Investigating the potential mechanism behind bumble bee preference for *Corydalis* flowers inhabited by nectar specialist yeast

Abstract

Flowering plants are in an evolutionary battle for the attention of pollinators to increase their fitness. However, microbes are increasingly recognized as key players in mediating interactions between plants and pollinators. Most studies of floral microbes have focused on the role of obligate nectar yeasts (e.g., *Metschnikowia reukaufii*), which increase flower preference in bumble bees through their ability to alter nectar chemistry and add fermentation volatiles to floral scent. However, these studies were conducted on a limited number of bumble bee species, so it is not known to what extent preference for yeast-colonized flowers is exhibited across a wider array bumble bee species. Furthermore, the chemistry of *Metschnikowia rekaufii* could alter the dynamics of mutualism by influencing the behavior of floral visitors such as legitimate visitors and nectar robbers. Therefore, we investigated how bumble bees with different foraging tactics reacted to yeast-associated volatile organic compounds (VOCs). By performing choice assays on wild-caught bumble bees with different foraging strategies using a Y-tube olfactometer, we found that facultative secondary robbers display a preference for yeast-inoculated *Corydalis caseana* flowers, whereas bumble bee pollinators did not. This suggests that yeast VOCs may only be utilized as a cue for the presence of nectar by secondary robbers. Further choice assays with the secondary robber bees showed that a synthetic, simplified blend of yeast-associated VOCs did not elicit the same preference and that they only preferred yeast volatiles when combined with floral volatiles. Ultimately, these results inform our understanding of how ecological context mediates bumble bee preference for yeast-colonized flowers.

Introduction

To successfully select for rewarding flowers, pollinators must interpret a variety of cues, from floral morphology to chemical signals, in order to assess the quality and quantity of nectar and pollen in flowers (1). Flowers are therefore in an evolutionary battle for the attention of pollinators to increase their fitness (2). A third party is increasingly recognized as a key player mediating plant-pollinator interactions: microbes (3). While our understanding of the interactions between flowers, bees and microbes is still in its infancy, these relationships have a long and complex history, where both parties have used cooperative and exploitative strategies (3). Understanding the dynamics and potential mutualism in this three-way relationship is therefore essential to better understand and conserve bee pollinator communities, which provide an estimated $195 to $387 billion in ecosystem services globally (4).

Various yeast species (mainly of the genus *Metschnikowia*) have evolved to colonize flowers by settling in the nectar, consuming a portion of the sugars, and scavenging amino acids (5). The yeast alters nectar properties by lowering the sugar and amino acid content, reducing the pH, and increasing the floral temperature. Additionally, plants naturally release volatile organic compounds (VOCs) - organic compounds that have a high vapor pressure, thus are commonly found in their gas state (6). However, fermentation of nectar by yeasts alters the composition of VOCs through the production of microbial VOCs, such as ethyl acetate and ethanol (3). Moreover, the altered floral VOCs are suspected to serve as a cue for nectar availability and quality. Insect pollinators are highly sensitive to shifts in VOC abundance and identity, which affects their foraging preferences and mediates learning (7). In particular, *Metschnikowia reukaufii*, a ubiquitous and well-characterized flower-colonizing yeast, has been found to attract honeybee and bumble bee species by luring them to flowers and extending

foraging time on flowers (8–10). Similarly, Schaeffer et al. (2014) demonstrated pollinator preference for yeast-inoculated nectar on flowers of *Delphinium nuttalianum* and *D. barbeyi*, highlighting the growing understanding of yeast-insect-plant interactions in wildflowers of the Colorado Rocky Mountains (11). However, current research has only characterized the yeast-mediated preference of a handful of bumble bee species *(B. impatiens, B. terrestris, B. vosnesenskii, B. appositus, and B. friseanus*), which are a small fraction of the existing bumble bee biodiversity (8–10, 12). As such, more studies are needed exploring the effect of *M. reukaufii* in nectar on other bumble bee species to understand how generally the attractant effect is on multiple species.

M. rekaufii-induced changes on nectar chemistry could alter mutualism dynamics by affecting the behavior of floral visitors such as legitimate visitors and nectar robbers. Nectar robbers can steal nectar by either creating a hole on the side of a flower (a primary robber) or take advantage of an existing hole (a secondary robber) (13). Furthermore, research has found which species can act as robbers, how they do so, and the costs incurred by plants (13). While nectar robbing has been widely documented to have primarily negative consequences for plant fitness, the underlying motive behind why pollinators choose to be either a primary or secondary robber instead of a legitimate visitor remains unknown (14). Given that the presence of yeast in nectar can alter a flower's reward chemistry along with floral visitor behavior, and the dependence of flower visitors to disperse yeast between flowers, the presence of yeast chemical cues may be a factor mediating floral visitor behavior (15, 16). However, bumblebee behavioral responses to yeast-inoculated nectar when using foraging strategies other than legitimate visitation have seldomly been studied.

We addressed whether bumble bee workers with different foraging tactics displayed a preference for yeast-associated volatile organic compounds (VOCs) within the floral reward. We performed behavioral observations on wild-caught bumble bee workers using a Y-tube olfactometer assay. Given that there is no prior literature establishing whether bumble bees prefer yeast-colonized *Corydalis caseana* flowers, we first established a baseline of whether bumble bee pollinators or facultative secondary robbers display preference for yeast-inoculated *Corydalis caseana* flowers, then yeast-inoculated nectar, and finally a blend of individual yeast-produced compounds inoculated in *Corydalis caseana* flowers. With these experiments we asked: (1) do legitimate visitors and facultative secondary robbers display a preference for yeast-inoculated flowers? (2) Is the response of these flower visitors more pronounced when we remove floral bouquet compounds and focus exclusively on yeast-inoculated nectar? And (3) could flowers treated with nectar containing a synthetic, simplified blend of yeast-produced compounds elicit preference?

Research on plant-yeast-bumble bee interactions in this study system in 2023 showed no significant preference for yeast-inoculated flowers by *B. appositus*, *Corydalis caseana*'s most abundant legitimate visitor (Souto-Vilarós et al. in prep). In contrast, facultative secondary robbers, *B. bifarius* and *B. flavifrons,* located robbing wounds more quickly on yeast-inoculated flowers (Souto-Vilarós et al. in prep), suggesting that yeast-mediated cues may be more attractive to nectar robbers. Thus, we predicted to see a pattern of preference by secondary nectar robbers for flowers and nectar containing yeast-associated volatiles throughout the study and no preference exhibited by legitimate visiting bumble bees.

Methodology

Flower and bumble bee collection and release

We conducted this study using *Corydalis caseana* flowers, from four large populations across Washington Gulch, near Crested Butte, Colorado, where nectar robbing by bumble bees is widespread. For the supply of *Corydalis* flowers for our experiment, we covered inflorescences in bud stage with mesh bags to prevent microbial contamination by flower visitors. On the days when the assays are carried out, we cut a flower stalk from two individual plants and placed them in transparent water containers to prevent the flowers from drying out. For our supply of native bumble bees, we collected two species of bumble bees: *B. appositus* workers*,* who primarily engaged in legitimate visitation (10), and *B. bifarius* workers that engaged in secondary robbing on *Corydalis* flowers (13, 17). For each bumble bee, we first observed 3 flower visits to determine the type of foraging tactic used by the bumble bee, and then captured them mid-forage using a net. Bumble bees were kept in pop-cap tubes with holes punched in the top and placed in a cooler containing an ice pack where they were starved until the testing began. Both the bumble bees and flowers were transported to the Gothic Research Center (GRC) and tested in the Wet Lab. After running through the assay, the bumble bees were placed and housed overnight within the lab individually in prepared deli cups filled with nectar analog and a couple of flowers to reduce stress. Before being released the next day at the site of capture, all the tested bees were marked with a nontoxic paint marker dot on their thorax to prevent recapture and retesting of the same individuals. In total, 16 inflorescences and 82 female bumble bee workers were used over the course of the three stages of experimentation with 40 bumblebees tested in stage 1 (19 *B. bifarius* and 21 *B. appositus*) and another batch of 21 *B. bifarius* bumble bees in stages 2 and again in stage 3. Each individual bumble bee was only tested once in a single stage.

Yeast Culture and Nectar Inoculation

A culture of *Metschnikowia rekaufii* collected from a *Corydalis* population in lower Washington Gulch in 2022 was used for this study and perpetually maintained through replating the strain from a freezer stock weekly and then replating the strain on yeast media agar plates supplemented with the antibacterial compound, chloramphenicol, every 48 hours. The *M. reukaufii* was used to inoculate a synthetic nectar at a concentration of 10^3 cells/µL, a cell density commonly observed in flowers in the field (18, 19), around 48 hours prior to performing trials. Flower inoculation first involved removing the nectar from each flower using a microcapillary tube, and then introducing 5µL of either sterile or *M. rekaufii*-inoculated synthetic nectar with a 50µL Hamilton syringe.

Two-Choice Behavioral Assay

To assess whether bumble bees that employ different foraging tactics exhibit a preference for yeast-inoculated nectar, we used a Y-tube olfactometer assay performed under red light. The olfactometer consisted of a 3-in long stem tube with two 3 in-long lateral arms with a 45^o angle at the Y-junction. An air pump pushed air through a charcoal filter for purification purposes at a rate of 100 mL per min^{-1} per arm (10). The purified air splits and passes through two vials containing either the control or treatment groups, carrying the respective scents into each branch of the Y-tube. The two streams of air collide in the “Decision Area”, defined as the area where the bumble bee must make a choice, and exit the olfactometer at the other end of the decision area. This process allows for continual supplementation of two streams of separate scents through the system (Figure S1). All connections in the olfactometer used inert plastic tubing.

The two-choice behavioral assays were done in 3 stages to untangle the impact of yeast-associated VOCs, where the treatment was the following: robbed flowers treated with yeast-inoculated nectar, the yeast-inoculated nectar alone, and robbed flowers treated with nectar containing a synthetic blend of four yeast-associated VOCs. In contrast, the control paralleled the context of the treatment with only a sterile nectar analog. A synthetic nectar

mimicking *Corydalis caseana* nectar amino acid and sugar composition was used across all assays. (20, 21).

To test for the treatment in stage 1, eight flowers of similar age (open 1-2 days) were removed from the same inflorescence. Each flower was inoculated with 5 µl of the previously prepared yeast-inoculated nectar, and finally placed in one of the two odor chambers of the olfactometer. For the control group in stage 1, the second chamber contained another eight flowers from the inflorescence from the same plant as treatment group and treated exactly like the treatment group except we added sterile nectar analog as a control. To prepare the Y-tube assay in stage 2, two 23.8 cm^2 piece of filter paper were loaded with 200 µl of either yeast-inoculated nectar analog or sterile nectar analog, and then placed in their respective odor chambers. To test for the treatment group in stages 3, eight flowers from the same inflorescence were inoculated with 5 µl of nectar each and placed in one of the two odor chambers of the olfactometer (10). The treatment nectar contained a blend composed of: 85,600 µM ethanol, 511 µM 3-methyl-1-butanol (isoamyl alcohol), and 0.0323 µM 3-methylbutyl acetate (isoamyl acetate). For the control group in stage 3, we repeated the same control procedure from stage 1. Each stage tested around 20 bumble bees of each species.

Before the start of an assay session, the olfactometer was cleaned with 2 rounds of hot water and soap, the airflow adjusted to (100 mL/min/arm), and the volatiles allowed to equilibrate for 3 minutes. At the start of each trial, the insect number and position of each VOC source was recorded, and individual were placed in the bottom of the Y-tube. We observed each bumble bee for 5 minutes, recording the time when the bumble bee passed the threshold (1" from the edge of the olfactometer), as its first choice, and the time it spends in each arm, also known as time latency (10). A successful trial was determined if a bumble bee chooses an arm within 3 minutes. The assay was repeated once per bumble bee. Between trials, the surface of the olfactometer was cleaned with hot water and flow rate checked. Additionally, the arm position of the control group was swapped after every trial to account for positional bias. To ensure a continual stream of chemical scents released from the control and treatment groups, the inflorescences or saturated filter paper was replaced after every 10 trials. Stages were done in succession one after another.

Statistical Analysis

All the data was analyzed with the R software (version 4.4.1) with the following packages: openxlsx, nlme, tidyr, ggplot2, and ggpubr (22–27). The Shapiro-Wilk test found that the normality assumption was not violated for our time latency data in stage 1 and 3 with *B. bifarius* ($p > 0.05$). Thus, these stages were analyzed using a one-way ANOVA with treatment as the independent variable and time spent per arm as the dependent variable. Stage 2 for *B. bifarius* and stage 1 for *B. appositus* were analyzed using a Kruskal-Wallis test due to non-normal data. The first-choice data were analyzed using a two-sided exact binomial test, where the hypothesized probability of success was set to 0.5 and our number of successes was defined as the number of first choices for treatment.

Results

For stage 1, *B. bifarius* secondary robbers spent significantly more time in the arm with the floral bouquet with yeast-inoculated nectar VOCs than the floral bouquet with sterile nectar VOCs (Figure 1a, $p = 0.0051$), while *B. appositus* pollinators showed no preference for either (Figure 1b, $p = 0.266$). However, for stages 2, *B. bifarius* secondary robbers did not spend significantly more time in either the yeast-inoculated nectar arm or the sterile nectar analog arm (Figure 1c, $p = 0.791$). Similarly, the *B. bifarius* secondary robbers in stage 3 did not spend more time in either the arm scented with *Corydalis* flowers containing nectar with a synthetic, simplified blend of yeast-associated VOCs or the arm scented with flowers treated with sterile nectar analog (Figure 1d, $p = 0.10$). Despite the preference for time spent in the yeast-

inoculated flower arm in the first stage of testing with *B. bifarius*, the first-choice results throughout all 3 stages of testing and for both bumble bee species did not show any significant difference for either treatment or control group, and thus no preference was indicated (Figure 2, p = 0.523, 0.359, 0.663, 0.663).

Discussion

Nectar-inhabiting microbes have a history of acting as a “double agent," either attracting or repelling insects, which can either be a service or disservice to the plant, and our findings are not an exception to this trend (28). The volatiles released by *M. reukaufii* uniquely shape plant-pollinator signaling, but the extent of influence is heavily context-dependent and can potentially fracture plant-pollinator mutualisms in the process (3). In stage 1, we assessed the effects of different foraging strategies on the bee’s preference for yeast-inoculated nectar within their natural context, i.e., in flowers. Based on our stage 1 time latency results, the difference in preference between secondary robbing and legitimately visiting species indicates that the yeast VOCs may serve as a foraging cue for locating nectar via robbing holes in the context of *Corydalis* flowers. Thus, this discovery could highlight one of the first known shortcuts utilized by robbing bumble bees to help discern which flowers and plants to rob or not within this system (29). However, the ability of yeast VOCs to attract nectar-robbing bumble bee species may cause no change *Corydalis* fitness, as *Corydalis* was more commonly visited by pollinators than nectar robbers and the few pollinators that visited the flowers saturated the stigmas enough to bear the full number of ovules per fruit (30). The finding in stage 1 also defies prior expectations, as other papers attributed the attractive nature of the yeast VOCs to being associated with an innate preference of bumble bees for yeast-inoculated nectar, but the lack of *B. appositus*’ preference for the yeast contradicts this perception (28, 31, 32). Schaeffer and Irwin (2014) found increased floral visitation and pollen dispersal from yeast-inoculated nectar on *Delphinium nuttalianum* by queen *B. appositus* foragers, the main pollinator of *D. nuttalianum* (9). Our analyses were run on bumble bee workers on *C. casseana* and although they appear to contradict this innate preference, the flower species and forager type context cannot be ignored, making it difficult to clearly tease out preference within the same bumble bee species (9).

A parameter that needs to be accounted for when considering this stage 1 results among the greater scientific literature is the inoculation time of the yeast. While other studies looking at the impact of yeast colonized nectar on preference had inoculated their nectar with yeast for 4-7 days, this study only incubated the nectar with yeast for 2 days (7, 10, 35). While this shorter inoculation time may have reduced the quantity of VOCs emitted by the yeast and the potential extent of preference displayed by the bumble bees, we believe that our incubation time more accurately represents the environmental conditions of the Corydalis nectar microhabitat. This is because the average Corydalis flower lasts approximately 4 days and pollinators are consistently visiting and draining the nectar from the flowers throughout the day, shortening the yeast-incubation time and weakening the effects of yeast VOCs (33). Thus, this may explain the difference in preference we are seeing in Schaeffer and Irwin’s 2014 paper.

We suspect that the distinct morphology of legitimate and robber bumble bees drives their foraging tactic strategy and either the utilization or not of yeast VOCs as a cue. On one hand, due to the short tongues of *B. bifarius*, they can’t easily assess the nectar produced deep within the corollas of *Corydalis* flowers, so they take advantage of pre-existing holes on the corollas, created by *B. mixtus* or *B. occidentalis* primary nectar robbers, to have easier access to the nectar (13). As *B. bifarius* approaches the robbing wounds on the corollas, they are exposed to yeast VOCs emitted from the nectar that become more potent as the bumble bee

secondary robber approaches the robbing wound due to the hole's proximity to the yeast-inoculated nectar, creating a preference for and a cue for these bumble bees to use in future forages. On the other hand, due to the long tongues of *B. appositus*, they can access and legitimately forage for the nectar deep within a *Corydalis* corolla (13). Thus, when they legitimately forage, they are mainly exposed to floral VOCs emitted from the petals that are especially concentrated at the floral opening, potentially acting as the main cue for legitimate foragers (34). While *B. appositus* is likely capable of recognizing yeast VOCs, they may not learn a preference for these scents because they do not assist them in legitimate foraging on *Corydalis caseana* (29). We suspect that the lack of preference for yeast VOCs was derived from lower exposure to yeast VOCs because legitimate foragers will visit both robbed and unrobbed flowers, diluting potential exposure to yeast VOCs. In contrast, *B. bifarius* would be more frequent exposed to yeast VOCs because robbed flowers have a higher chance of yeast contamination from previously visited bumble bees (13). However, we are unsure why *B. appositus* wouldn't develop a preference for control nectar because robbed corydalis flowers tend to have less nectar than unrobbed flowers, and yeast VOCs seem to be a cue for robbed flowers (14). Ultimately, more research is needed to unravel the reasoning behind why either a preference or lack of preference has emerged for either species.

In contrast, stages 2 and 3 assessed the importance of the floral context and the roles of specific yeast-produced VOCs in the preference of *B. bifarius* for yeast-inhabited flowers. However, the lack of preference from *B. bifarius* during stages 2 and 3 tests exemplified the importance of a specific proportion and combination of both floral and nectar VOCs to create a scent profile that elicits a preference. Past literature on floral VOCs with other pollinator species has shown the importance of specific compounds and ratios of compound blends for creating the necessary scent profile that either attracts or repels a target species (35). Thus, the findings from stages 2 and 3 cement this common trend within a bumble bee context and raise questions about what VOCs from the floral context could be added into our synthetic blend in stage 3 to create the presence seen in *B. bifarius* in stage 1.

However, the lack of preference towards only yeast-inoculated nectar was particularly surprising, as a prior study from Schaeffer et al. in 2017 found that bumble bees showed a significant initial preference for yeast-inoculated artificial flowers, which wouldn't have emitted floral VOCs (7). However, the difference in results may be attributed to the experimental design and sourcing of the bumble bees. Their study utilized lab-raised bumble bees that were not exposed to floral volatiles because they were fed with nectar feeders rather than flowers. The familiarity of wild-caught bumble bees with floral VOCs may have contributed to the lack of preference we saw in our wild bumble bee experimental group for yeast-inoculated nectar alone in stage 2 (7). We also suspect that the preference seen in this prior study was enhanced by the longer inoculation time for the yeast in the nectar (7 days vs. 2 days), which could have enhanced the intensity of the yeast VOCs and led to increased preference (7).

Initially for our VOC blend in stage 3, we chose to include ethanol, isoamyl alcohol, and acetoin in our blend because they are compounds that are highly produced in yeast-inoculated nectar, are fragrant, and have some role in attracting animals to a flower or fruit (35). Additionally,, we choose isoamyl acetate, despite the relatively lower quantity present in yeast-inoculated nectar in comparison to our other three selected VOCs, because it's a fruity ester identified as an alarm pheromone by honeybees and was found to be highly detected by bumble bees through an electro-antennogram analyses (35). However, the blend could be enhanced for future trials on a C*orydalis/B. bifarius* study system through the inclusion of more yeast VOCs, such as acetic acid or dimethyl sulfide (Martin et al., unpublished). By adding more yeast VOCs, we could add the necessary VOCs that may adapt the scent to be what bumble bees prefer by

either masking avoidance compounds to bring out a desired scent or enhancing the effect of attractant compounds enough to bring out preference.

Olfactometer assays are a useful tool to test animal preference for VOCs (9, 10), nevertheless, individual's behaviors must be accounted for. In our trials, some bumble bees were clearly engaging as intended by clearly antennating at the decision chamber, or even by actively trying to feed from the flow holes. Contrastingly, some clearly tired or agitated bumble bees displayed less clear behavior and could potentially skew the time estimates on each arm. Similarly, the use of first choice as an indication of preference can also be misleading. In stage 1 of our arrays, the first choice and time spent on each arm for *B. bifarius* appear contradicting. From our observations, it was clear that some bumble bees were unable to settle in a single arm of the y-tube until after visiting both VOC sources or refused to visit the other arm. Similarly, Schaeffer et al. 2019 reported a similar trend with time latency showing a significant preference while first choice did not (10).

In the future, this study can be expanded in multiple different directions, including looking at different study systems or yeast VOCs. Regarding different study systems, we aim to repeat the first stage of experimentation with two other floral species, *Mertensia* and *Linaria*. Prior unpublished work in our lab has found that there are a series of VOCs emitted by yeast-inoculated nectar that are shared among different floral species, but others that are unique to either one or a few floral species (Martin et al., unpublished). For example, yeast-inhabiting *Corydalis* flower has been found to exclusively produce an abundance of acetic acid in addition to other VOCs, while aldehydes are only seen in the VOCs of yeast-inhabiting the nectar of *Mertensia* and *Ipomopsis* (Martin et al., unpublished). Thus, with the shift in VOC composition among different floral species, this then begs the question: Does *B. bifarius* preference shift with different yeast VOCs composition or is the yeast VOC cue different among different contexts? Additionally, the reality that *B. bifarius* employs different foraging strategies to differing degrees depending on the flower species adds another layer of complexity and understanding as to how and why context drives the perception and preference for yeast-colonizing nectar in bumble bees (36). Another direction that could be taken is by looking at the effects of yeast VOC compounds, such as ethanol or acetoin, on bumble bee preference. By individually and then collectively looking at yeast VOC compounds, we can start to tease these individual roles, whether they are attractant, avoidant, or masking compounds, and how the compound synergizes together to create a blend that attracts bumble bee in a particular context. Additionally, given the wide range of inoculation time for yeast in nectar used in different studies assessing bumble bee preference, a study looking at the threshold of yeast VOCs needed to produce bumble bees' preference would help to better understand and compare results between studies. Lastly, given that *B. bifarius* showed a preference for yeast-inoculated nectar in stage 1, we could explore the development of this preference, such as whether this preference is innate or learned. A study by Schaeffer et al. in 2017 found some possibility that bumble bee preference for yeast-inoculated nectar could be innate but modulated over time with learning (7). Thus, a series of experiments comparing the preference of *B. bifarius* for yeast-colonized nectar before and after they learned how to nectar rob within a controlled environment could help to tease out the plasticity of this preference.

Overall, bees navigate a chemosensory landscape partially shaped by microbes inhabiting floral hosts, including our study yeast, *M. rekaufii*. Our olfactometer assay revealed that bumble bees' preference for yeast-inoculated nectar, both within and without floral context, is heavily context-based and more variable than initially expected. Yeast-inoculated nectar in *Corydalis* flowers appears to have a foraging tactic-dependent effect, where the altered VOCs

serve as a cue for nectar via robbing holes that nectar-robbing bumble bees utilize within this study system. While our results indicate that the scent profile needed to create preference is highly specific, involving a specific ration of floral and yeast VOCs, the possibility of creating a blend that can capture this scent profile opens new doors in the realm of agriculture biocontrol. For example, these chemical blends could be key for attracting and stimulating more native bumble bee pollination, which is crucial as pollinators are experiencing declines across the world (37).

Figures

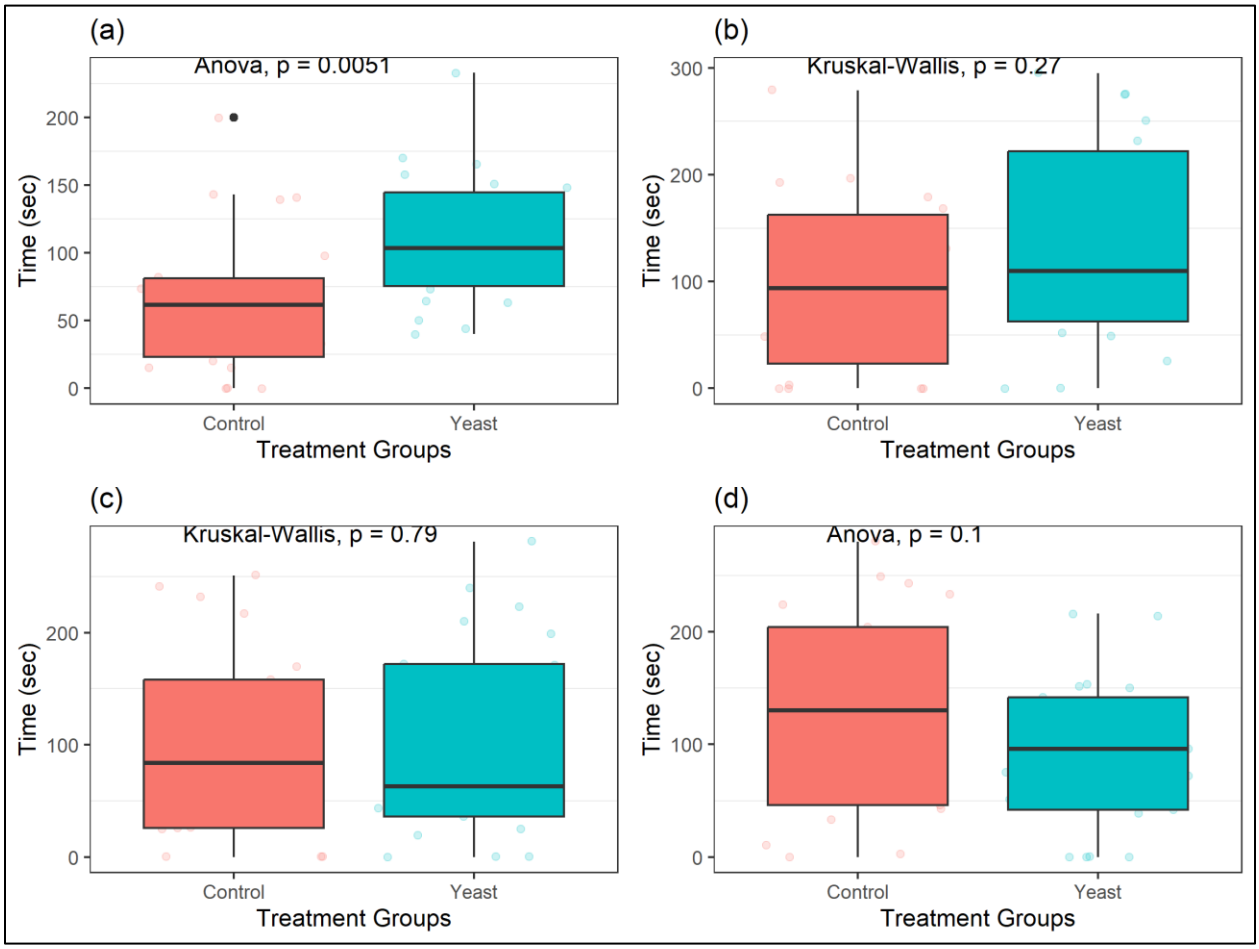


Figure 1**:** Behavioral preference of bumble bees, either *B.appositus* (b) or *B.bifarius* (a, c, and d) for a nectar analog that was either inoculated by *M. reukaufii* and injected into *Corydalis* flowers (a and b), only inoculated with *M. reukaufii* (c), or inoculated with a blend created from four known VOCs released from *M. reukaufii* inhabiting *Corydalis* and injected into *Corydalis* flowers (d). All figures show the total time each bumble bee resided in the arms of the olfactometer.

Treatment Stage	Species	Choice for: Treatment	Control	p	n	No response
Stage 1	*B. bifarius*	56	39	0.523	23	4%
	B. appositus	33	57	0.359	19	9.50%
Stage 2	*B. bifarius*	43	57	0.663	21	0%
Stage 3	B. *bifarius*	43	57	0.663	21	0%

100 75 50 25 0 25 50 75 100

Figure 2**:** Behavioral preference of bumble bees, either *B.appositus* or *B.bifarius,* for a nectar analog that was either inoculated by *M. reukaufii* and injected into *Corydalis* flowers for stage 1, only inoculated with *M. reukaufii* for stage 2, or inoculated with a blend created from four known VOCs released from *M. reukaufii* inhabiting *Corydalis* and injected into *Corydalis* flowers for stage 3. All figures show the first choice of arm visitation for each bumble bee in the olfactometer.

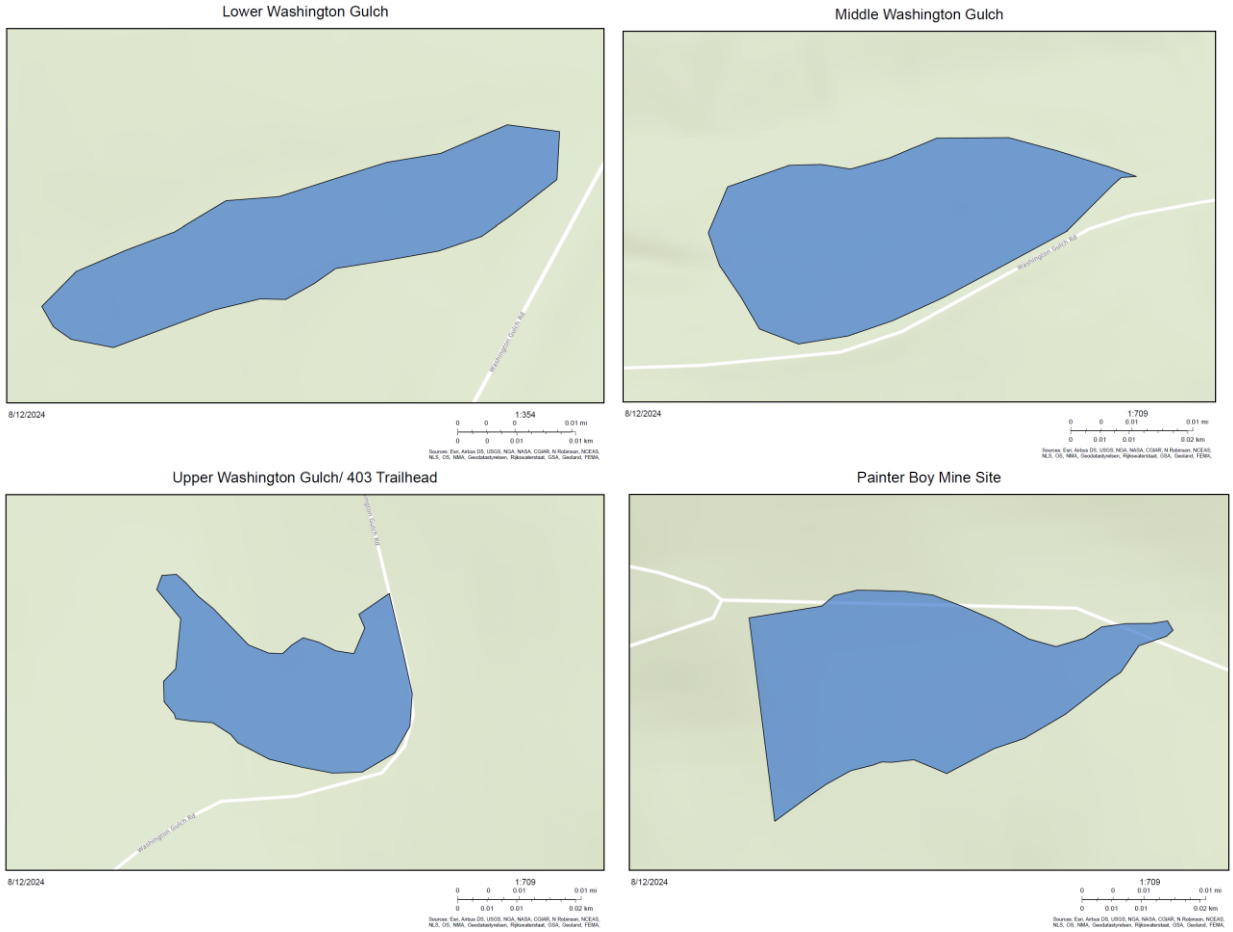
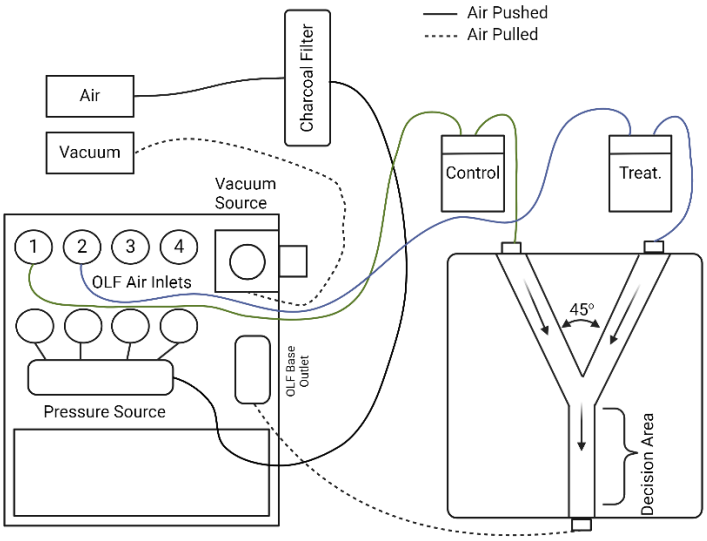


Figure 3: The following four maps are the four sites across Washington Gulch where bumblebees were caught, tested, and returned for my experiment.

Supplemental Figure



Supplement Figure 1:
Schematic drawing of the Y-tube olfactometer with the external machinery to facilitate function (By Bailey Crowley). The air enters through the section labeled “Air” and was purified through the charcoal filter before entering and being split into two streams at the “Pressure Source” and “OLF Air Inlet” sections. Each stream of air was directed into one of two vials containing either the control or treatment samples. Afterward, each stream of air was directed through an arm of the olfactometer and combined in the decision area, where the air exited at the end of the “Decision Area”, entering the OLF Base Outlet and being pumped out through by a vacuum. Bumble bees were placed at the bottom of the “Decision Area” and given 5 minutes to make their first choice and spend time in either arm under red light.

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