Development of Novel *Varroa* Mite Control Methods from Attractants and Arrestants Isolated from Brood Host Volatiles

Project No.: **10-POLL6-CARROLL**

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Objectives:

1) Evaluate other volatiles emitted by brood hosts as potential semiochemical ("signaling chemical") cues and as potential synergists for CA and CB (known attractants/arrestants).
2) Develop an in-hive attracticide trap for Varroa mite using identified attractants and arrestants isolated from brood host volatiles.
3) Develop in-hive flooding techniques using identified semiochemicals isolated from brood host volatiles.

Interpretive Summary:

The Varroa mite is a leading cause of mortality for honey bee colonies worldwide. This parasitic mite feeds on the hemolymph of honey bee adults and capped brood during pupation. One approach for the control of Varroa mite is the identification of odor volatiles that the mite uses to find a host bee. Key host odors could be used to lure mites to a trap or to interfere with mite host seeking behaviors. During cell invasion, a female mite detects and moves into the cell of an older bee larva just before the cell is capped by adult bees. Mites are able to distinguish between older host larvae and non-host younger larvae by odor alone. We previously identified two volatiles from older bee larvae named CA and CB that excite and halt female mites at high odor concentrations. We have begun to investigate other odor chemicals associated with older bee larvae to determine if these chemicals affect Varroa mites by themselves or in combination with CA and CB. We evaluated the movements of mites exposed to chemical odors in a circular mesh floor arena with the EthoVision behavioral analysis system. One volatile specifically associated with non-host larvae, termed CC, acts as a repellent to Varroa mites at high concentrations. The limited responsiveness of mites to lower concentrations indicates that these three compounds probably affect mite behavior toward larval hosts at contact or near-contact distances. In Arizona, our spring and summer

research was delayed by severe drought and hard freezes which, surprisingly, affected mite responses to larval hosts. Mites from colonies experiencing severe dearth conditions (lack of nectar and pollen, from May to July) were not nearly as responsive to odors from host larvae as mites from colonies with reliable access to floral resources (September and October). This decrease in the number of responsive mites may be due to reduced interactions with host larvae as the number of bee larvae present in the colonies declined during the dearth.

We will continue to work on identification of mite signaling chemicals with fully responsive mites during fall 2011. We will also continue our efforts to develop CA and other signaling chemicals as flooding agents and trap lures to control mites in the hive environment.

Materials and Methods:

Mites and bees

Varroa mites were reared in colonies headed by European queens of mixed Italian and Carniolan lineage. Phoretic (animals using hosts for transport) Varroa mites were removed from adult bees by the powdered sugar shake technique. Sugared mites were transfered by brush to a damp paper towel and lightly rinsed with DI water to remove the sugar. To maintain activity, mites were kept at high humidities and temperature (34°C) before use in assays.

In spring and summer 2011, our Varroa mite research was interrupted as southeastern Arizona experienced an extended extreme drought and record freezes that decimated honey bee colonies. The near total failure of most bee nectar and pollen forage sources, including citrus, spring wildflowers, and mesquite, affected Varroa mite populations and cell invasion behaviors as much as their host bee colonies. Our local mite populations declined absolutely by August to 27% of the early March population. We decided to test for mite receptivity toward brood hosts (older 5^{th} instar larvae) to detail the effects of severe drought on mite host seeking behaviors.

Brood host receptivity bioassay

Only a small proportion of phoretic mites on adult bees are receptive to brood hosts at any given time. Female mites that emerge from a cell with their new adult bee host require several days to mature before they are ready to seek a new larval host. Adrian Duehl initially developed a brood host receptivity bioassay to preselect mites that responded to the presence of larval hosts for bioassays. Mark Carroll adapted this bioassay to test for mite receptivity toward brood hosts during the drought.

Single mites were placed in the center of a mesh arena that had ten capping bee larvae pulled out of cells located in one quadrant. Mites that moved over the brood host within 30 minutes and remained were considered receptive. Mites that did not stop over the brood host were not considered receptive. Between 90 and 120 mites were removed from six colonies and evaluated to check receptivity toward brood hosts during each month tested. Receptivity bioassays were conducted in a warm (33°C), humidified environmental chamber to simulate colony conditions.

Mite responses to brood volatiles - dual choice bioassay

Mite responses to synthetic brood volatiles were evaluated by tracking their movements in a dual choice arena. The arena consisted of a nylon mesh floor glued to the bottom of a 9.0 cm Plexiglass ring. Odors were presented in a four quadrant Petri dish located directly below the mesh floor – odors from each quadrant wafted up from the odor source through the mesh. In the dual choice bioassays, mites were given a choice between two quadrants of water (control) and two quadrants of a volatile compound in water (volatile). A starting pin (1 cm long insect pin) was placed in the center of the mesh floor as a neutral starting point. Video of the arena was recorded by a near IR camera with four near IR LED light banks. The entire arena and apparatus were placed at colony temperatures (34°C) and high humidities to simulate colony conditions.

To start a trial, a single mite was placed on the starting pin. Mites that did not walk down the pin within two minutes were considered non-responders and eliminated. Mite movement about the arena was recorded for five minutes after contact with the mesh floor. The time spent over each odor source (volatile or control) was calculated by an EthoVision XT behavioral analysis software program (Noldus, Inc., Leesburg, VA).

Results and Discussion:

Female Varroa mites displayed poor attraction to bee larval host odors in bioassays during the period of severe drought (see **Figure 1**). From May through July, a far smaller proportion of mites were responsive to bee larva host odors (1% to 6%) than the proportion normally observed in summer mites (approximately 30%). The poor responsiveness of mites during this dearth period may have been due to reduced cues from larval hosts as bee larva numbers declined. Varroa mite cell invasion behavior is known to decrease sharply during prolonged periods of low brood rearing by honey bees, such as mid-winter. Lower female mite reproductive rates have also been reported during extended periods of hot and dry weather (Harris et al., 2003). Phoretic mites from the summer dearth period were sampled and frozen for later molecular and biochemical comparisons against receptive summer mites from well-fed bee colonies. A better understanding of the mechanisms that govern mite receptiveness toward brood hosts may provide a target for future supression of mite cell invasion behaviors. We avoided use of these mites in bioassays during the dearth period because of their poor responsiveness.

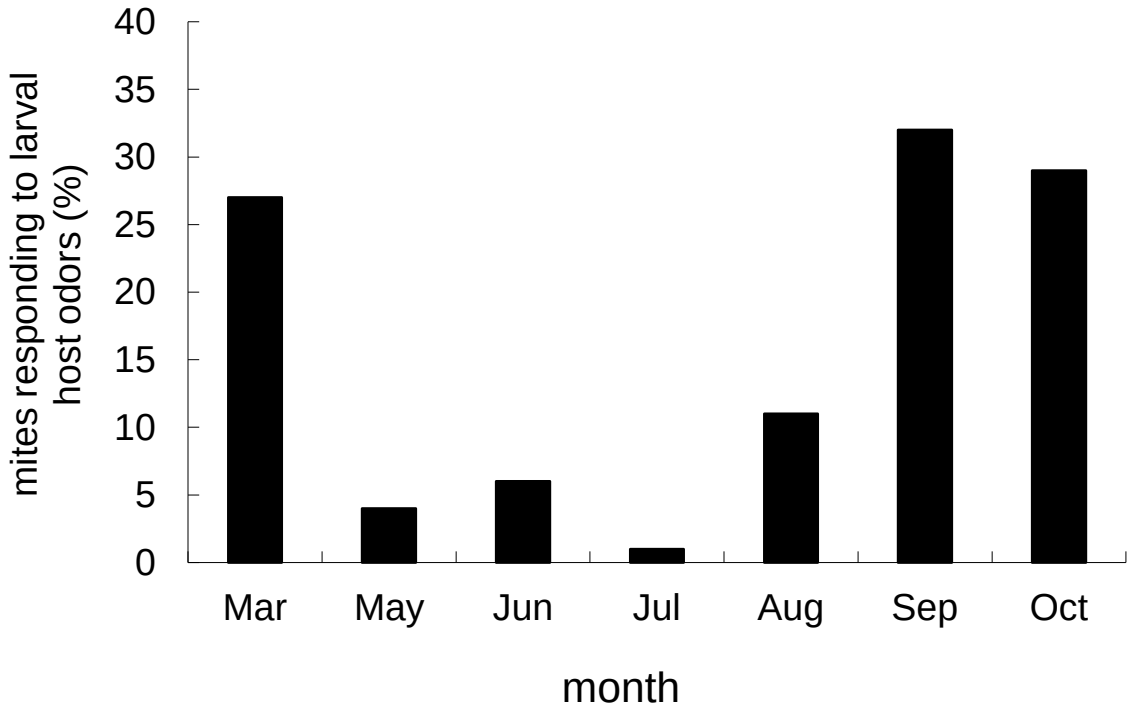

Figure 1. Proportion of mites responding to odors from brood hosts in a mesh arena bioassay. Severe drought conditions occurred from October 2010 through July 2011.

We focused first on testing mite responses to brood volatiles because of the relatively low numbers of mites required for these experiments. Of the brood volatiles examined thus far, we have discovered one brood volatile (termed compound C or CC) that acts as a repellent to Varroa mites at high concentrations (see **Figure 2**). Unlike CA or CB, this brood volatile is specifically emitted at high levels by non-host larvae that Varroa actively avoid. Like CA and CB, CC only affects the behavior of female mites at extremely high concentrations such as would be expected with a contact or near contact host signaling chemical. Varroa mites detect and respond to their brood at relatively close distances (within a bee's body length)(Boot et al., 1994). A repellent volatile such as CC might be useful in supressing mite cell invasion behavior if sufficient quantities of the compound are added to the air around capping brood.

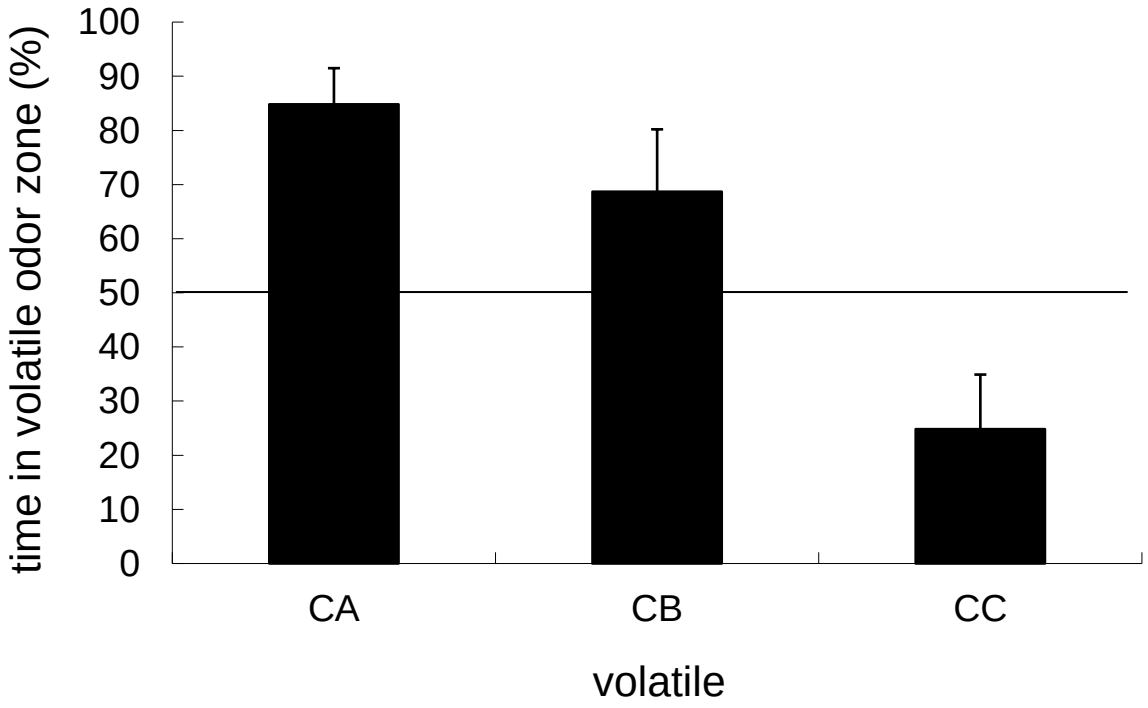

Figure 2. Response of mites (mean and standard error) to synthetic brood volatiles presented in a dual choice bioassay. Mites were given a choice between a volatile odor source and water (control). CA and CB are known volatile attractants and arrestants, while CC is a volatile strongly associated with non-host brood.

Now that our drought has ended and we have responsive mites, our next step is to continue evaluating other brood volatiles for their effects on mite behaviors. Active volatiles will be tested at different concentrations to determine the active range that triggers mite behaviors. We will then use this knowledge of active dosage to develop in-hive trap and flooding strategies against the Varroa mite in the hive environment.

Research Effort Recent Publications:

United States research patent (USSN 61/377,533, D.N. 0124.09) on use of volatiles CA and CB as mite attractants is in the final stages of approval. This patent is based on previous research.

References Cited:

Boot, W.J., Beetsma, J., and J. Calis. 1994. Behaviour of Varroa mites invading honey bee brood cells. Experimental and Applied Acarology 18: 371-379.

Harris, J.W., Harbo, J.R., Villa, J.D., and R. G. Danka. 2003. Variable population growth of *Varroa destructor* (Mesostigmata: Varroidae) in colonies of honey bees (Hymenoptera: Apidae) during a 10-year period. Environmental Entomology 32: 1305-1312.