Development of novel Varroa mite control methods from attractants and arrestants isolated from brood host volatiles

Mark J. Carroll[1], Adrian Duehl[2] and Peter E. A. Teal[2]

[1] USDA-ARS, Carl Hayden Bee Research Center, Tucson, AZ ,[2] USDA-ARS, CMAVE Chemistry, Gainesville, FL

Introduction The Varroa mite (*Varroa destructor)* is the most destructive parasite of honey bees worldwide. The recent spread of mite resistance to miticides and concerns about the effects of miticides on bees has lead to increased interest in alternative control methods. One approach for the novel control of Varroa mites centers on chemical cues that mediate cell invasion, or larval host acquisition, by adult female (phoretic) mites (Figure 1). During cell invasion, a female mite moves from its adult bee host into a brood cell containing a bee larva near cell capping. Female mites are able to distinguish between preferred larval hosts (drone and worker larvae near capping) and non-hosts (younger larvae). The fact that blind female mites are able to detect and acquire a larval host without direct contact suggests that volatile host cues may be involved.

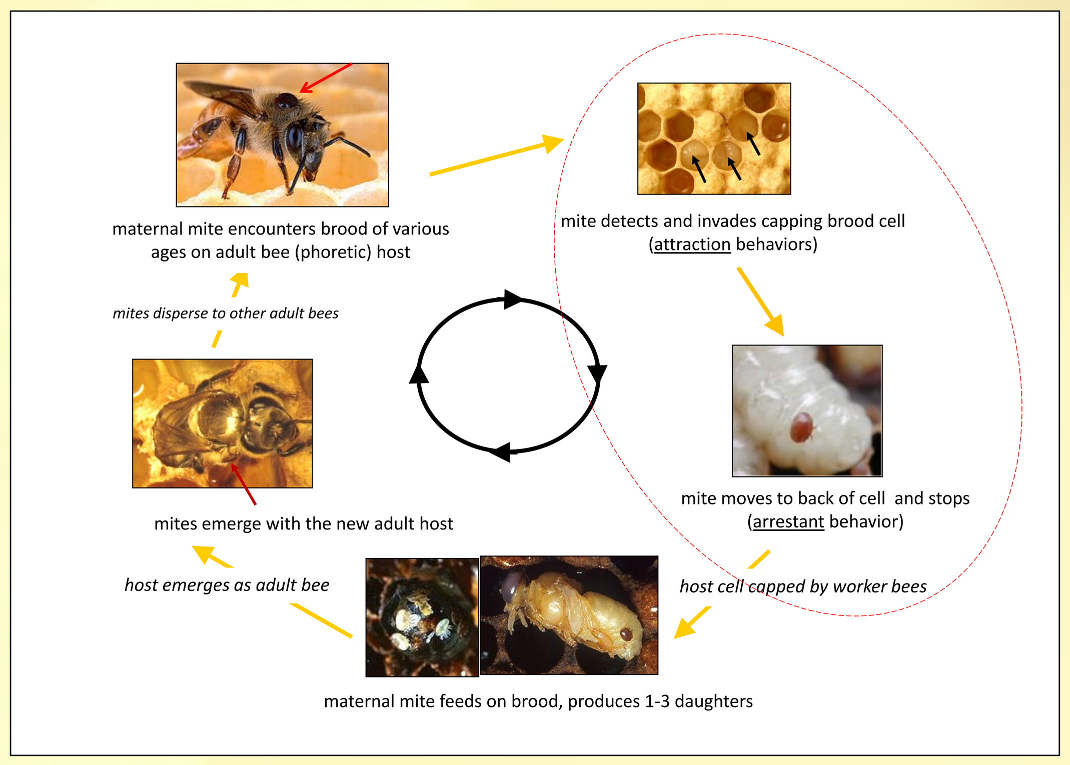

Figure 1. The life cycle of the Varroa mite, with cell invasion (brood host acquisition) phase circled.

We have identified volatiles present in capping larvae on brood comb that serve as short-distance attractants and arrestants to female mites. To sample volatiles from relatively undisturbed larvae, we enclosed larvae and attending adult workers on intact comb in an observation frames and sampled the frame airspace with a volatile collection system (Figure 2) . We have identified over 120 compounds present in active brood comb by GC-MS (gas chromatography-mass spectrometry) analysis, 31 of which varied consistently with brood age around cell capping (Figure 3). Adult female mites exposed to two of these compounds (referred to as CA and CB, patent pending) leave their adult bee host in significant numbers. Mites show responses to CA that are consistent with the known sequence of behavior during cell invasion, where a mite becomes excited adjacent to the larval host cell, moves off the adult bee to the bottom of the brood cell, and stops underneath the larval host.

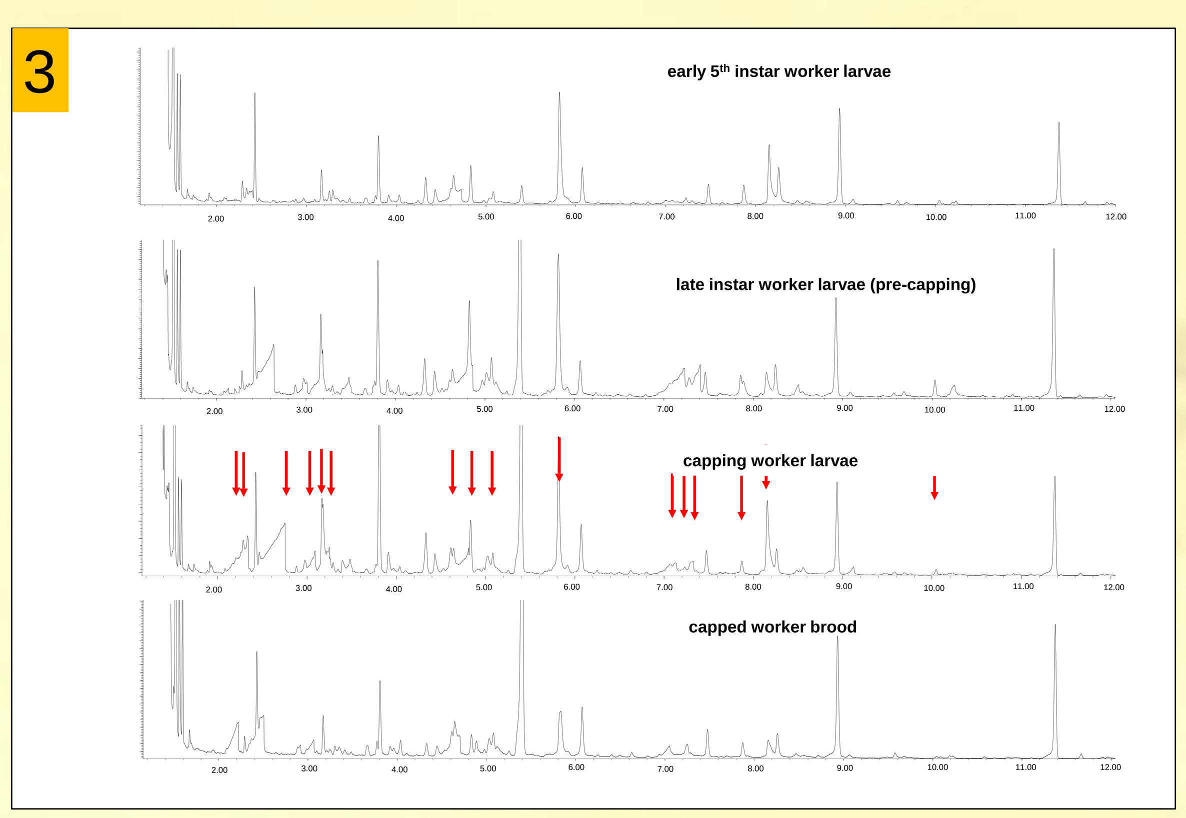

Figure 2. Volatile collection system used to trap odors from bee frames.
Figure 3. GC chromatogram of volatiles from different stages of brood. Volatiles that vary with developmental stages (host stage) are highlighted by red arrows.

We intend to use our understanding of host acquisition semiochemicals either to trap and kill phoretic mites, or to disrupt cell invasion behaviors (flooding). Here, we describe the approaches that we will employ in Spring and Summer 2011.

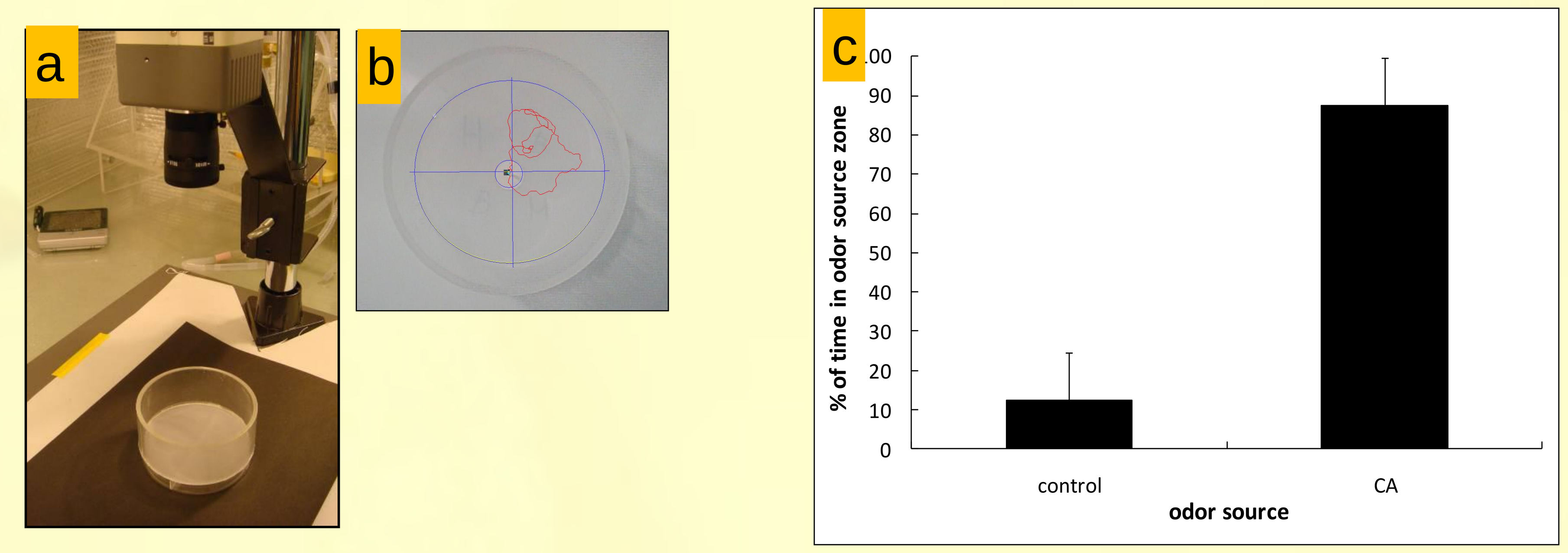

Figure 4. A) Ethovision behavioral analysis system setup, showing the bioassay arena. B) A single mite is placed in the center of the arena and its movements in response to chemicals below the arena are tracked by the computer. C) Mites are strongly attracted to CA odor volatiles in these arenas.

Objective 1) Screen other bee brood volatiles as potential semiochemical ("signaling chemical") cues and synergists of CA and CB We will evaluate brood volatiles other than CA and CB both individually and in combination with CA and CB using Ethovision behavioral analysis software system (Figure 4). Responses of free-roaming mites to synthetic volatiles will be evaluated in a mesh-floor arena suspended over a volatile-releasing solutions. Each mite will be introduced to an odor-neutral zone (a vertical pin placed through the center of the arena floor) and observed for movement and mobility. Mite movements will then be analyzed and compared against controls (mites in arenas without synthetic volatile release).

Figure 5. Synthetic versions of attractive brood volatiles may be used either to A) trap mites or B) disrupt their senses by flooding the airspace with the chemical cue. Both technologies will be pursued in Spring and Summer of 2011.

Objective 2) Develop an in-hive attracticide trap for Varroa mite using attractants and arrestants isolated from brood hosts volatiles
Our goal is to incorporate identified attractants and arrestants as synthetic baits in an in-hive trap in the brood box of honey bee colonies. Our preferred frame-based prototype is designed to replace a brood frame in a standard Langstroth configuration **(**Figure 5A). We will evaluate trap efficacy and effects on colonies containing traps with synthetics (treatments), traps without synthetics (synthetic controls), and constructs without traps at all (trap controls) . Colonies will be monitored over a four week period to follow mite trapping efficacy and bait effects on normal honey bee behaviors (brood rearing, oviposition) changes over time.

Objective 3) Develop in-hive flooding techniques using semiochemicals isolated from brood host volatiles
Chemically-mediated behaviors can be disrupted by overwhelming the senses with excess amounts of stimulatory chemical cues. Preliminary experiments with glass-enclosed brood frames have indicated that mite cell invasion can be significantly reduced by flooding the brood comb airspace with high concentrations of synthetic CA volatiles without disrupting normal honey bee behaviors (Figure 5B). Volatile concentrations that reduce mite cell invasion but do not interfere with normal bee colony behaviors will then be evaluated in colonies in the field.

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Potential Benefits to Beekeepers and the Almond Industry
Our goal is to make successful volatile technology available to commercial beekeepers as soon as feasible. In-hive traps and flooding designs that reduce mite infestations in nuc colonies will be rapidly tested in full size commercial colonies in the field. We seek to develop applications that are immediately available to future industry collaborators once our patent on Varroa attractants and arrestants has been approved.