Use of a brood-derived volatile attractant as a fumigant to control Varroa mite

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Introduction Successful treatment strategies against parasites exploit differences in chemical sensitivities by selecting chemicals that harm or disrupt the parasite but not the host. One set of chemicals that parasites are potentially sensitive to are host chemicals. Deployment of attractive host chemicals at artificially high concentrations can overstimulate and overwhelm the parasite. We describe the adaptation of a host attractant for use as a fumigant against the Varroa mite, the leading cause of parasitic honey bee mortality worldwide. Female Varroa mites are excited by a volatile odor (termed CA) specifically emitted by their capping larval hosts. Mites use chemical odors to locate both larval hosts for reproduction and adult bees for feeding and transportation (the **phoretic host**). Here, we use extremely high concentrations of synthetic CA volatiles to disrupt and overstimulate Varroa mites exposed on adult bee phoretic hosts. Critically, we also noted the effects of CA fumigants on the adult bee hosts.

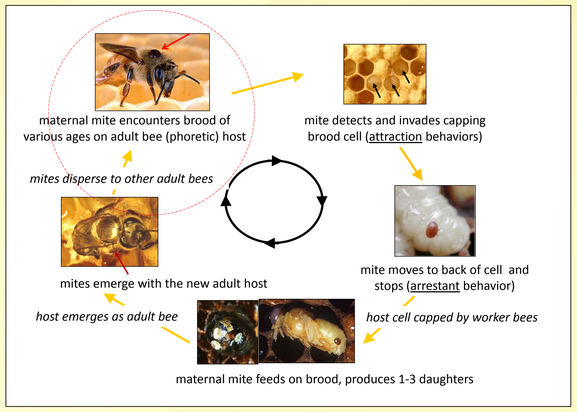

Figure 1. The life cycle of the Varroa mite, with the phoretic adult bee host phase circled. The mite uses the phoretic host both for food and transportation.

Objectives

1) Determine the effect of CA fumigants on mite drop rates in a container arena bioassay
2) Examine the effects of CA fumigants on the ability of isolated mites to reacquire an adult host
3) Determine the effect of CA fumigants on mite drop rates in a colony environment

Methods

1) Effects of fumigation on phoretic mites in containers

The effects of exposure to CA fumigants on Varroa mite drop rate off the phoretic host were tested using a flow-controlled air system. For each replicate, approximately 20 phoretic mites were isolated on 40 worker bees in an air tight container with a mesh floor. Air and vacuum flow through the container was made through ports drilled through the lids. Each container was infused with one of five CA fumigant volatile treatments (100%, 50%, 25%, 12% or 0% (control)) by bubbling the airstream airflow through a CA solution. Bees and mites were exposed to the fumigant treatment for 5 minutes. At the end of each trial, we noted the number of mites that remained on the host and the number that were off the host. Mites that fell completely off the host were trapped in a layer of water or powdered sugar beneath the screen floor. Mites that remained on adult bee hosts were rinsed off and counted in an alcohol wash.

2) Effects of fumigation on mite acquisition of an adult bee host

Isolated mites that fail to acquire an adult or larval host die within a few hours in the colony environment. The effect of CA fumigant exposure on the ability of an isolated mite to maintain and then reacquire an adult host was evaluated with a simple arena assay. A single bee with a phoretic mite was isolated in a sealed 45 mL tube. Air and vacuum flow through the tube was made through ports drilled through the lid. Each mite and adult bee host was exposed to CA fumigant or air only (control) for 5 minutes, then clean air for 20 minutes to allow affected mites a chance to reacquire a host bee. We recorded whether the mite remained on the bee both at the end of the 5 minute treatment exposure and 20 minutes after exposure to clean air.

3) Effects of fumigation on phoretic mites in a colony environment

The effect of CA fumigants on colony Varroa mites was tested using five frame nucleus colonies. Each nucleus colony contained approximately 5,000 adult bees infested with phoretic Varroa mites . A mite trap consisting of a screen bottom over a powder sugar board was placed between the nuc box and bottom board. Each nucleus colony was sealed to control the flow and concentration of the CA fumigant. The sealed colony was then fumigated for 20 minutes with an airflow containing CA volatiles or air only (control). Mites that dropped completely off their adult host passed through the screen bottom to the trap below. Mites that remained on their adult bee hosts were removed and counted separately after a powdered sugar shake of adult bees.

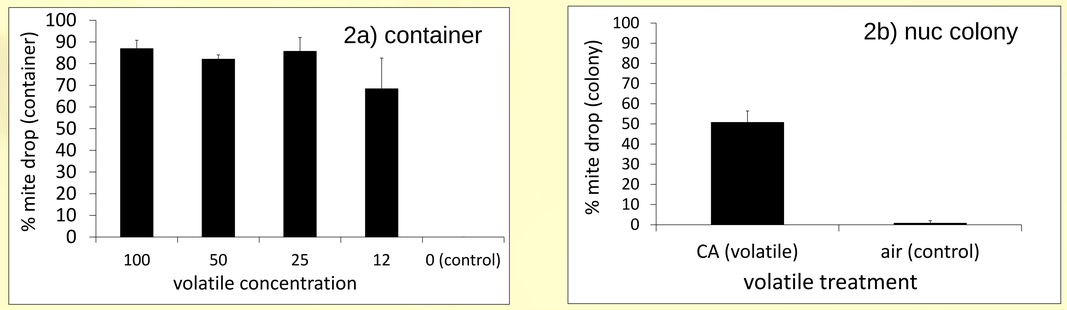

Figure 2) Drop rates of phoretic mites from their adult bee hosts into a screen bottom trap in a) the container assay and b) the nuc colony assay (error bars are standard error).

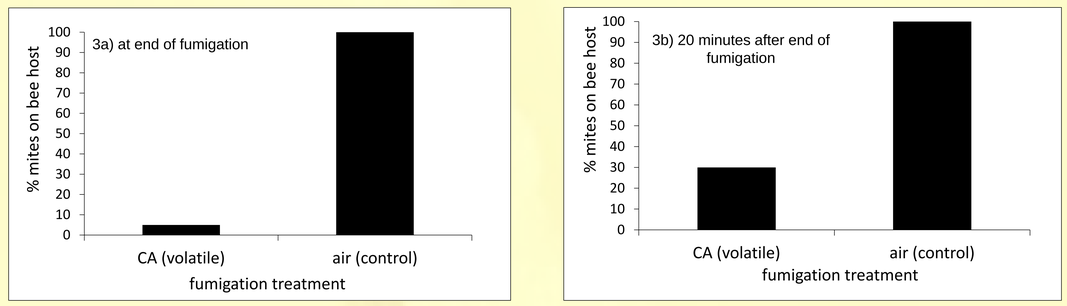

Figure 3). Proportion of mites remaining on an adult bee host a) at the end of a 5 minute fumigation treatment period and b) 20 minutes after removal of fumigant by clean air (during host reacquisition period).

Results and Discussion

Mites exposed to different concentrations of CA fumigation in the container assay dropped from their hosts between 67 % to 87% of the time (Figure 2a). Affected mites responded to CA fumigation by walking constantly and rapidly both on and off the adult bee hosts. Most mites were not killed outright, but seemed unable to recognize adult bees as hosts during fumigation. In the reacquisition assay, over 90% of phoretic mites lost their adult bee host within 5 minutes of exposure to CA (Figure 3a). Fumigated mites simply walked right over adult bees rather than stopping on and attaching to the bee host (as in unfumigated mites). High concentrations of CA volatiles may irritate the mites such that they cannot engage in normal host seeking behaviors. However, some mites (~30%) were able to reacquire an adult bee host after CA fumigation ceased (Figure 3b).

Adult honey bees exposed to moderate ly high CA fumigation engaged in rapid wing fanning, movement, and continuous grooming behavior during the duration of the exposure. Bees became wet during bouts of constant grooming. Adult bees exposed to moderately high concentrations of CA recovered within 2-5 minutes after fumigation ended (approximately the time required for the fumigant to fully dissipate).

Colony mites exposed to CA fumigation experienced lower rates of mite drop than the mites in the container assay (Figure 2b). The hive structure interfered with the efficacy of the screen trap in the colony environment. In the colony, dislodged mites often landed on the comb rather than dropping completely to the ground. Once fumigation had ceased for a while, some of these mites recovered and reacquired a new adult bee host. However, CA fumigation was effective for isolating mites that fell completely through a screen bottom.

Conclusion

Fumigation with high concentrations of synthetic CA volatiles rapidly separates phoretic mites from their adult bee host during the fumigation period. We now intend to find an agent that can neutralize the dislodged mites before they reacquire their adult bee hosts.

Future studies

1) Optimize fumigant delivery and concentrations to maximize mite drop and minimize colony disturbance.
2) Determine if lower concentrations of CA volatiles can be effectively employed as a flooding agent to block odor-driven acquisition of new adult bee hosts. The mite might not be able to find a new host if it can't smell it.
3) Examine the sublethal effects of CA exposure on critical colony functions such as egg laying and brood rearing.
4) Combine CA fumigation with other fumigant agents to permanently immobilize or kill knocked-off mites.

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