

Volatile organic compounds as signals for pollinators and their consistency across years.

**Student: Lucas Sharrett
Mentor: Diane Campbell
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Abstract

Volatile organic compounds (VOCs) are emitted by every part of a plant and are believed to work as possible olfactory signals, drawing in pollinators and antagonists. This study was designed to address whether VOCs served as a signal to pollinators, indicating the size of the nectar reward. This part of the study focused on two populations of *Ipomopsis aggregata* plants. Another part of the 2019 study measured floral VOCs for six species in a dry meadow also studied in 2017 and 2018 to address the consistency of emission of VOCs across three years differing in environmental conditions including date of snowmelt. The total emission of volatiles by flowers of *I. aggregata* did not correlate detectably with the production of nectar. In the dry meadow experiment, the composition of volatile emissions changed significantly across the three years, particularly for *Galium boreale* in the late snowmelt year of 2019.

Introduction

Floral signals attract pollinators to transfer pollen (the male gamete) to another flower often on another individual. As such, the ability to attract pollinators is a major selection pressure causing floral signals to evolve. Changes in plant morphology and visual display are often the key focus for such adaptation. As one example, in *Ipomopsis aggregata*, visitation frequency of hummingbirds, the main pollinator, increases as corolla length, width, and nectar production increase (Campbell et al. 1991; Campbell et al. 1997). However, flowers do not use only visual signals to communicate with pollinators for this process, but olfactory ones as well. Volatile organic compounds (VOCs) are produced by various biosynthetic processes in every part of the plant. Selection pressures on the VOCs can originate from various pollinators, antagonists, or even changes in the environment (Schiestl 2015). Emission of floral volatiles can change over the course of a day, allowing for attraction by day and night pollinators using different volatile signals (Bischoff et al. 2014). Visual and olfactory traits can also act in combination. For example, the combination of blue color with an olfactory cue allow the bee species *Hoplitis adunca*, to find flowers of *Echium* (Burger et al. 2010). In the sexual relationship of flowers and pollinators, there are often three major factors that select for flower volatile traits (Schiestl 2015). These include honest signals, specific host flower recognition, and sensory exploitation.

The primary factor observed in this study is honest signaling, or the association between VOCs and the quantity and quality of rewards available in the flower. A major component to this signaling is the quantity of nectar. What is not fully understood in the study of honest signaling is whether VOCs can serve as a proxy for signaling the quantity and quality of nectar in a plant. In at least one bee-pollinated plant, the concentration of VOCs as an olfactory signal correlates with the quantity and quality of nectar as an honest signal from flowers (Knauer and Schiestl 2015).

To determine whether such volatiles are used as honest signals by pollinators, this study measured the correlation of floral volatiles with nectar production. The main study species for this experiment was *I. aggregata*, a monocarpic herb which grows in subalpine conditions. *I. aggregata* is mainly pollinated by nectar feeding organisms such as the hummingbirds *Selasphorus platycircus* and *S. rufus* with pollination in some years also by swallowtail butterflies and the hawkmoth species *Hyles lineata* (Campbell 2003; Bischoff et al. 2015).

Flowers are subject to attack by the seed predator *Delia* sp. (Diptera: Anthomyiidae) whose larvae consume seeds inside the fruit (Campbell et al. 2002). Pollinators, antagonists, and environmental conditions can selectively favor various floral traits in *I. aggregata*. Corolla width and sepal width have been found to correlate with oviposition by *Delia* flies (Campbell et al. 2002). Hawkmoths prefer narrower corollas in contrast to hummingbirds which prefer longer and wider corollas (Campbell et al. 1997, Campbell et al. 1991). Also, longer style lengths and exerted stigmas can capture more pollen, increasing pollinator efficiency (Campbell 1989). Pollinators and antagonists are major factors selecting for floral VOCs and flower morphology, but another factor is the environmental conditions which could influence volatile emissions.

Environmental factors can play a big part in plant-pollinator interactions. Climate change has been altering environmental conditions at an increasingly unnatural rate and has affected such components like timing of snowmelt in subalpine regions (IPCC 2014; Fyfe et al. 2017). Early snowmelt has even been correlated to decreased seed production, leading to population declines in *I. aggregata* (Campbell 2019). In a study on the effect of drought and herbivory on floral traits, drought conditions tended to decrease visual floral traits in forb plant species, making flowers smaller in general and in turn, decreasing pollinator attraction (Burkle and Runyon 2016). Along with changes in floral morphology, floral volatiles differed in diversity, composition, and concentration with increasing drought conditions, for several species, including *I. aggregata* (Burkle & Runyon 2016; Campbell et al. 2019). However, little is known about consistency of VOCs across years. Some consistency is required for pollinators or seed predators to evolve the use of VOCs as cues. A second study was the observation of the variability in floral volatile emissions across years that differ in environmental conditions.

The main questions addressed in this study are: (1) Do floral volatile emissions correlate with nectar production, such that volatiles can serve as an honest signal to pollinators? (2) How variable are floral volatile emissions across years that differ in environmental conditions? I hypothesized that with an increase in nectar production, a higher concentration of VOCs would be emitted, allowing floral volatiles to be used as honest signals for pollinators. Second, I hypothesized that a different composition of floral volatiles would be emitted between the dry and wet years, as different floral compounds can respond differently to drought (Burkle & Runyon 2016; Campbell et al 2019).

Methods

Study System

In total, 69 individuals were sampled for nectar production, corolla size, and floral volatile emissions from each of two populations of *Ipomopsis aggregata*. One population was located on Rocky Mountain Biological Laboratory (RMBL) property, known as Vera Falls, where 23 individuals were sampled. The second population was in Poverty Gulch, CO, located up the Slate River drainage at 2900 m elevation (Figure 1).

For observation on the variation of floral volatiles across years that differ in environmental conditions, VOCs for several plant species in a dry meadow community that was also studied in 2017 to 2018 were collected (Barth, unpub). The date of snowmelt at RMBL was May 24, May 5 and June 6 in 2017, 2018, and 2019 respectively (www.gothicwx.org). Therefore, the soil during

June was likely the driest in 2018 and the wettest in 2019. The meadow study site is a dry subalpine meadow located off the 401 trail to Judd Falls and was the site of a study on plant-pollinator networks by CaraDonna et al. (2017) (Figure 1). The species analyzed for this study spanning three years were: *Arenaria congesta*, *Achillea millefolium*, *Campanula rotundifolia*, *Eriogonum subalpinum*, *Galium boreale*, and *Heterotheca villosa*. Approximately four samples (range = 2 to 5) were taken per species in each year.

Volatile Sampling Methods

Floral volatiles were sampled using dynamic headspace sampling, as described by Bischoff et al. (2014) for both the *Ipomopsis* study and the community-wide study. The scent samples were collected using a 7x10 cm food-grade Reynolds© oven bag heat-sealed using an impulse sealer, a Spectrex© PAS-500 personal air sampling pump connected to a section of Tygon tube and a glass pipette, a 2 cm section of silicone tubing placed on the very tip of the pipette, and a scent trap built to collect the volatile emissions. Scent traps were each made from a 28 mm section of glass capillary tube filled with 5 mg of Tenax TA© absorbent polymer trapping agent. The ends of the capillary tube were each capped with glass wool and stored in a clean 20 ml glass vial with a glass liner insert. For each sample, an oven bag was placed around a portion of inflorescence and the bag was then sealed with a twist tie. The floral tissue was oriented in the center of the bag with room to allow headspace emissions to build up for 40 minutes in the case of the community-wide study and 30 minutes for *Ipomopsis*. Then, a small incision was made in the bag and the scent trap inserted. The bag was pumped using a personal air sampling pump and the headspace volatiles were collected across the scent trap for 20 min (or 15 minutes for the *Ipomopsis* study) at a flow rate of 100 ml/min. Before floral scent samples were collected, the height of the plant and the volumetric water content (%VWC) of the surrounding soil were recorded using a meter stick and a Campbell Scientific HydroSense II sensor respectively. Soil moisture during sampling averaged 7.1% in the late snowmelt year of 2019 compared to 3.3% in the early snowmelt year of 2018 (Barth, unpub.). An ambient air scent baseline was sampled using the same technique for floral scent once at the beginning of data collection and once more at the end of data collection. The floral volatile samples were only collected between 9:00 am and 1:00 pm. In both studies, to standardize emission rate, the flowers utilized for floral VOC collection were severed from the plant and the dry mass obtained to calculate the emission of each floral volatile per gram of dry mass. To estimate the dry mass of the plant collected, the floral tissue samples were baked in a gravity convection oven at 60°C for 2.0 hours and immediately weighed afterwards. Individuals selected for assessment of floral volatile emissions were flagged to avoid repeated sampling.

Measurements of Floral Traits

Nectar production and concentration for *I. aggregata* were measured for approximately 2 flowers (range = 1 to 4) per plant on a 48-hour time scale using capillary tubes and a refractometer as described in Campbell et al (1991). Values for nectar were averaged across flowers on the plant to obtain a single trait value for the plant for use in statistical analysis. For the *Ipomopsis* study, floral fragrance was measured during the day for one flower each on every plant. Methods are as described above for the community study except that flowers were only bagged for 30 minutes, and pumping was only for 15 minutes. Floral scent samples were then processed using the GC-MS equipment to determine presence and concentration of floral VOCs.

Volatile Analysis

To analyze the concentrations and identities of various volatile floral scent compounds, a gas chromatography-mass spectrometry (GC-MS) machine was used onsite at RMBL. The GC-MS is a Shimadzu GC-MS QP2020 with a DB-5 all-purpose, non-polar GC column (30m/.25mm/0.25 μ m) and a Markes thermal desorber system with a Unity 2 attached to an ultra-auto loader with sampling tubes, using ultra-high purity helium gas as the carrier. Each of the scent traps were placed within a Markes sampling tube and within the autosampler, where it underwent thermal desorption. The first stage of this process occurred for five minutes at a temperature of 250°C. The second portion of thermal desorption was the re-trapping of the floral scent samples on a Tenax-filled cold trap set to 25°C, which was then rapidly heated to 200°C for three minutes. The gas chromatography oven cycle program involved an initial re-trapping of the floral volatile compounds for two minutes at 40°C, then a temperature ramp of 10 degrees per minute to 250°C, then 30 degrees per minute to 275°C, with a final hold for three minutes. The chromatograms for the floral samples were compared to those of the air controls to filter out contaminants, with assistance from a program written in R (see Data Analysis section). Once the set of compounds was determined, the area under the peak for a compound was used as a relative measure of emissions during the fixed sampling period.

Data Analysis

The Shimadzu GCMS Postrun Analysis software was used to identify compounds. The standards from the NIST14 library (National Institute of Science and Technology) were used for the compound similarity search by retention times and ion breakdown in the mass spectra. The chromatogram information was parsed into R-Studio using the `read.shimadzu` custom function created by John Powers, forming a matrix of values including the file name, retention time, peak area, peak number, similarity index, and the first compound search result for every peak in every sample (R Core Team 2018). Using the Bouquet Package written in R 3.4.2 by John Powers, Katherine Eisen, Diane Campbell, and Robert Raguso (2019), meta data for each sample including the filename, species, type of sample (floral, ambient, blank), and biomass of each sample were first imported into a metadata object to be used in the bouquet functions. Then, the GC-MS data were filtered by retention time with a minimum of four minutes and a maximum of twenty minutes, similarity index with a minimum of 80%, and frequency in floral samples by species with a minimum of 20%, and a total sum peak area minimum for each compound of 400,000 across all samples. Two T-tests with an alpha value of 0.001 were performed comparing the peak area of the various compounds in the floral samples to the ambient and the blank samples and only the compounds that passed were used for estimating scent compounds for each species. Contaminants that passed filters were identified manually as nonfloral compounds and excluded from the results output. The final data set of volatiles were confirmed as floral volatiles when found to be reported in www.pherobase.com or in a comprehensive review by Knudsen et al. (2006).

For question 1, the correlation coefficient between total emission of 25 key *Ipomopsis aggregata* floral volatiles previously identified by Campbell et al. (2019) and the reward of nectar production, expressed as μl per 24 hours, were calculated. The total peak area was log-transformed prior to analysis. For question 2, the six plant species sampled in 2017 and 2018 as well as 2019 were the main focus of the study. Peak areas were then converted into proportions of the total peak area for each sample. I then used a permanova in the Vegan Package of R to test for differences in the multivariate composition of volatiles due to the plant species, year, or the interaction between species and year. Any differences were visualized with non-metric dimensional scaling (NMDS), also available in the Vegan package (Oksanen et al. 2019).

Results

For *Ipomopsis aggregata*, the 25 compounds included 3 aliphatics, 3 benzenoids, 8 monoterpenes, 1 nitrogen containing compound, and 9 sesquiterpenes (Campbell et al. 2019). No correlation was found between the emission of compounds and the production of nectar ($p = 0.28$) (Figure 2). Floral biomass and soil moisture also did not detectably correlate with total emission rate ($p = 0.151$, $p = 0.39$) (Figure 3; Figure 4).

For the dry meadow experiment, 74 confirmed floral volatile organic compounds were identified for the six species of the community data set (Table 1). The compound classes include 22 aliphatics, 2 benzenoids, 29 monoterpenes, and 21 sesquiterpenes (Table 1). The relative proportions of floral volatiles differed significantly across the six species ($p < 0.001$) and years ($p < 0.001$), judging from permanova (Figure 5, Figure 6). In addition, the effect of year depended upon the species (interactions, $p < 0.001$) (Figure 7). On a per species basis, only *Galium boreale* showed detectable differences across years once p-values were corrected for multiple comparisons using a Bonferroni correction with comparison-wide alpha value of 0.0083 since there were six tests (Figure 8). Volatile composition appeared different in the late snowmelt year of 2019 from the other two years. The years differed most along the NMDS 2 axis, and the compounds with highest scores on the axis were β -copaen-4 α -ol, methyl-3-hexenoate, and (Z)-3-Hexenyl 2-methyl-2-butenate. Compounds with large negative scores included (Z)-caryophyllene, 2,2,6-trimethyl-1,4-cyclohexanedione, and 2,6-dimethyl-3,7-octadien-2,6-diol.

Discussion

The total emission of floral volatiles in *Ipomopsis aggregata* did not correlate detectably with nectar production in the flower. A few samples had no floral volatiles detected while showing unusually high levels of contaminants and may not be representative. With that caveat, total floral volatiles therefore do not act as an honest signal in terms of nectar produced, however, they could still act as honest signals in terms of the concentration of sugar in the nectar. It is also possible that a particular compound, rather than total volatiles, is used to attract pollinators. In a study on honest signaling in the plant species *Brassica rapa*, bumble bees displayed preference for the floral compound phenylacetaldehyde, which was positively correlated to the concentration of nectar sugar and pollen (Knauer & Schiestl 2015). It is possible that honest signaling of rewards is less important in a plant species, such as *I. aggregata*, that is visited often

by hummingbirds as well as insects, as there is more evidence that insects use scent in choosing flowers (Raguso 2008). Hummingbirds, specifically, have been known not to have a strong preference on headspace emissions when selecting flowers for nectar feeding, however, they can show positive responses to some sesquiterpenes, including caryophyllene, a constituent of *I. aggregata* scent (Kessler & Baldwin 2006). It would be interesting to extend this study to examine correlations of floral volatiles with traits, such as sepal width, that the fly seed predator of *Ipomopsis* responds to (Campbell et al. 2002). Nectar concentration could also be another factor to look at in the honest signaling of *I. aggregata* when compared to floral volatile emissions (Knauer & Schiestl 2015).

Even though floral volatile composition was quite variable among the six species across years, the only species with a significant change in composition across the three years was *Galium boreale*, which increased especially the emission of the monoterpene Terpinen-4-ol in 2019. From the NMDS analysis, there was a clear difference in composition of the volatile mixture across multiple years varying in environmental conditions. The samples taken during 2019 seem to have the greatest difference from 2017 and 2018, leading me to hypothesize that late snowmelt, and the higher soil moisture seen in 2019, may influence the composition of floral volatile emissions. These results are interesting given that water availability has been proven to play a major role as an environmental factor in total volatile emissions. In the artificial dry down experiment of Campbell et al. (2019), drought stress increased total volatile emissions and shifted composition in *I. aggregata*. In an artificial drought experiment including two of the same study species we used, *C. rotundifolia* and *H. villosa*, the former doubled its total volatile emissions in drought, shifting in its diverse array of compounds with decreased water availability. On the other hand, *H. villosa* had no significant change in floral volatile emissions with drought conditions (Burkle & Runyon 2016). In the dry meadow experiment, the total volatile emissions of *C. rotundifolia* were not observed to react in a similar fashion to differences among years that differed in extent of natural drought conditions (Figure 9). *H. villosa* seemed to have acted in the same manner as the artificial dry down experiment, having no significant change in floral volatile emissions across the years varying in environmental conditions (Figure 10). *I. aggregata* floral volatiles showed no correlation to volumetric water content in this field study (Figure 4), unlike in the artificial dry-down experiment, where low volumetric water content was positively correlated with total amount of floral volatiles (Campbell et al. 2019). Overall, it might be concluded that the late snowmelt of 2019 and early snowmelt of 2018 in Gothic, Colorado may not have differed in environmental conditions drastically enough to produce an effect on floral volatile emissions in most species based solely on water availability.

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Tables and Figures

Table 1. All 74 known floral compounds filtered from chromatograms of the six species analyzed for the dry meadow study. Organized by class of compound.

Species	Class	Compound Name
<i>Arenaria congesta</i>		
	Aliphatic	(E)-2-Hexenal (E)-3-Hexenyl acetate (Z)-2-Hexenol 2-Heptanol 2-methylbutyl acetate Isohexanol
	Monoterpene	2,6-Dimethyl-1,7-octadien-3,6-diol 4-Oxoisophorone D-Limonene
	Sesquiterpene	Caryophyllene
<i>Achillea millefolium</i>		
	Aliphatic	(E)-2-Hexenal (E)-3-Hexenyl acetate (Z)-2-Hexenol (Z)-3-Hexenyl butanoate (Z)-3-Hexenyl isobutyrate (Z)-3-Hexenyl propanoate (Z)-3-Methylbutylalldoxime 2-Methylbutanoic acid 2-methylbutyl acetate Heptyl acetate Isobutylalldoxime Isohexanol Pentyl acetate Prenol
	Monoterpene	(E)-beta-Ocimene (E)-beta-Ocimene epoxide (E)-Pinocarveol (E)-Sabinene hydrate (Z)-3-Hexenyl (E)-2-methyl-2-butenate 2,6-Dimethyl-1,7-octadien-3,6-diol 2,6-Dimethylocta-1,5,7-trien-3-ol 2-Methyl-6-methylene-1,7-octadien-3-one 4-Oxoisophorone Allo-Ocimene alpha-Pinene alpha-Thujene beta-Myrcene Camphene Car-3-en-5-one Carvacrol Cosmene D-Carvone D-Limonene gamma-Terpinene Limonene Oxide Rose furan Terpinen-4-ol
	Sesquiterpenes	(E)-alpha-Bergamotene alpha-Bergamotene alpha-Copaene beta-Elemene beta-Selinene beta-Sesquiphellandrene Borneol Caryophyllene Caryophyllene oxide Copaene gamma-Elemene gamma-Murolene Sesquisabinene
<i>Campanula rotundifolia</i>		
	Aliphatic	(E)-2-Hexenal (E)-3-Hexenyl acetate (Z)-2-Hexenol Cyclosativene
	Benzenoid	Anisole
	Monoterpene	2,6-Dimethyl-1,7-octadien-3,6-diol alpha-Pinene D-Carvone D-Limonene Terpinen-4-ol Linalool Oxide
	Sesquiterpene	(Z)-Caryophyllene beta-Bourbonene beta-Copaen-4-ol Caryophyllene
<i>Eriogonum subalpinum</i>		
	Aliphatic	

	(E)-3-Hexenyl acetate (Z)-2-Hexenol (Z)-3-Hexenyl butanoate (Z)-3-Hexenyl isobutyrate 2-methylbutyl acetate	(Z)-3-Hexenyl 2-methylbutanoate Isobutylaldoxime Pentyl acetate Prenol Methyl 3-hexenoate
Benzenoid	2-Phenylethanol	Anisole
Monoterpene	(E)-beta-Ocimene Allo-Ocimene 2,6-Dimethyl-1,7-octadien-3,6-diol alpha-Pinene	D-Limonene Car-3-en-5-one Cosmene Carveol
Sesquiterpene	beta-Bisabolene Caryophyllene	Caryophyllene oxide
<i>Galium boreale</i>		
Aliphatic	(E)-2-Hexenal (E)-3-Hexenyl acetate (Z)-3-Hexenyl 2-methylbutanoate Isobutylaldoxime	2,2,6-Trimethyl-1,4-cyclohexanedione Prenol Tricyclene
Monoterpene	(E)-beta-Ocimene epoxide (E)-Pinocarveol 2-Methyl-6-methylene-1,7-octadien-3-one 4-Oxoisophorone (E)-beta-Ocimene Allo-Ocimene 2,6-Dimethyl-1,7-octadien-3,6-diol alpha-Pinene D-Limonene alpha-Thujene	beta-Myrcene Car-3-en-5-one Terpinen-4-ol Carveol Linalool Oxide gamma-Terpinene Limonene Oxide Rose furan p-Mentha-1,3,8-triene Verbenone
Sesquiterpene	Caryophyllene	gamma-Elementene
<i>Heterotheca villosa</i>		
Aliphatic	(E)-2-Hexenal (E)-3-Hexenyl acetate (E)-3-Hexenyl butanoate (Z)-2-Hexenol (Z)-3-Hexenyl butanoate (Z)-3-Hexenyl propanoate 2-Methylbutanoic acid	2-Methylbutyl acetate (Z)-3-Hexenyl 2-methylbutanoate Isobutylaldoxime Isohexanol 2-Heptanol Pinocamphone
Benzenoid	2-Phenylethanol	Anisole
Monoterpene	2,6-Dimethyl-3,7-octadien-2,6-diol (E)-Sabinene hydrate 2,6-Dimethylocta-1,5,7-trien-3-ol (E)-Pinocarveol 2-Methyl-6-methylene-1,7-octadien-3-one Allo-Ocimene D-Carvone alpha-Pinene	D-Limonene alpha-Thujene beta-Myrcene Cosmene gamma-Terpinene Limonene Oxide p-Mentha-1,3,8-triene Verbenone Terpinolene
Sesquiterpene	alpha-Cubebene beta-Copaene beta-Dihydroagarofuran beta-Bourbonene epsilon-Murolene (E)-alpha-Bergamotene alpha-Bergamotene alpha-Copaene beta-Selinene	Borneol beta-Copaen-4 α -ol beta-Bisabolene Caryophyllene oxide Copaene gamma-Murolene Caryophyllene gamma-Elementene

Figure 1. Map displaying the location and coordinates of the sites used to study volatile organic compound emissions in 2019. *Ipomopsis aggregata* experiment: Site-Vera Falls and Site-Poverty Gulch. Dry meadow community experiment: Site-Dry Meadow. Gunnison County, CO.

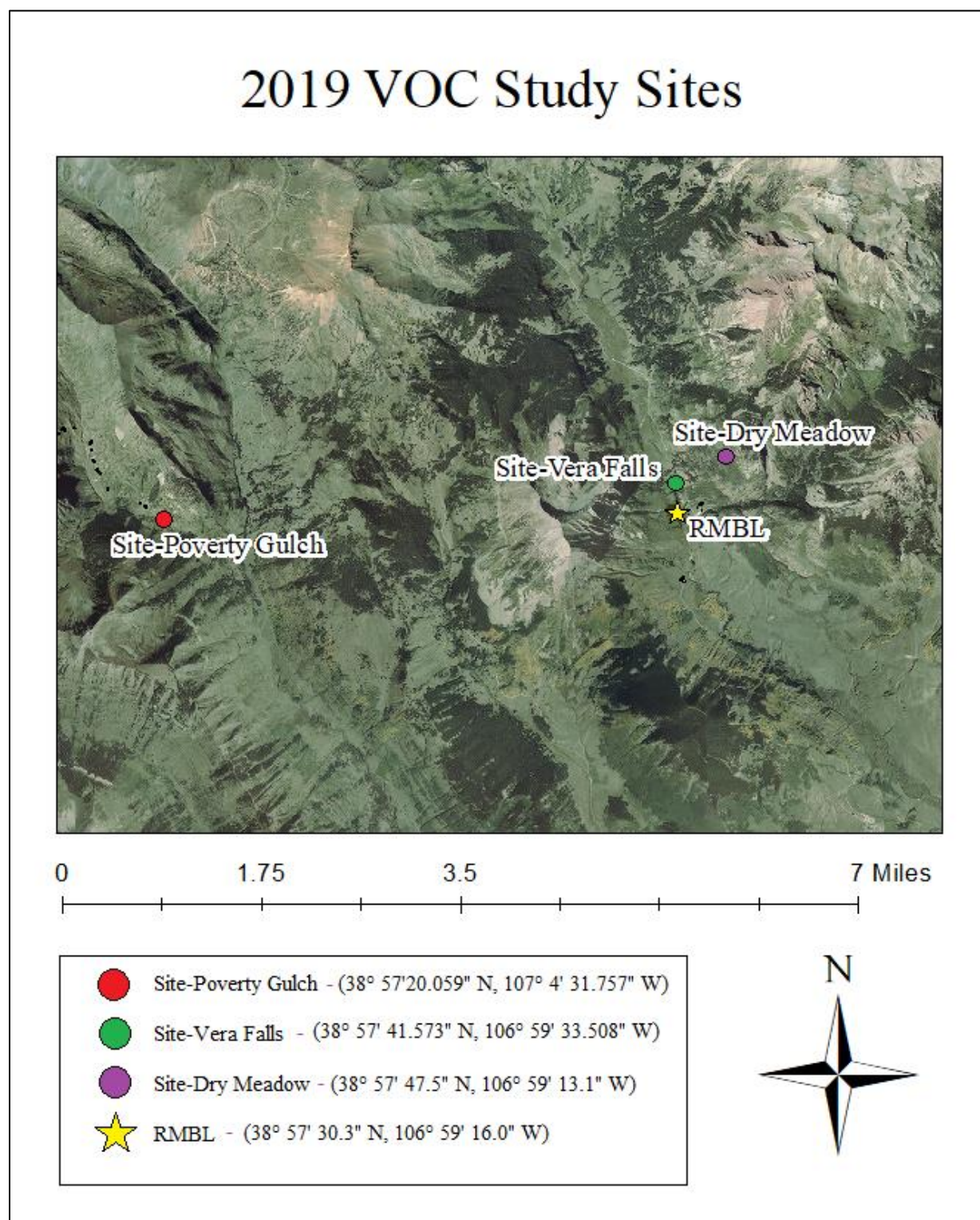


Figure 2. Scatterplot expressing the comparison between the log of the total peak area (quantifying the emission rate per sample) and the 24-hour nectar production in μL , $p = 0.28$. L = site “L” at Poverty Gulch, VF = site at Vera Falls.

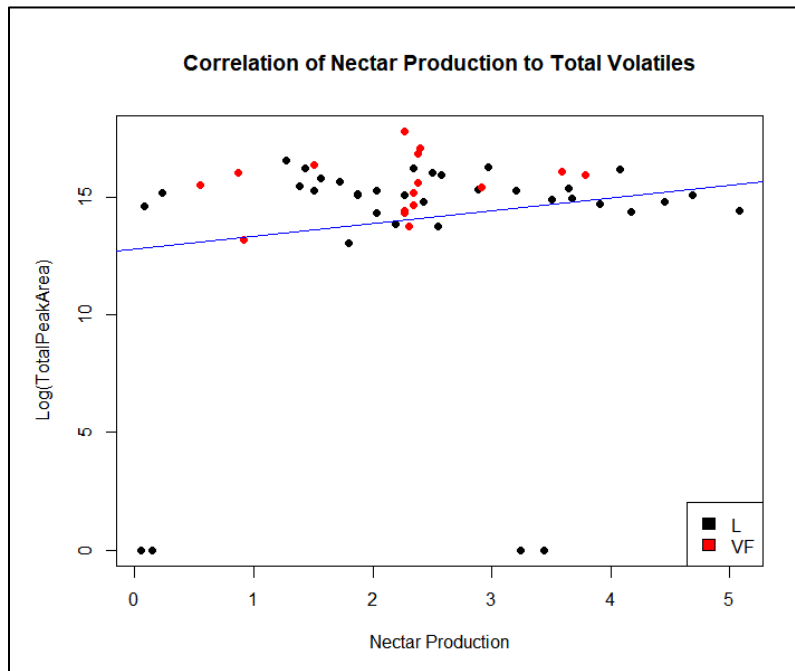


Figure 3. Scatterplot expressing the comparison between the log of the total peak area and the biomass in grams per sample, $p = 0.151$. L = site “L” at Poverty Gulch, VF = site at Vera Falls.

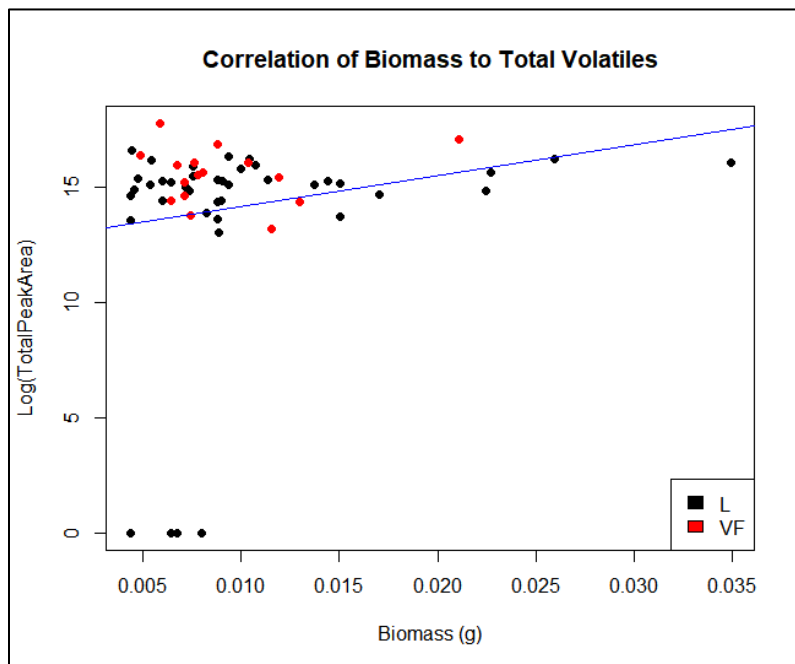


Figure 4. Scatterplot expressing the comparison between the log of the total peak area and the soil moisture in volumetric water content percent per sample, $p = 0.39$. L = site “L” at Poverty Gulch, VF = site at Vera Falls.

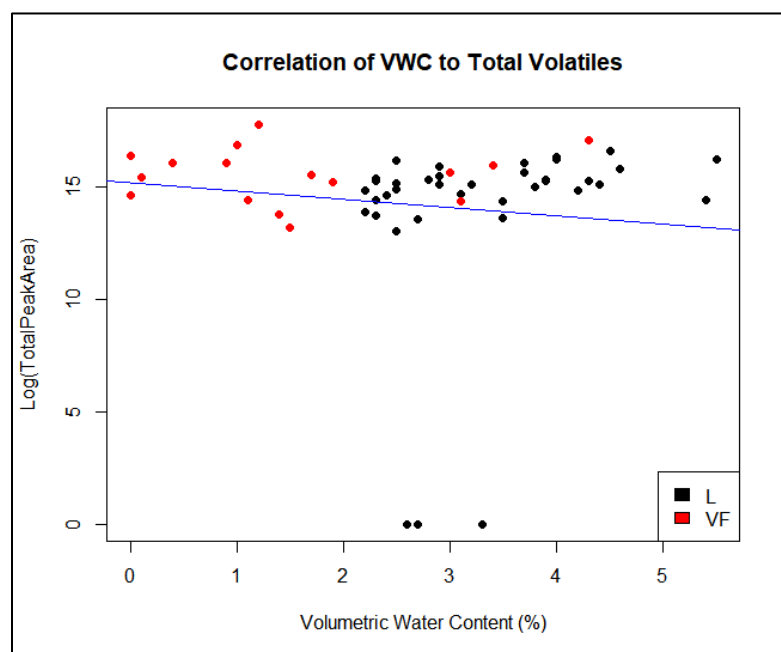


Figure 5. Nonmetric Multidimensional Scaling (NMDS) comparison of the proportion of volatile emissions across six plant species over the years of 2017 to 2019. Dissimilarity in chemical composition and proportion emitted by each species is represented as distance from one another in the NMDS, $p < 0.001$. Labels AC = *A. congesta*, AM = *A. millefolium*, CR = *C. rotundifolia*, ES = *E. subalpinum*, GB = *G. boreale*, and HV = *H. villosa*.

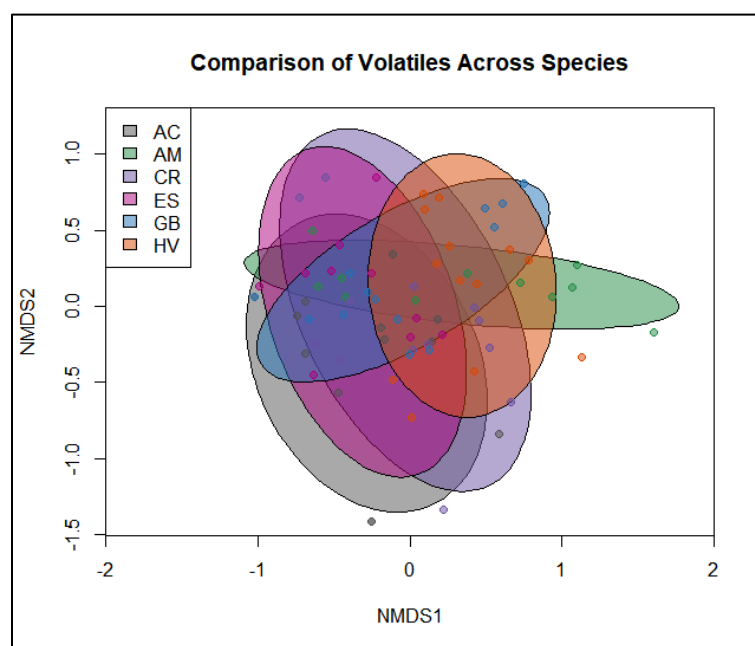


Figure 6. Comparison of the proportion of volatile emissions of six species in a dry meadow community across three years varying in environmental conditions. Dissimilarity in chemical composition and proportion emitted by the community per year is represented as distance from one another in the NMDS, $p < 0.001$. Labels are the years 2017, 2018, and 2019.

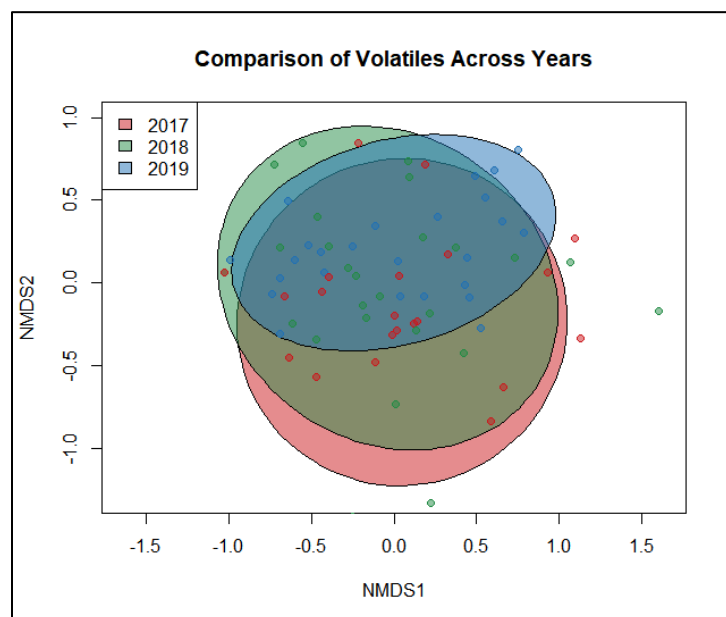


Figure 7. Comparison of volatiles across species and years using NMDS. Dissimilarity in chemical composition and proportion emitted by each species per year is represented as distance from one another in the NMDS, $p < 0.001$. Labels with 7, 8, and 9 equate to 2017, 2018, and 2019 respectively. AC = *A. congesta*, AM = *A. millefolium*, CR = *C. rotundifolia*, ES = *E. subalpinum*, GB = *G. boreale*, and HV = *H. villosa*.

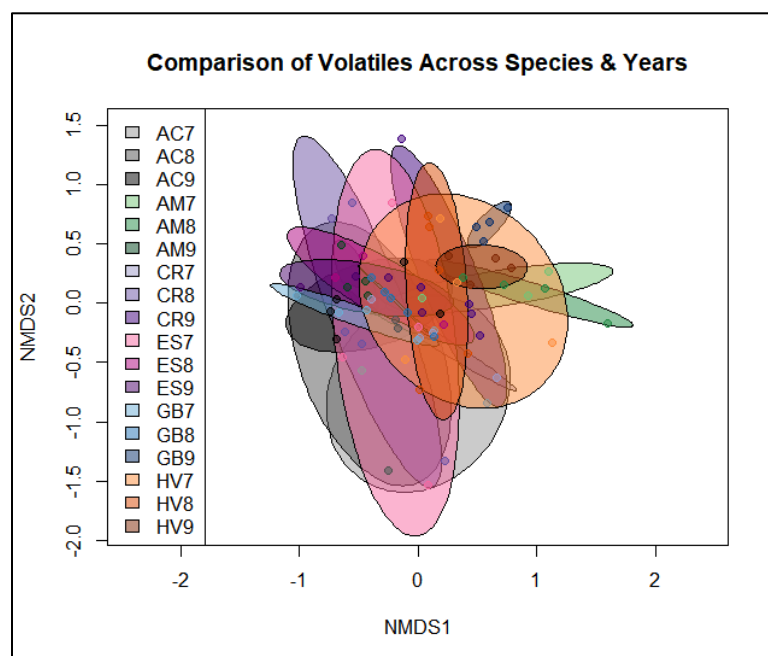


Figure 8. NMDS of all *G. boreale* samples grouped by year. Dissimilarity in chemical composition emitted by each *G. boreale* sample is represented as distance from one another in the NMDS. Composition and proportion of volatiles compared across years, $p = 0.002$.

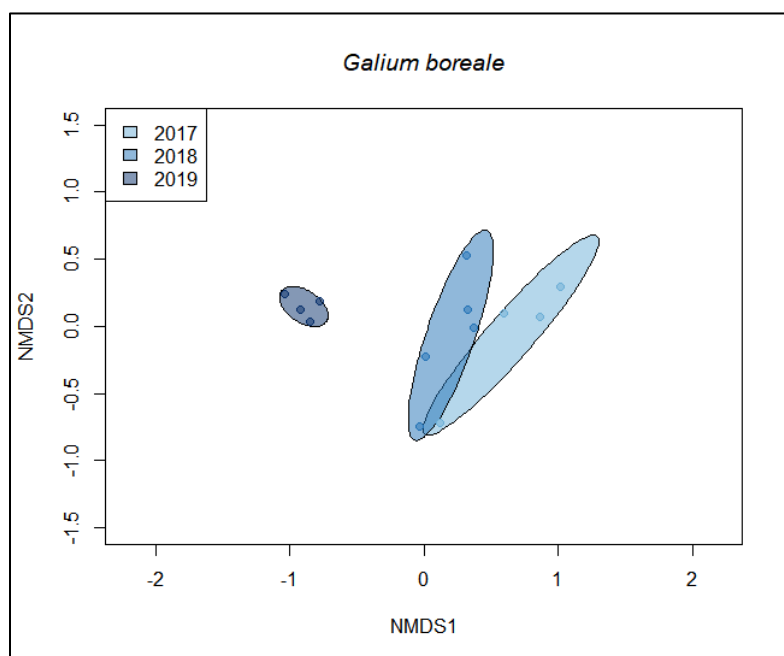


Figure 9. NMDS of all *C. rotundifolia* samples grouped by year. Dissimilarity in chemical composition emitted by each *C. rotundifolia* sample is represented as distance from one another in the NMDS. Composition and proportion of volatiles compared across years, $p = 0.012$.

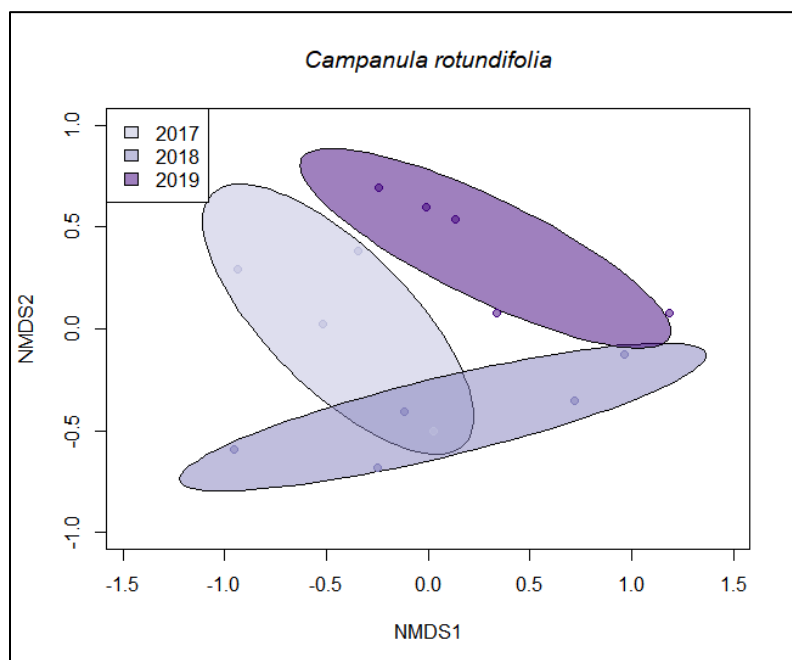


Figure 10. NMDS of all *H. villosa* samples grouped by year. Dissimilarity in chemical composition emitted by each *H. villosa* sample is represented as distance from one another in the NMDS. Composition and proportion of volatiles compared across years, $p = 0.121$.

