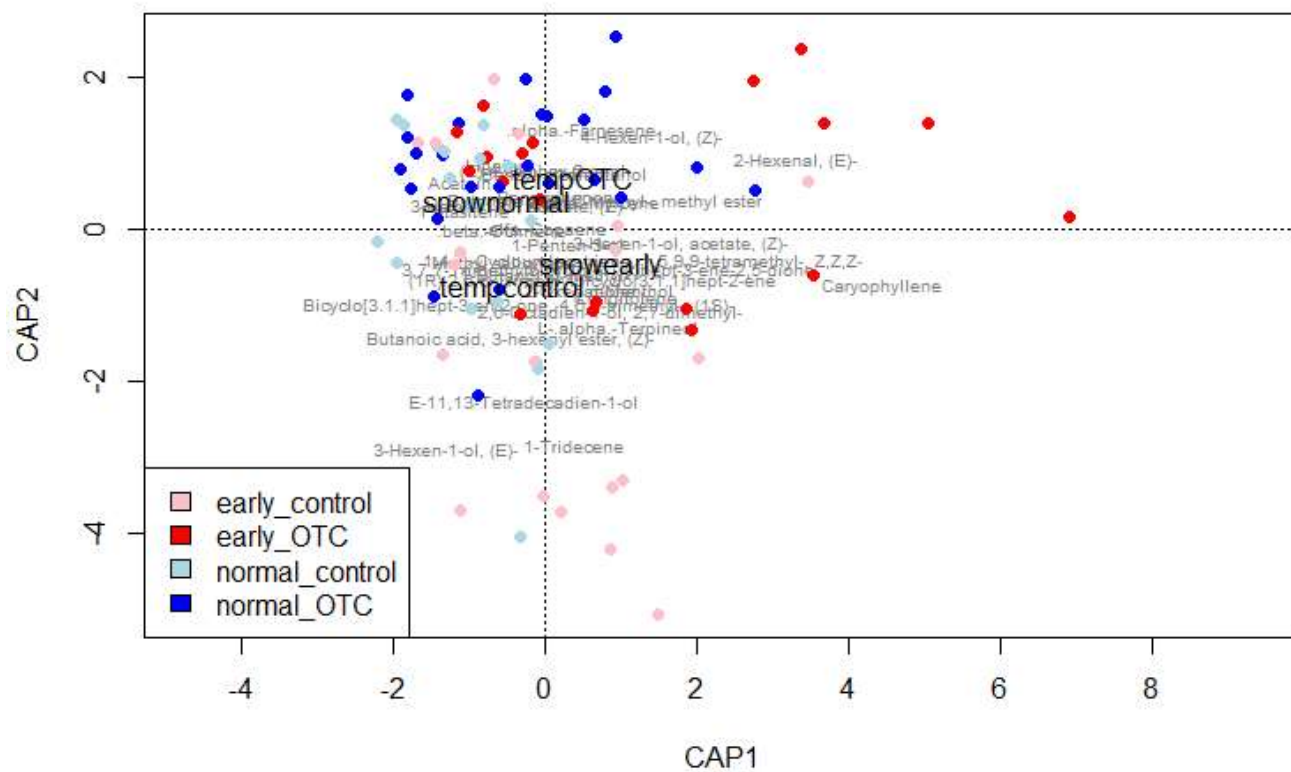


Figure 12. Effect of higher temperature and early snowmelt on identity of VOCs released. Treatment labels given in dark black lettering give centroid position of individuals that received respective treatments. Volatile compound labels given in light gray lettering give scores of the respective compounds.

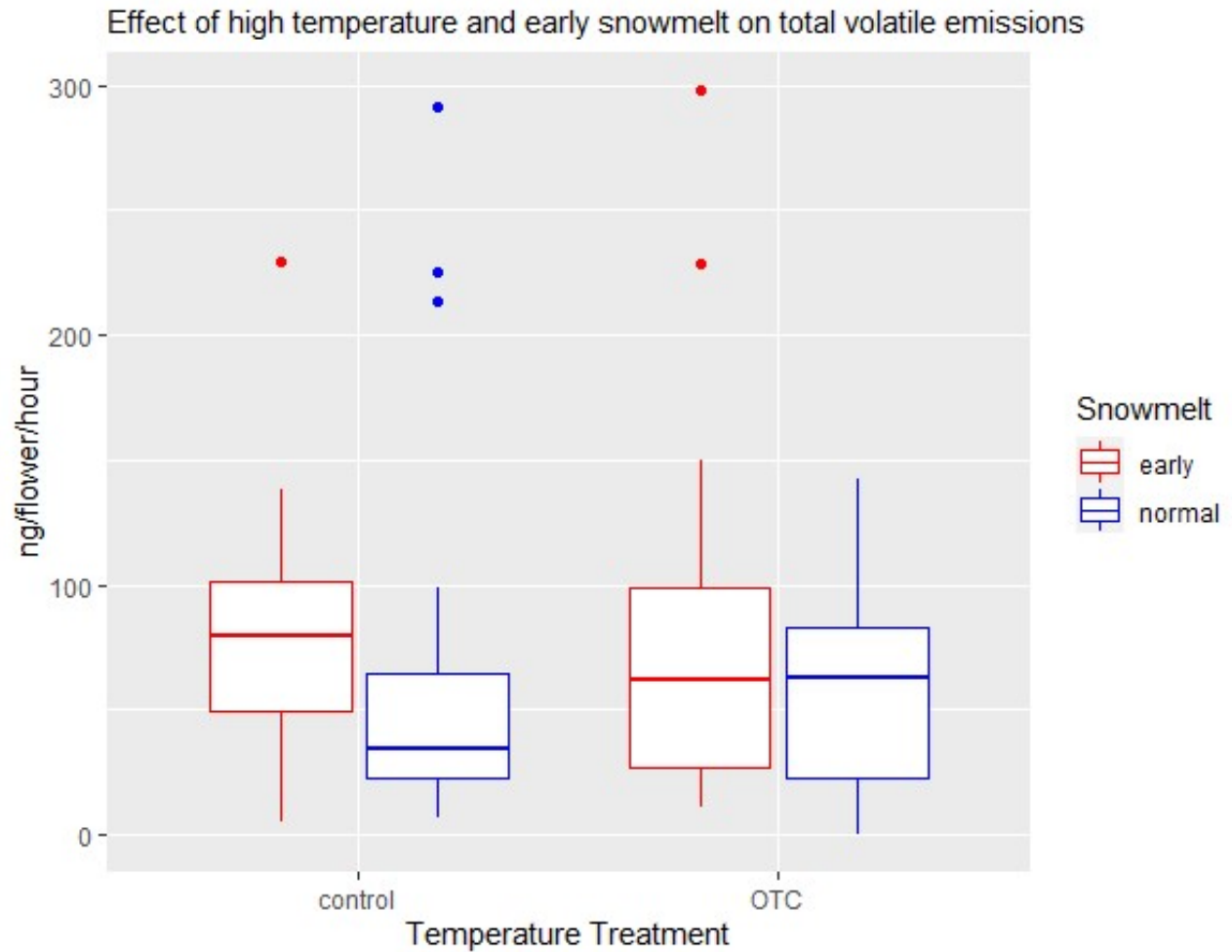


Figure 13. Effect of higher temperature and early snowmelt on total volatile emissions. Middle line of box depicts median. Each half of the box contains 25% of data spread. Whiskers on either side of box depict min and max points of dataset. Any points beyond whiskers depict possible outliers.

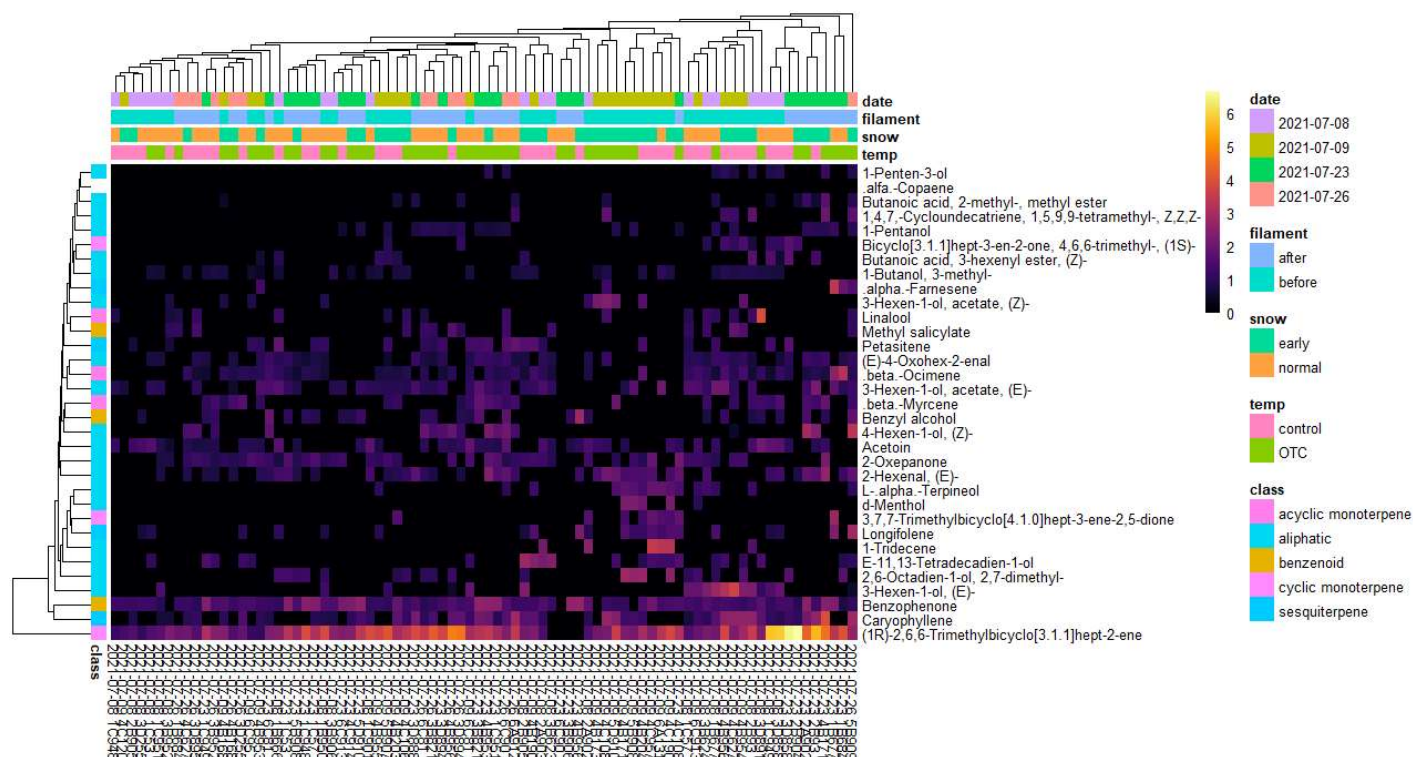


Figure 14. Effect of higher temperature, early snowmelt on VOC identity and quantity. Bottom axis labeled with sample name. Right axis labeled with all compounds detected after filtration. Left axis labeled with class of compounds. Top axis labeled (from top to bottom) with date sample was taken, whether the sample was run in the GC-MS before or after filament change on 2021-07-21, snowmelt treatment, and temperature treatment. Color of internal box refers to the relative quantity of compounds found in that sample on a scale of 0-6; 6 meaning very high relative quantity found in the sample and 0 meaning none of the compound found in sample. Brackets on top of chart show clustering of samples, brackets on left side of chart show clustering of compounds released.

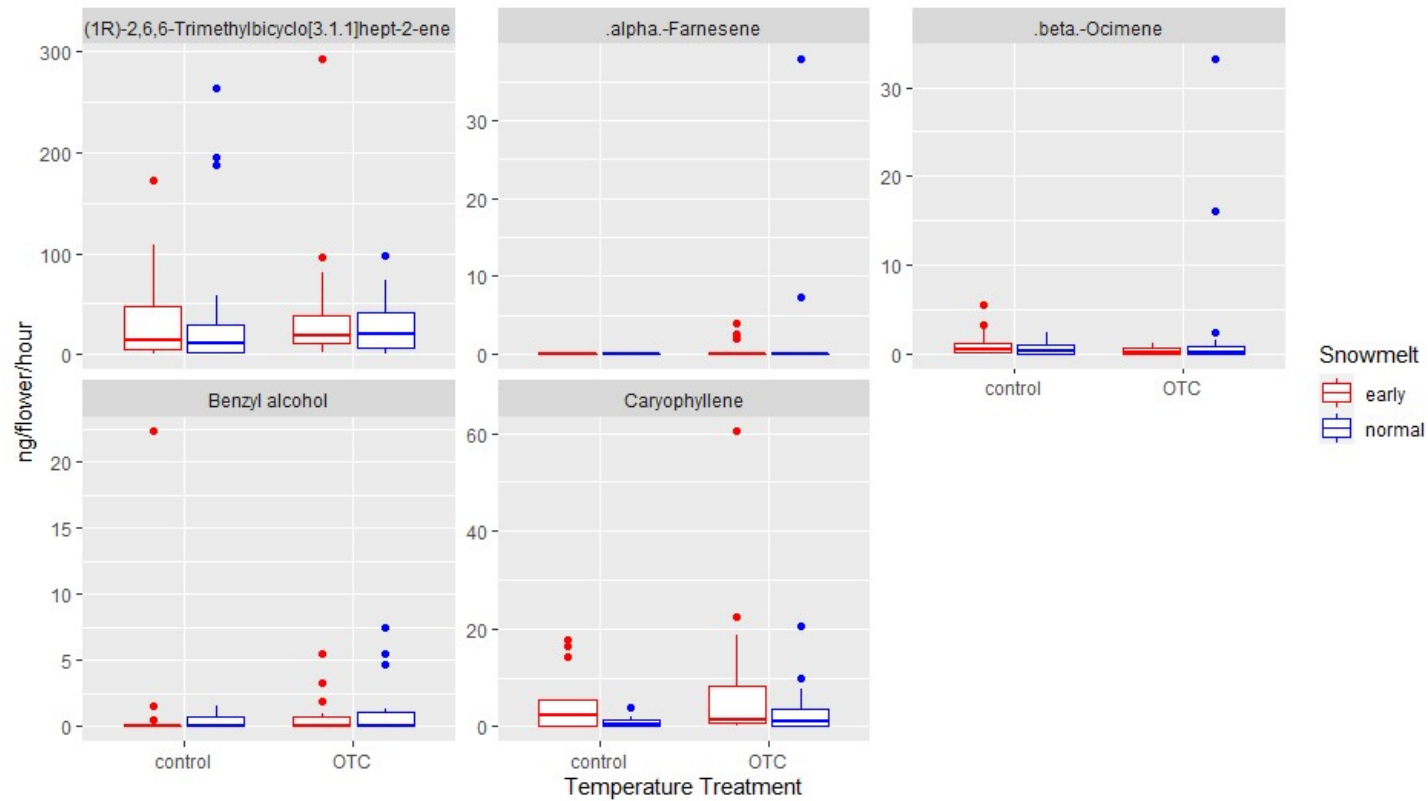


Figure 15. Effect of higher temperature and early snowmelt on emissions of (1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene (α -pinene), α -farnesene, β -Ocimene, benzyl alcohol, and caryophyllene (ng/flower/hour). Middle line of boxes depicts medians. Each half of the boxes contain 25% of data spread. Whiskers on either side of box depict min and max points of dataset. Any points beyond whiskers depict possible outliers.

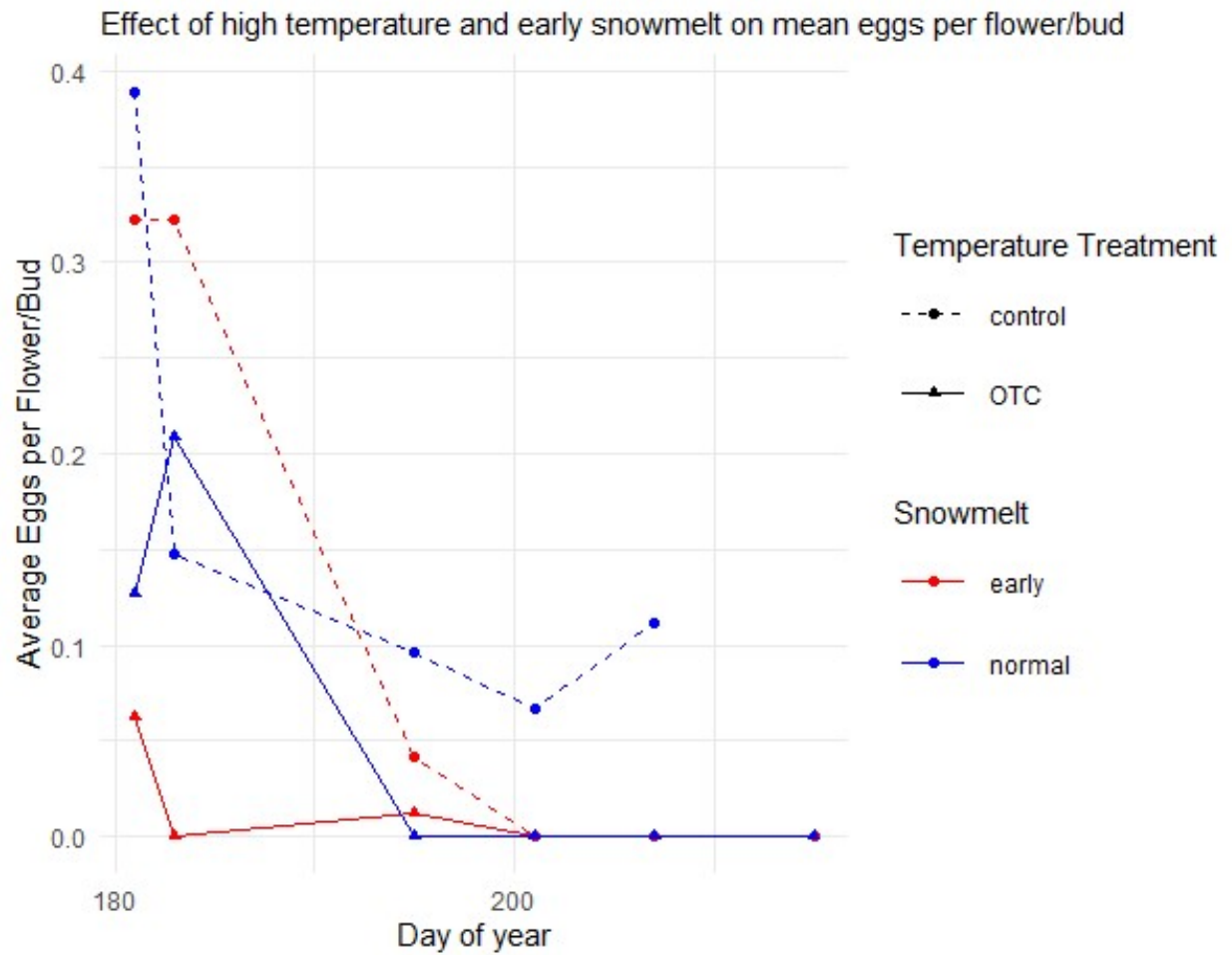


Figure 16. The effect of higher temperature and early snowmelt on the mean number of eggs per flower or bud. Eggs were counted on all open flowers as well as buds on plant at time of data collection.