Comparing Deposition Patterns and Survival of Fecal-Orally Transmitted Bee Pathogen on Two Chemotypes of Wild Bergamot

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Introduction

Infectious diseases are a concern for both commercial and wild bees and have been implicated in bee population decline [1]. The trypanosomatid pathogen *Crithidia bombi* infects *Bombus impatiens* and several other bumblebee species at rates of up to 80% [1]. Consequences of infection include impaired cognition, decreased foraging efficiency, and reduced reproduction [1,2]. Diverse phytochemicals have been shown to reduce pathogen infection in bees, such as monoterpenes produced in the trichomes of *Monarda fistulosa*. These chemicals serve purposes ranging from toxins and herbivory deterrents to pollinator attractants [3]. Phytochemicals (such as monoterpenes) in nectar and pollen can inhibit *C. bombi* growth [2]. *M. fistulosa* genotypes, termed "chemotypes," produce predominantly a single monoterpene, such as linalool or thymol, that differ in their ability to reduce *C. bombi* infection [4].

Goals

1. Determine where fecal deposition occurs on *M. fistulosa* inflorescences, which could affect transmission. To see whether there is a difference in deposition location and amount between thymol and linalool chemotypes and to see whether this differs between infected and uninfected bees.
   - **It is predicted that *B. impatiens* infected by *C. bombi* will defecate on thymol more than linalool chemotypes due to thymol's infection reduction. Uninfected bees will forage more on the sweeter linalool chemotype since there is no benefit to forage on thymol if not infected.**
2. Assess *Crithidia bombi* viability on different locations and chemotypes of *M. fistulosa* inflorescences.
   - ***C. bombi* deposited on thymol inflorescences will stay viable for a shorter time than those deposited in linalool inflorescences because thymol has a greater infection reduction ability than linalool. *C. bombi* will be less viable when deposited on the bract than on the flower because the bract is assumed to contain a higher concentration of phytochemicals to fight off infection compared to the flower which has a lower concentration to attract pollinators.**

Model Organisms

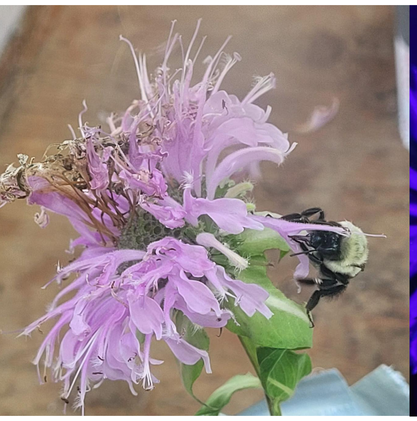

Figure 1. *Bombus impatiens*, the common eastern bumblebee, a generalist native to North America and used in commercial pollination [2].

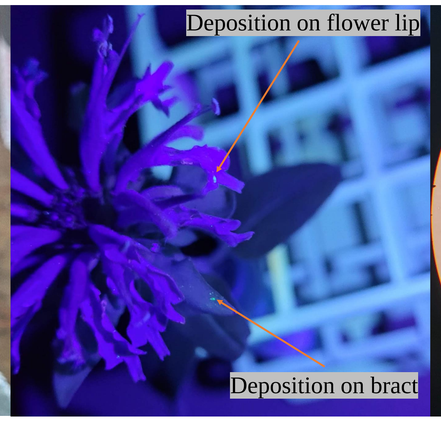

Figure 2. *Monarda fistulosa*, or wild Bergamot, that has several chemical phenotypes, or chemotypes [3]. We used linalool and thymol chemotypes.

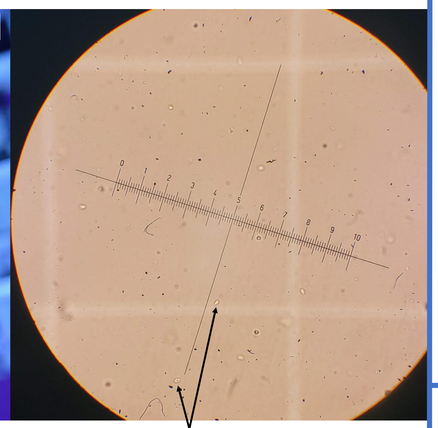

Figure 3. *Crithidia bombi*, gut pathogens that are transmitted fecal-orally between bees [2].

Methods

1. Fecal Deposition

- **Infected** and **Uninfected** bees are fed fluorescent dye 24 hours prior to foraging
- Bees are placed in foraging cages containing one inflorescence of each chemotype
- Bees forage for 3 hours, and then inflorescences are checked for bee feces by shining UV light; deposition amount and location is recorded
- Bee guts are dissected, to verify infection status

2. Pathogen Viability

- *Crithidia bombi* inoculum is placed on inflorescences on the bract and the flower, two locations with high deposition during the deposition trials (See Figure 2)
- Each trial includes both thymol and linalool chemotypes and 4 time periods (30, 75, 120, and 165 minutes)
- At the end of each period, viability is checked by pipeting remaining inoculum into a hemocytometer to record the presence or absence of *C. bombi*

Results

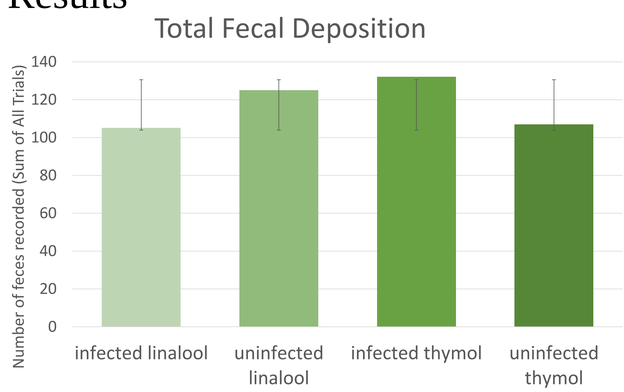

Figure 4. **Infected bees defecated more on thymol inflorescences than on linalool, and uninfected bees defecated more on linalool inflorescences than on thymol** ($\chi 2$=4.5, P = 0.2).

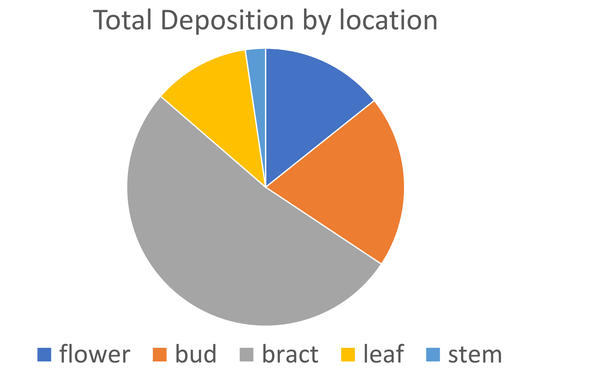

Figure 5. Regardless of chemotype and infection, **deposition differed in location**, with the most deposition occurring on the **bract, bud, and flower** ($\chi 2$=339.0, P > 0.0001).

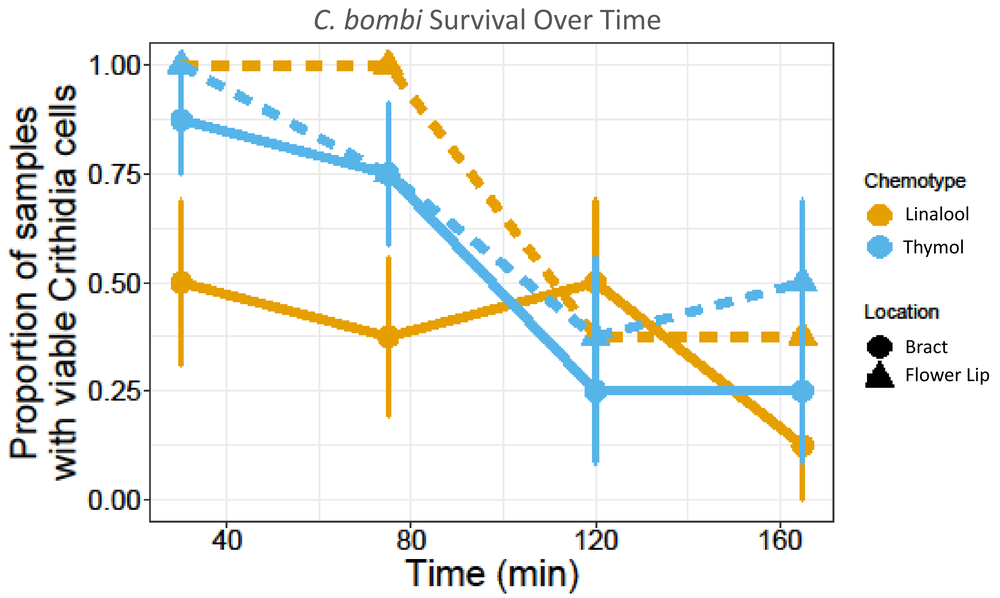

Figure 6. *C. bombi* survival declined over time, regardless of chemotype. The flower lip and bract of linalool inflorescences had the most pronounced differences in survival, with low survival in the linalool bract as early as 30 minutes. Survival on thymol did not differ greatly by location.

Discussion

- Deposition location is not affected by chemotype or *C. bombi* infection. However, deposition amount differs slightly between infected and uninfected bees, as predicted.
  - Infected bees defecated more on thymol than linalool chemotypes, suggesting there was a foraging preference for thymol over linalool. This correlates with the fact that thymol reduces *C. bombi* infection more than linalool [4].
  - Uninfected bees defecated more on linalool than thymol chemotypes, suggesting a preference for linalool over thymol.
- Defecation occurs most often on the bracts, buds, and flowers, suggesting that these are likely locations for disease transmission.
- *C. bombi* survival declines over time, so the likelihood of disease transmission will decline as time passes.
- For linalool chemotypes, deposition on the flower may increase the risk of disease transmission compared to the bract.

Implications

- Understanding where defecation occurs and how long *C. bombi* stays viable gives a better picture on the mechanism of interspecies disease transmission.
- Planting thymol varieties of *M. fistulosa* may help reduce *C. bombi* transmission between *B. impatiens* and other bee species [1]. This may improve the health of native bee ecosystems and of commercial colonies.

Further Study

- Acquisition trials would give the complete picture of disease transmission from infected bee to plant to uninfected bee. Inoculum would first be placed on the bracts and flowers of thymol and linalool inflorescences. Then, bees without infection will be let to forage on them for three hours. After one to two weeks, they would be dissected to check for infection levels.

References

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