Attractiveness of Solitary Bee Nesting Cues to a Cleptoparasite

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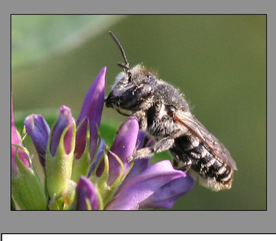
Background

Megachile rotundata F. (Hymenoptera: Megachilidae), the alfalfa leafcutting bee, is a solitary bee commonly used in the northwestern United States (including Utah and Idaho) for pollination of alfalfa for seed production. Managing solitary bees presents challenges, including low seasonal return of commercial populations and low rates of offspring survival. One major factor causing the low return rate is parasitism (Pitts-Singer 2007; James and Pitts-Singer 2013).

Megachile rotundata is not a native species to the United States, and is heavily affected by parasitism of the native cleptoparasitic wasp *Sapyga pumila* (Cresson, Hymenoptera: Sapygidae). *S. pumila* exhibit a high rate of parasitism, and a relatively small population can parasitize a high number of *M. rotundata* offspring. The high rate of parasitism is partly due to the long life span of the *Sapgya* wasp, which gives it the ability to parasitize nests for the entire flight period of the alfalfa leafcutting bee. Because a small population is capable of a parasitizing many *M. rotundata*, it is difficult for bee managers to trap and kill enough *S. pumila* to significantly reduce rates of parasitism. Effective traps are essential in order to achieve an effctive reduction of parasitism (Torchio 1974).

Figure 1. *Megachile rotundata* female and cocoon wrapped in leaf pieces.

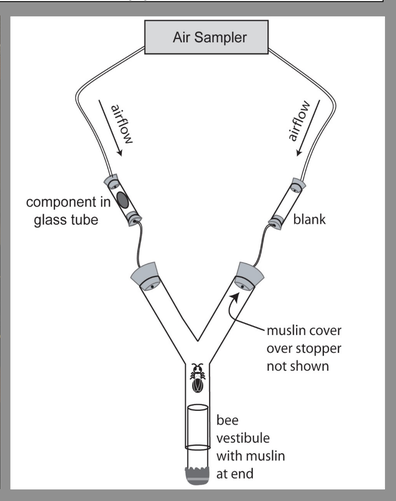


Approach and Objectives

The purpose of this experiment is to isolate organic compounds that can be used as attractants in traps specific to *S. pumila*. Olfactory cues derived from the cocoons of another managed solitary bee species, *Osmia lignaria* (Megachilidae) have proven to be an effective attractant of its parasite, *S. elegans* (Pitts-Singer 2007; Pitts-Singer et al. 2014; Malan and Pitts-Singer, unpublished). We predict that *S. pumila* will also be attracted to the same compounds that were attractive to *S. elegans* (odor cue 1). If *S. pumila* are not attracted to the same compounds as *S. elegans*, we predict that *S. pumila* will be attracted to olfactory cues derived from the cocoon of *M. rotundata* (whole cocoon). We tested the attractiveness of odor cue 1 and the whole cocoon extract in two different trials.

Whole cocoon olfactory cues were created using a methanol chloroform extraction of 100 *M. rotundata* whole cells from which bees had already emerged (see extraction protocol for cocoon extract). This cue was tested for attractiveness against a blank in a Y tube apparatus (Fig 2).

Figure 2. Left: Sam Malan observing wasp during Y tube bioassay. Right: Diagram of Y tube apparatus.



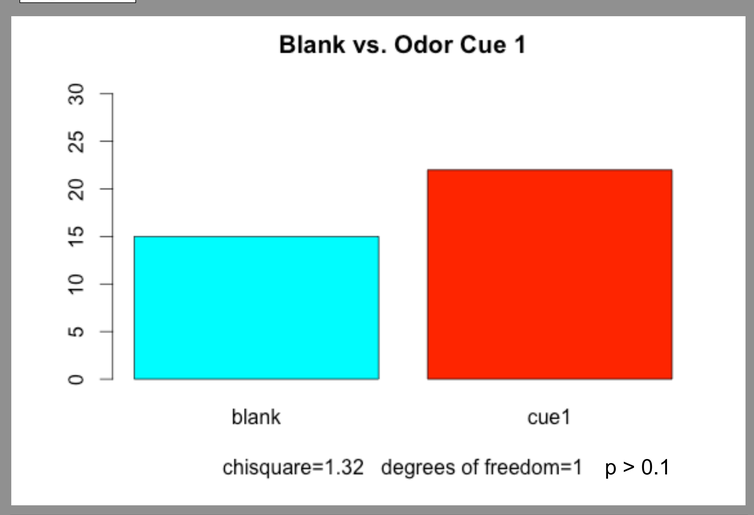
Experimental Design

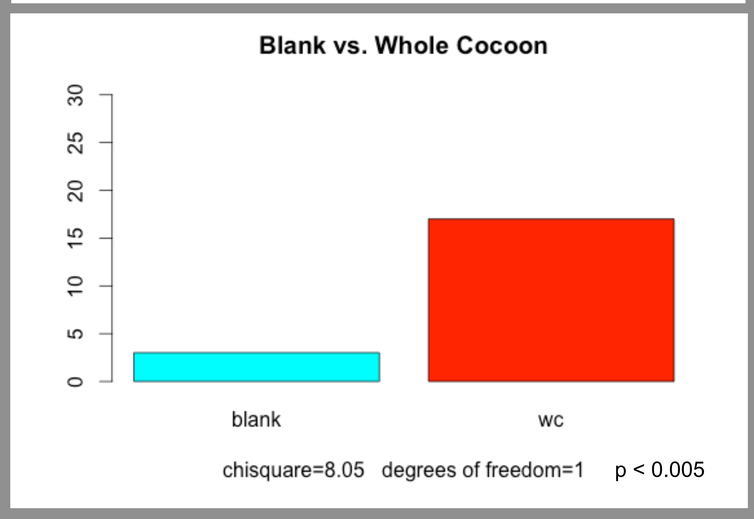
Bioassay: To test the attractiveness of olfactory cues on *Sapgya pumila,* diapausing wasps were found among nest cells of *M. rotundata.* They were reared in an incubator at 29 °C. *S. pumila.* Emerged wasps were placed in a benchtop Plexiglas cage so that males and females were allowed three days to mate and ovary maturation could occur before being females were tested. During this waiting period, the *S. pumila* were maintained at 29°C and fed a mixture of 90% water and 10 % local honey. This was done to ensure that females were mature and actively seeking a host. On the third day after emergence, cocoon extract was applied to filter paper and allowed to dry before use on the same day. For trials, each wasp was introduced to a Y tube apparatus (Fig. 3) within which it was allowed to walk about freely. Each apparatus was enclosed by a large cardboard box equipped with a full spectrum light to exclude external visual cues that may have biased wasp movement. The Y-tube apparatus itself consisted of an air pump that drew air through a charcoal filter, which purified the air of other potential cues in the environment. The air was passed over the cues (in vestibules) and then through the arms of the Y-tube before reaching the wasp. The wasp was then observed for up to 30 minutes to see which cue it followed. The first choice of cue was recorded. A choice was constituted by the insect touching the extremity of the Y-tube nearest one cue or the other. Significant differences between choices were determined using a Chi Square analysis.

Chemical Extraction: Extracts were made using 100 empty cocoons and associated leaf pieces. The cocoons were placed in a glass bottle, and 25ml of a 2:1 chloroform:methanol mixture was added (25ml is the minimum volume needed to cover all cocoons). The glass bottle was then agitated by hand for 5 minutes. After agitation, large pieces of cocoon were filtered from the extract via rough glass fiber filtration. Extracts were stored in glass scintillation vials.

For dosage of whole cocoon extracts, a 1 cocoon equivalent (CE) was used for Y-tubes. 250ul of extract was applied to the filter paper.

Figure 3.





Results

In the first trial (blank vs. odor cue 1), 15 wasps chose the blank and 22 chose odor cue one. In the second trial (blank vs whole cocoon), 4 wasps chose the blank and 17 chose the whole cocoon odor plume (Fig 3).

Statistical analyses reveal that olfactory cues derived from the whole cells of *M. rotundata* are significantly attractive to *S. pumila,* while odor cue 1 is not. Further analysis will allow researchers to isolate the organic compound contained in the cocoon that is most attractive to *S. pumila*.

Discussion

Further research is necessary to isolate the compounds that are most attractive to *S. pumila* from the *M. rotundata* cocoon. Separation of the components that make up the cocoon odor will allow for comparing the attractiveness each component. *S. pumila* may be attracted to volatile compounds released by the leaves of the alfalfa that surround the cocoon of *M. rotundata,* or to compounds found within the cocoon itself.

M. rotundata is not a species that is native to the United States, and we speculate that *S. pumila* is not attracted to an odor contained in the cocoon of *M. rotundata* that is unique to *M. rotundata.* Instead, we speculate that *S. pumila* is attracted to volatile compounds released by freshly cut leaf pieces, as this is a more reliable indicator of a potential host (Torchio 1974).

Once individual attractive components are revealed, it may be possible to improve the efficacy of traps to reduce populations this parasite where *M. rotundata* are used as commercial pollinators. However, because both whole cell and leaf piece extracts also are attractive to *M. rotundata* (Pitts-Singer 2007), trap designs using odors of these substrates must be developed to prevent accidental capture of *M. rotundata*.

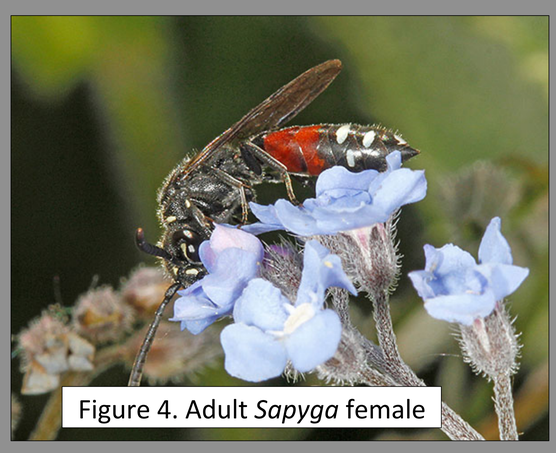


Figure 4. Adult *Sapyga* female

Acknowledgements

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References

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notes

Figure 5. *Sapyga* trap and nesting site for *M. Rotundata*

- Ongoing research to improve the efficacy of the pollinators known commonly as the blue orchard bee (Osmia lignaria) and the alfalfa leafcutter bee (Megachile Rotundata) is necessary to render both species a viable option for large-scale agricultural pollination.
- *Megachile rotundata* is responsible for two-thirds of the pollination of alfalfa in the United States, and *Osmia lignaria* is used as a commercial pollinator of tree fruit.
- However, certain obstacles must be overcome in order to achieve effective large-scale pollination using *O. lignaria* and *M.* rotundata. One of these obstacles is parasitisim.
- Both bees are parasitized by wasps of the genus *Sapyga,* leading to reduced numbers of bee offspring. This decreases the population size and the rate of pollination.
- In a previous study, olfactory cues derived from *O. lignaria* cocoons were isolated and tested for efficacy in attracting the natural enemy of *O. lignaria* known as *Sapyga elegans.* Two cues were tested extensively and determined to be attractive to *Sapyga elegans*. However, research must still be done to isolate compounds that are attractive to Sapyga *pumula*, the natural enemy of the alfalfa leaf cutter bee. The purpose of this research is to find attractants of *Sapyga sp.* That can be used in traps near nesting sites of *M. rotundata*.
- Testing started with two olfactory cues that were previously shown to be attractivie to *Sapyga elegans.* These olfactory cues did not attract *Sapyga pumula,* and researchers moved on to test odors extracted from the cocoons of *M. rotundata*.