

Talking Yeast: The characterization of bumblebee- and nectar- specialist yeast volatiles in
Corydalis caseana
Natalie Rodriguez / Post Bacc. Program at RMBL

Abstract: I explored the role of microbial volatile organic compounds (mVOCs) in pollination processes, particularly focusing on a comparison of a nectar-specialist yeast species (*Metschnikowia reukaufii*) and a bumblebee-specialist yeast (*Starmerella bombi*). The paper aims to understand whether these yeasts produce different mVOC profiles in the same nectar environment, how nutrient availability affects their volatile profiles, and whether their metabolism interacts with floral volatiles. I conducted both *in vitro* and *in planta* experiments. The *in vitro* experiments involved examining mVOCs emitted by the two yeast species in synthetic nectars with varying amino acid compositions. Surprisingly, removing individual amino acids did not significantly impact volatile emissions. The results revealed distinct mVOC profiles for the two yeast species, validated through non-metric multidimensional scaling (NDMS) and permutational multivariate ANOVA (PerMANOVA) analyses. The *in planta* experiment assessed mVOCs produced by wildflower inflorescences inoculated with each yeast. *Corydalis caseana* flowers were inoculated with sterile nectar or nectar containing one of the yeast species. The volatile profiles of these yeast-inoculated flowers were then analyzed and compared with ambient and vegetative controls. This part of the study is ongoing, and the results are not yet fully analyzed. The study's findings suggest that the yeast species have distinct mVOC profiles and that amino acid removal might not drastically affect volatile emissions. This sheds light on the complex chemical interactions occurring during pollination, with potential implications for understanding plant-pollinator relationships, bee behavior, and floral ecology. Further analysis of the *in planta* experiment will likely provide more insights into the roles of these yeasts in the chemical ecology of pollination.

Introduction: Pollination is a way for pollen to travel from one flower to another, via animal pollinators like bees or birds, ultimately reaching its desired target on another plant of the same species. This is a convenient way for these sessile organisms to reproduce with plants other than their (often closely related) direct neighbors (Real, 2012). Pollination is important for most flowering plants (and thereby all terrestrial ecosystems) because it maintains genetic diversity, sustains healthy populations, and increases seed production. For these same reasons, pollination plays a crucial role in most forms of crop production (Gill et al., 2016, Khalifa et al., 2021).

In the past century, we have discovered that other organisms can influence plant-pollinator interactions. These other organisms comprise microbial communities that inhabit flowers. They play a role in influencing pollination through the production of volatiles: microbial volatile organic compounds (mVOCs). Volatile production is a form of chemical communication that serves as a universal language in terrestrial and aquatic ecosystems. Organisms use this form of communication in order to interact with one another with attractive, repellent, or defensive scents (Bagnères & Hossaert-Mckey, 2016). Microbial volatiles are perceived by the pollinators, thus modifying their perception of overall floral volatile profiles and influencing their behavior (Real, 2012 and Vannette, 2020). Microbes such as fungi, especially yeasts, and bacteria have been widely studied and recognized to influence an insect's association of scent (nectar chemistry) with the quality of foraged resources (nectar quality) (Hausmann et al., 2017, Schaeffer et al., 2019, Vannette, 2020, Wright & Schiestl, 2009).

Nectar is composed of sugars and amino acids, which are essential in the chemical production of volatiles. Microbes inhabiting nectar, particularly fermentative ascomycetous yeasts, produce a wide range of volatile compounds that can in some cases attract pollinators (Crowley-Gall et al., 2021, Dzialo et al., 2017). Recent studies have found that nectar-specialist yeast species sometimes participate in a three-way positive interaction: these yeasts inhabit the nectar of the flower, luring in pollinators with their attractive scents in order to be picked up and dispersed by the pollinator to new flowers, the plant benefits by pollinator visitation to disperse their pollen to others. The pollinator benefits from finding tasty food (Januário da Costa Neto & Benevides de Moraes, 2020, Sobhy et al., 2018). Many of these studies examine nectar-specialist yeasts like *Metschnikowia reukaufii*, however there are few studies that look at other specialist yeasts involved in pollination systems, such as bumblebee specialist yeasts like *Starmerella bombi*. The *Starmerella* genus has been described to be mostly isolated from bees (Januário da Costa Neto & Benevides de Moraes, 2020). This particular yeast over-winters in the digestive tracts of queen bumblebees and inhabits honey pots constructed in spring by the queen. Both yeasts have been found to be involved in plant-bumblebee mutualisms (Brysch-Herzberg, 2004), but these two species likely produce different volatiles, and may utilize amino acids and sugars differently (V. Martin, personal communication).

To broaden our understanding of different yeast species and their influence on the chemical ecology of pollination, this project looked at mVOC production by both the nectar-specialist yeast, *Metschnikowia reukaufii* and the bumblebee specialist yeast, *Starmerella bombi*. We made the following predictions:

- a) The two yeast species will produce different mVOC profiles in the same nectar environment,
- b) a yeast's volatile profile is dependent on nutrient availability, so removing key amino acids will change each yeast's volatile profile,
- c) both yeast species' profiles will be observed alongside floral volatiles in yeast-inoculated wildflower inflorescences, and
- d) both yeasts will reduce plant specialized metabolites in nectar and floral displays.

To test these predictions, we conducted *in vitro* experiments that examined mVOCs through the use of lab-made nectar (specifically testing hypotheses A, B, and D), as well as conducted an *in planta* experiment to compare volatiles of those in the lab with yeast-inoculated wildflower inflorescences (specifically testing hypotheses C and D).

Methods:

Study System:

In this experiment, we created a nectar mimic of the perennial herb, *Corydalis caseana* ssp. *brandegeei*. Historically, the reproductive biology, pollination ecology (including nectar robbing), and floral microbiome of *C. caseana* populations near the RMBL have been studied (Maloof, 2000, Irwin & Maloof, 2002). Field work was conducted at Washington Gulch ([38.940202](#), [-107.023029](#)) near the 403 trailhead (Figure 1). Prior to inoculation, *C. caseana* inflorescences (those budding and had not yet opened) were bagged with organza bags to ensure limited visitation by insects.

Experimental design:

To compare scent from nectar yeasts, their utilization of nectar nutrients, as well as their volatile profiles, we used two species of yeast: a bumblebee specialist yeast (*Starmerella bombi*) and a nectar specialist yeast (*Metschnikowia reukaufii*). The *S. bombi* strain was isolated from *Ipomopsis aggregata* near the RMBL and the *M. reukaufii* strain was isolated from *Corydalis caseana* in Washington Gulch in 2022.

Nectar mimics contained one or neither of these yeasts. The experiment was divided into an *in vitro* and an *in planta* experiment. In the *in vitro* experiment, we characterized volatile profiles from *M. reukaufii* nectar and *S. bombi* nectar in the lab, then in the *in planta* experiment, we characterized volatile profiles in yeast-inoculated wildflower inflorescences with *M. reukaufii* nectar, or *S. bombi* nectar at our field site in Washington Gulch. This allowed us to compare the lab volatile profiles and the field volatile profiles and fully characterize both yeasts.

In vitro experiment - amino acid removal:

Amino acids are essential components found in nectar and thought to be important for scent production by yeasts. To investigate the utilization of amino acids by these two different yeast species and characterize their volatile profiles, we created 21 nectars: a complete nectar and twenty additional synthetic nectars, each with one amino acid removed. In the lab, we first made our synthetic nectar that consisted of 35% sugar: fructose, sucrose, and glucose; this is equal to 11.667 g/100 mL. We made 300mL of synthetic nectar, so each sugar was measured out to 35 grams each. We added sterile deionized water to the sugar up to the volume of 300 mL and heated up this mixture until all the sugar dissolved. Each amino acid was added at a concentration of 4.3 mg/mL.

To collect the nectar scent, we put 1mL of nectar into the corresponding 22 mL labeled vial with a PTFE septum. Those with yeast (vials labeled *M. reukaufii* or *S. bombi*) were inoculated with the corresponding yeasts; at 1,000 cells/uL, incubated in the sealed vials at room temperature for 2 days to allow for growth and equilibration of volatiles in the headspace within the vial. On the day of scent collection, we put a clean charcoal filter 5 mm above the nectar for incoming air (made with a pasteur pipette, glass wool, and activated charcoal, to allow for clean air into the headspace) and a clean scent trap just peeking from below the seal top (Figure 2a.) through the top of the PTFE septum. These scent traps are 4 mm outer diameter tubes (2.6 mm inner diameter) made with 10 mg of Carboxen (40-60 mesh), 20 mg of Tenax TA (60-80 mesh), and silanized glass wool (Figure 2b.) (Rering et al. 2020). When ready to sample, the exposed part of the scent trap was connected to a micro air sampler (Supelco PAS-500). Scent samples were collected with a flow rate of 20 mL min⁻¹ for 10 minutes.

In vitro experiment - comparison of mVOC profiles emitted by *M. reukaufii* and *S. bombi*:

We did not find any evidence of effects of amino acid removal on the volatile emissions by these two yeast species (see Results). Thus, we pooled the approximately 20 samples of each yeast species in this experiment and compared the mVOC profiles of the two species.

In vitro experiment - reduction by yeasts of plant volatile emissions from nectar

0.1 uL/mL of acetophenone, beta-ocimene, and phenylacetaldehyde were added individually to aliquots of *Corydalis* nectar mimic (see the following section for *Corydalis* nectar nutrient composition). These volatile compounds were previously detected from natural *Corydalis* and *Mertensia* nectar samples (V. Martin, personal communication). Three 1 mL replicates of each nectar were inoculated with *M. reukaufii* at a concentration of 1,000 cells/uL in

22 mL vials and incubated for two days at room temperature. An additional three replicates were kept sterile and incubated for the same two-day period. Scent collection was completed using the same materials and methods as for the amino acid removal *in vitro* experiment.

In planta experiment:

To test hypotheses C and D, we characterized volatiles produced from both yeasts alongside floral volatiles in yeast-inoculated wildflower inflorescences and assessed whether both yeast species reduce plant specialized metabolites in nectar. We inoculated all open flowers of a *Corydalis caseana* inflorescence (15-40 flowers) with 5 μ L of sterile nectar, *M. reukaufii* nectar, or *S. bombi* nectar with a 50 μ L Hamilton syringe. Nectar mimics were made with 35% sucrose concentration with amino acid concentrations mirroring natural *C. caseana* nectar and either kept sterile or inoculated with *M. reukaufii* or *S. bombi*. For each treatment, we inoculated five replicates each day for two days. After inoculation, treated inflorescences were re-covered with the organza bag for at least an hour to allow for yeast volatile production and any plant response to the introduction of yeast cells to the flower. Once an hour had passed, treated inflorescences were enclosed in a Reynold's oven bag. Headspace volatiles were allowed to equilibrate for 15 min. Once equilibrated, a clean scent trap was inserted into the bag. The exposed end of the scent trap is connected to tubing that is connected to a micro air sampler (Supelco PAS-500). The micro air sampler is attached to a tripod to avoid breaking the stems of the plant and stabilizing the scent trap so that it doesn't come out of the oven bag when sampling. Scents were sampled at a flow rate of 100 mL min⁻¹ for 10 minutes. The components of the scent traps are the same as the *in vitro* experiment (Campbell et al. 2019).

Volatile Analysis:

All scent samples were analyzed using the GC-MS (Gas Chromatograph-Mass Spectrometer) that is located at Rocky Mountain Biological Laboratory. Scent traps were placed into stainless steel desorption tubes and put into the Markes autoloading system, where the compounds collected on the scent trap undergo two-stage thermal desorption. First, the scent traps were heated to 220°C in a Markes desorption tube with helium flowing at a rate of 75 mL/min through the tube. The compounds are released from the scent trap into the helium gas and transferred to the Markes cold trap, a glass tube packed with Tenax adsorbent held at -30°C. The Markes machine then heats the cold trap to 220°C at a rate of 40°C/min and transfers the sample via helium gas to the GC column (30 m HP5 nonpolar column). Here, heat steadily increases in the column oven as the compounds travel through the column matrix. As each compound exits the end of the column, it is fragmented and ionized in the mass spectrometer, which then counts the number of each fragment ion size (in our method, ions between 33-300 Daltons) multiple times per second, creating a mass spectrum every 0.05 minutes. Compounds were tentatively identified using Shimadzu GCMS Postrun software to match spectra with those from the National Institute of Standards and Technology (NIST) mass spectral reference library.

Data Management:

The Shimadzu GCMS Postrun software displays a chromatogram of the sample and mass spectrums of all compounds detected. Compound emissions were measured as peak area, which is recorded on an excel sheet with unique compounds as rows. The columns will correspond to each of the samples.

Statistical Analysis:

All analyses were done in R (4.2.1). To filter the compounds and remove contaminants, we filtered with an 80% match and contaminants were removed using the R package, *bouquet* (Powers et al. 2021). The volatile profiles for the ambient air and vegetative controls were compared to the yeast volatile profiles using the function *bouquet* to dismiss those from the analysis that appeared in similar quantities. To explore peaks list from GC-MS and extract certain patterns, we used *enviGCMS*. PermaNOVA was used to compare volatile profiles and determine whether species matter for scent using the *adonis* function in *vegan*, and their volatile profiles were visualized using non-metric multidimensional scaling (NMDS) based on Bray–Curtis dissimilarities. The mean of the ranked dissimilarities between the control and two yeast species were compared to the mean of ranked dissimilarities within groups through an analysis of similarities test (DESEQ2) using the *deseq2* function in *vegan*.

Results:

In vitro experiment - amino acid removal:

We observed no differences in volatile emissions from either of the yeast species when single amino acids were eliminated from the synthetic nectars. While we did not test enough replicates to be able to do a statistical comparison, the peak areas were approximately the same for the detected yeast metabolites across all amino acid removal nectars.

In vitro experiment - comparison of mVOC profiles emitted by *M. reukaufii* and *S. bombi*:

In total, we collected microbial volatiles from 60 nectar vials (plus blank vials); each set of vials (3) were missing one amino acid and were either inoculated with *M. reukaufii*, *S. bombi*, or were sterile. A non-metric multidimensional scaling (NDMS) plot revealed the separation of these two yeast species based on the volatiles they produced (Figure 3). To further validate that there was a separation of emissions between these two yeast species, we ran a permutational multivariable ANOVA (PerMANOVA) statistical analysis. The PerMANOVA (Table 1) validated that the emission of volatiles from both yeast differ significantly ($p < 0.001$). The sampling of the nectar vials yielded a total of 14 compounds identified in nectar inoculated with *M. reukaufii* or *S. bombi* after filtering out any contamination and compounds in the blank vials and traps (Table 2). A DESEQ2 test for the two yeast species further resulted in seeing variances in emission abundance of certain compounds from the 14 identified to be significantly different. (Figure 4).

In vitro experiment - reduction by yeasts of plant volatile emissions from nectar

M. reukaufii greatly reduced the emissions of phenylacetaldehyde, acetophenone, and beta-ocimene from nectars. While we did not have enough replicates for meaningful statistical comparisons, the peak areas of each plant VOC standard were reduced approximately 10-fold in *M. reukaufii*-inoculated nectars compared to sterile nectars that were incubated for the same two-day period.

Discussion:

The combination of the NDMS plot and PerMANOVA analysis concluded that *M. reukaufii* and *S. bombi* have separate volatile profiles, yet they do share some volatiles (14 identified). This is interesting considering the two yeast come from different clades. Between the 14 shared volatiles, 8 were found to be significantly different in emission abundance. Differences in emission abundance refers to the peak area of the identified compound. A higher peak area

corresponds to higher emissions of that volatile compound by the yeast culture. The yeast *M. reukaufii*, was found to have emitted more 1-pentanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 2-methyl-1-propanol, 3-methyl-1-butanol acetate, 2-methyl-1-butanol acetate, ethyl ester, acetic acid, and butyl ester volatiles. Uniquely, *S. bombi* emitted more 4-ethoxy-benzoic acid volatiles. Future studies regarding this piece of information can further investigate its effects on bee behavior and what volatiles attract them.

An aspect that we aimed to investigate was the utilization of nutrients by yeast. Yeast utilize nutrients like sugars and amino acids to perform fermentation, which results in the production of compounds, many being volatiles. More specifically, amino acids have direct correlations to higher alcohol formation through the Ehrlich pathway: three-step process of transmission, decarboxylation, and reduction. I hypothesized that if we removed an amino acid from our synthetic nectar, that we would see a reduction in volatile emissions, specifically higher alcohols. However, we did not see this. In fact, we saw no differences in emission if one amino acid was eliminated. This could be because when in short supply, pathways will favor anabolic routes (Dzialo et al., 2017).

Another finding was the reduction of three plant VOCs due the presence of *M. reukaufii*. Specifically, we saw reductions in phenylacetaldehyde, acetophenone, and beta-ocimene from nectars. Uniquely, microorganisms that inhibit flowers have the ability to reduce or increase certain floral volatiles (Rering et al., 2021, Vannette & Fukami, 2016). Additionally, changes in secondary metabolite concentrations can have impacts on the attractiveness to pollinators. Reductions of these metabolites have been found to influence pollinator consumption of the nectar, thus indirectly affecting foraging behavior (Vannette & Fukami, 2016).

Ongoing Analysis

We are still in the process of analyzing the samples from the *in planta* experiment. All scent traps have gone through the GC-MS and produced a chromatogram to which we have manually gone through the first 22 minutes of the sample and identified all peaks present in two ambient samples, a vegetative sample, and one yeast-inoculated inflorescence sample that has our target compounds at higher concentrations. After that, we compared the compounds we identified with the compounds the Shimadzu software identified during automatic peak integration and removed any automatically detected compounds that were not manually verified. We are currently going through the compounds that were not present on the automatically computed list and adding them manually by providing the ion ratios of the three highest ions present from each compound. We will then create a batch list with all *Corydalis* inflorescence samples and apply our compound identification and quantification method. This process results in a .txt file output that will then be converted in R to a .csv file of all compounds found in each sample. We can then complete background subtraction (removing volatile compounds found in the blanks at the same concentrations) and then perform statistical analyses.

Conclusion and Future Directions:

In conclusion, this study aims to connect microbial volatile organic compounds (mVOCs) and their influence on pollination dynamics. By examining common yeast species like *M. reukaufii* and not so well studied yeast species like *S. bombi*, we can get a better understanding of microorganisms' role in shaping these plant-pollinator interactions. Through the combination of *in vitro* and *in planta* experiments, we can conclude that the two yeast species possess distinct mVOC profiles. Surprisingly, the removal of individual amino acids from synthetic nectars did

not significantly alter volatile emissions, challenging conventional assumptions about the direct correlation between amino acids and higher alcohol formation via the Ehrlich pathway. Also, we can conclude the impact of *Metschnikowia reukaufii* on plant volatile emissions. This nectar-specialist yeast was found to substantially reduce the emissions of specific plant volatile compounds, potentially influencing the chemical makeup of nectar and subsequently affecting pollinator attraction and behavior.

The next step is to finish the data analysis for the *in planta* volatile samples. From there, we can identify the top microbial volatiles and conduct an olfactometer experiment. Here, we will be using volatile standards/different yeast species and investigating their effects on bee behavior (choice experiment). With a notable difference in volatile profiles from the two yeast species used in this experiment, our next step is to compare different yeast species and characterize those volatiles. This will allow us to ask questions regarding whether differences or similarities between volatile emissions are phylogenetic.

Acknowledgements:

Thank you to Valerie Martin, Daniel Souto, Nick Dabagia, Drew Freshour, Jade Stryker, Becky Irwin, and Robert Schaeffer for all the support, mentorship, and help throughout this project. Thank you to the Rocky Mountain Biological Laboratory for the opportunity to conduct research here this summer and the National Science Foundation for funding the project and traveling. Finally, thank you to my friends and family for the never-ending love and support.

References:

- Adler, L. S., Michaud, K. M., Ellner, S. P., McArt, S. H., Stevenson, P. C., & Irwin, R. E. (2018). Disease where you dine: Plant species and floral traits associated with pathogen transmission in bumble bees. *Ecology*, 99(11), 2535–2545. <https://doi.org/10.1002/ecy.2503>
- Bagnères, A.-G., & Hossaert-Mckey, M. (2016). *ChemicalEcology_2016.pdf*. ISTE Ltd and John Wiley & Sons, Inc.
- Brysch-Herzberg, M. (2004). Ecology of yeasts in plant–bumblebee mutualism in Central Europe. *FEMS Microbiology Ecology*, 50(2), 87–100. <https://doi.org/10.1016/j.femsec.2004.06.003>
- 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>
- Crowley-Gall, A., Rering, C. C., Rudolph, A. B., Vannette, R. L., & Beck, J. J. (2021). Volatile microbial semiochemicals and insect perception at flowers. *Current Opinion in Insect Science*, 44, 23–34. <https://doi.org/10.1016/j.cois.2020.10.004>
- Dzialo, M. C., Park, R., Steensels, J., Lievens, B., & Verstrepen, K. J. (2017). Physiology, ecology and industrial applications of aroma formation in yeast. *FEMS Microbiology Reviews*, 41(Supp_1), S95–S128. <https://doi.org/10.1093/femsre/fix031>
- Gill, R. J., Baldock, K. C. R., Brown, M. J. F., Cresswell, J. E., Dicks, L. V., Fountain, M. T., Garratt, M. P. D., Gough, L. A., Heard, M. S., Holland, J. M., Ollerton, J., Stone, G. N., Tang, C. Q., Vanbergen, A. J., Vogler, A. P., Woodward, G., Arce, A. N., Boatman, N. D., Brand-Hardy, R., ... Potts, S. G. (2016). Chapter Four - Protecting an Ecosystem Service: Approaches to Understanding and Mitigating Threats to Wild Insect Pollinators. In G. Woodward & D. A. Bohan (Eds.), *Advances in Ecological Research* (Vol. 54, pp. 135–206). Academic Press. <https://doi.org/10.1016/bs.aecr.2015.10.007>
- Hausmann, S. L., Tietjen, B., & Rillig, M. C. (2017). Solving the puzzle of yeast survival in ephemeral nectar systems: Exponential growth is not enough. *FEMS Microbiology Ecology*, 93(12), fix150. <https://doi.org/10.1093/femsec/fix150>
- Irwin, R. E., & Maloof, J. E. (2002). Variation in nectar robbing over time, space, and species. *Oecologia*, 133, 525–533.
- Januário da Costa Neto, D., & Benevides de Moraes, P. (2020). The vectoring of *Starmerella* species and other yeasts by stingless bees in a Neotropical savanna. *Fungal Ecology*, 47, 100973. <https://doi.org/10.1016/j.funeco.2020.100973>
- Khalifa, S. A. M., Elshafiey, E. H., Shetaia, A. A., El-Wahed, A. A. A., Algethami, A. F., Musharraf, S. G., AlAjmi, M. F., Zhao, C., Masry, S. H. D., Abdel-Daim, M. M., Halabi, M. F., Kai, G., Al Naggar, Y., Bishr, M., Diab, M. A. M., & El-Seedi, H. R. (2021). Overview of Bee Pollination and Its Economic Value for Crop Production. *Insects*, 12(8), Article 8. <https://doi.org/10.3390/insects12080688>
- Maloof, J. E. (2000). Reproductive biology of a North American subalpine plant: *Corydalis caseana* A. Gray ssp. *brandegei* (S. Watson) GB Ownbey. *Plant Species Biology*, 15(3), 281–288.
- Real, L. (2012). *Pollination Biology*. Elsevier.
- Rering, C. C., Vannette, R. L., Schaeffer, R. N., & Beck, J. J. (2020). Microbial Co-Occurrence in Floral Nectar Affects Metabolites and Attractiveness to a Generalist Pollinator. *Journal of Chemical Ecology*, 46(8), 659–667. <https://doi.org/10.1007/s10886-020-01169-3>

- Schaeffer, R. N., & Irwin, R. E. (2014). Yeasts in nectar enhance male fitness in a montane perennial herb. *Ecology*, 95(7), 1792–1798. <https://doi.org/10.1890/13-1740.1>
- Schaeffer, R. N., Rering, C. C., Maalouf, I., Beck, J. J., & Vannette, R. L. (2019). Microbial metabolites elicit distinct olfactory and gustatory preferences in bumblebees. *Biology Letters*, 15(7), 20190132. <https://doi.org/10.1098/rsbl.2019.0132>
- Sobhy, I. S., Baets, D., Goelen, T., Herrera-Malaver, B., Bosmans, L., Van den Ende, W., Verstrepen, K. J., Wäckers, F., Jacquemyn, H., & Lievens, B. (2018). Sweet Scents: Nectar Specialist Yeasts Enhance Nectar Attraction of a Generalist Aphid Parasitoid Without Affecting Survival. *Frontiers in Plant Science*, 9. <https://www.frontiersin.org/articles/10.3389/fpls.2018.01009>
- Powers, J., Eisen, K., Campbell, D., & Raguso, R. (2021). bouquet: Filter and Augment Biological GC-MS Data. R package version 0.0.0.1. <https://github.com/jmpowers/bouquet>
- Vannette, R. L. (2020). The Floral Microbiome: Plant, Pollinator, and Microbial Perspectives. *Annual Review of Ecology, Evolution, and Systematics*, 51(1), 363–386. <https://doi.org/10.1146/annurev-ecolsys-011720-013401>
- Wright, G. A., & Schiestl, F. P. (2009). The evolution of floral scent: The influence of olfactory learning by insect pollinators on the honest signaling of floral rewards. *Functional Ecology*, 23(5), 841–851. <https://doi.org/10.1111/j.1365-2435.2009.01627.x>

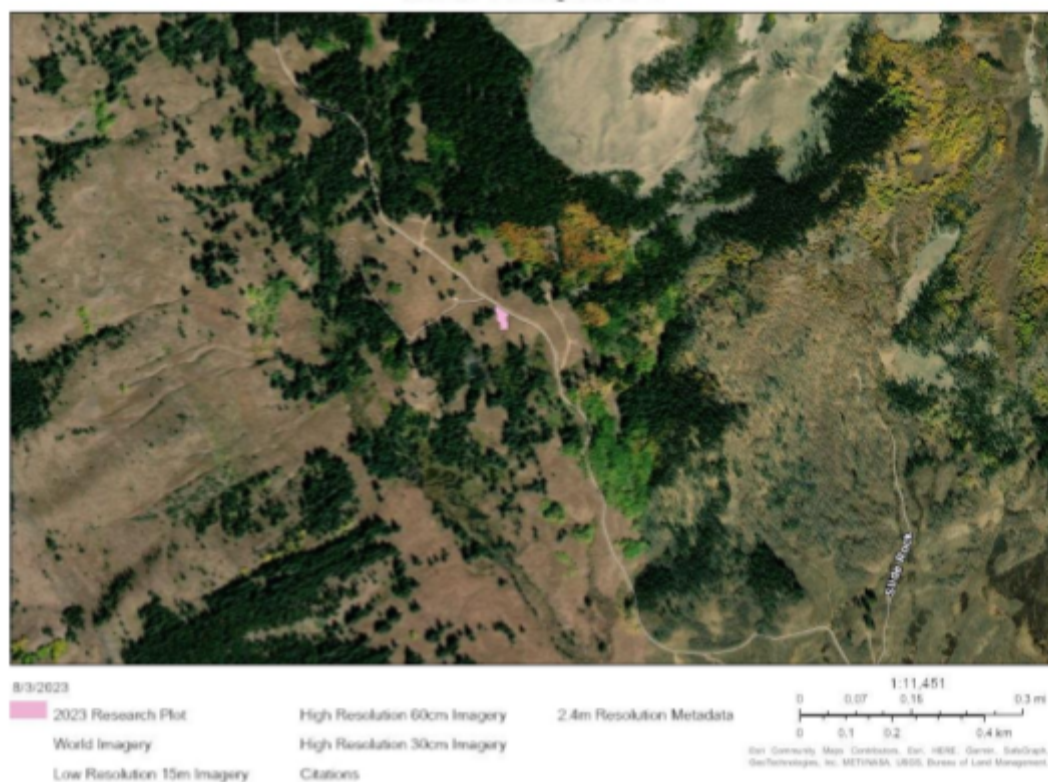


Figure 1: Map of *Corydalis caseana* population used in research.

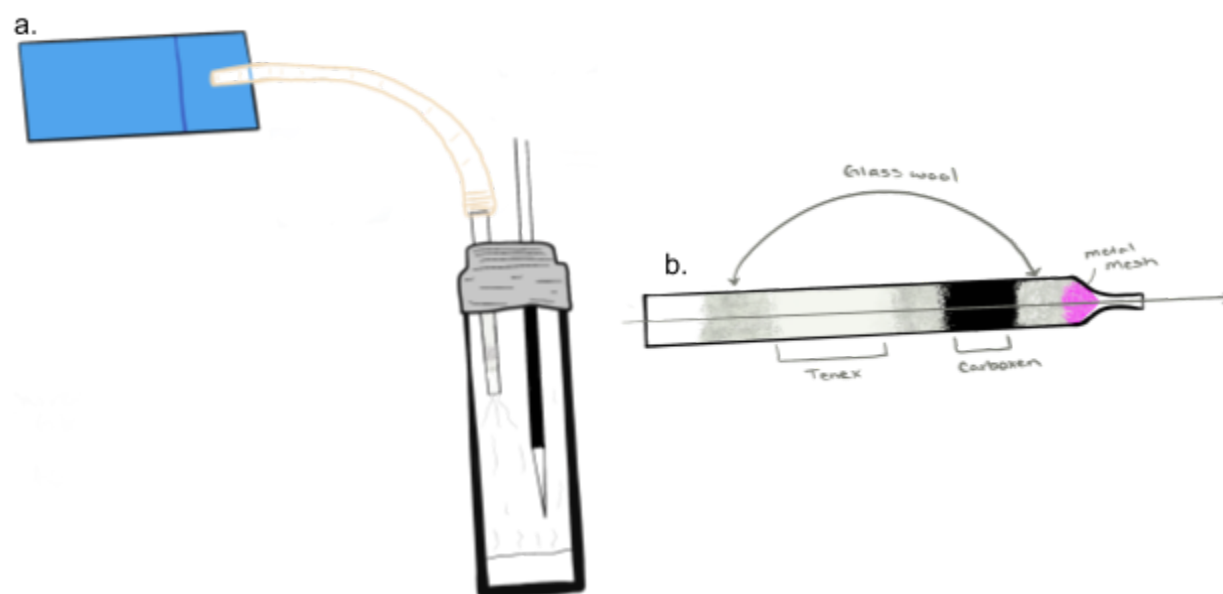


Figure 2: Nectar Scent Collection, a) nectar scent collection onto a scent trap from 1 mL of nectar in a glass vial, with clean air entering the vial during sampling via a charcoal-filled pasteur pipet, b) scent trap visual with direction of airflow.

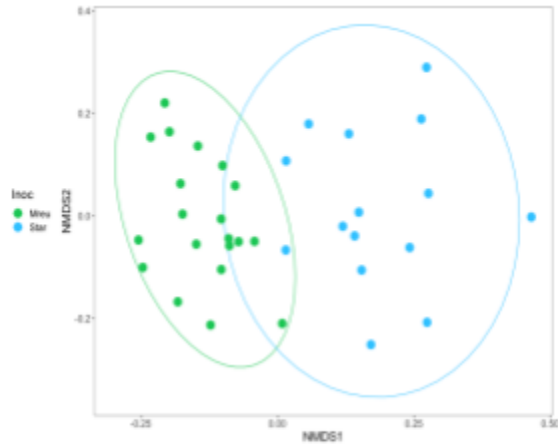


Figure 3: Microbial volatiles from *Metschnikowia reukaufii* (green) and *Starmerella bombi* (blue) were sampled from vials of nectar that were missing an amino acid to investigate the effects of amino acids on volatile emissions. A Non-metric multidimensional scaling (NDMS) plot allowed us to visually see the separation of volatiles emissions from both yeast.

Table 1. PerMANOVA statistical analysis of microbial volatiles produced by *Metschnikowia reukaufii* and *Starmerella bombi* inoculated nectars in vials.

	Df	Sum of Squares	p-value
Yeast Species	1	1.1831	0.001 ***
Residual	33	1.2693	
Total	34	2.4525	

Statistical analysis supports the NMSD visualization of the two yeast species and their prospective volatile profiles; volatile profiles differ significantly ($p < 0.001$). Sample size ($n=70$).

Table 2. Compounds Identified (14) in Nectar Inoculated with *Metschnikowia reukaufii* or *Starmerella bombi*

Alcohols	Esters	Other Compounds
*2-methyl-1-propanol	*3-methyl-1-butanol acetate	2,4-dimethyl-1-heptene
*3-methyl-1-butanol	*2-methyl-1-butanol acetate	Propanoic acid, 2-methyl-, anhydride
*2-methyl-1-butanol	*Benzoic acid, 4-ethoxy-, ethyl ester	Unidentified compound
*1-pentanol	*Acetic acid, butyl ester	
Phenylethyl alcohol		
3-Hexanol, 5-methyl		
2-Ethyl-1-hexanol		

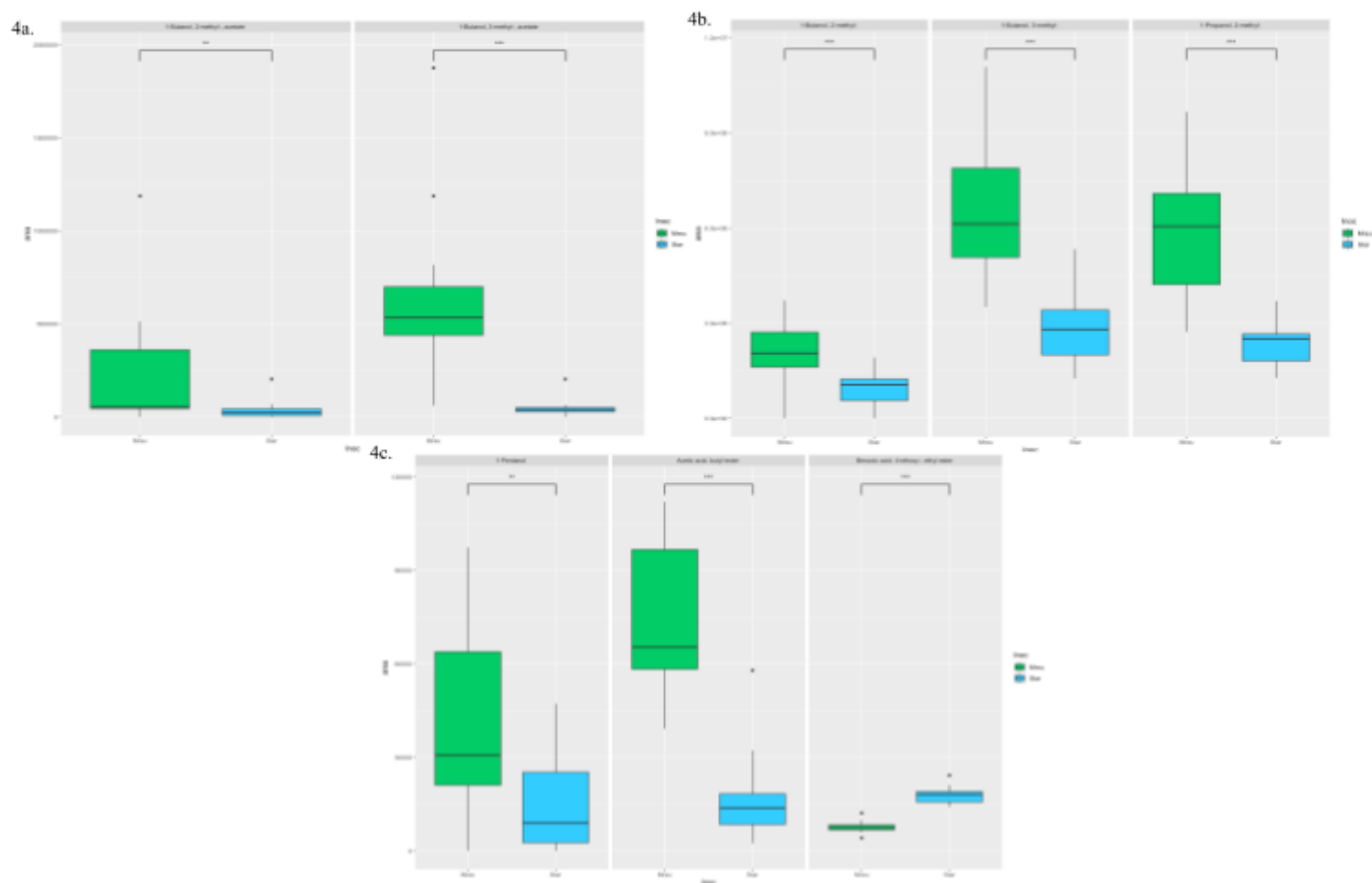


Figure 4. Variances of acetate (4a.), alcohol (4b.), and other compounds (4c.) volatile emissions between *Metschnikowia reukaufii* (green) and *Starmerella Bombi* (blue).