Varroa mite attractants: potential solution for Varroa mite/ viral challenges to honey bees

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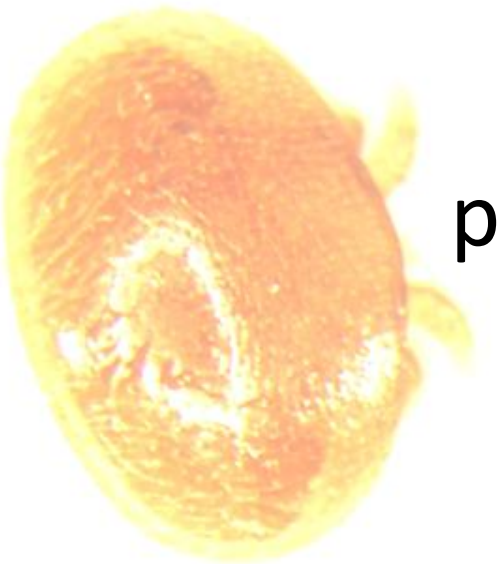


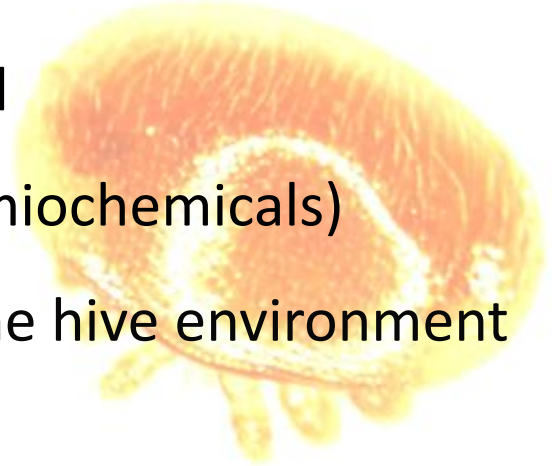
Varroa mite attractants: potential solution for Varroa mite/ viral challenges to honey bees

I. Volatile (odor) collections in the hive environment

II. Comparisons of volatiles from bee brood

III. Responses of mites to host volatiles (semiochemicals)

IV. Using host volatiles for mite control in the hive environment



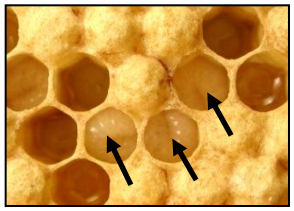
Varroa mite
(*Varroa destructor*)

- parasite of capped bee brood and adult bees
- recent host switch from the Asian honey bee *(Apis cerana)* to the western honey bee *(Apis mellifera)*
- western honey bees are highly susceptible
- leading known cause of colony mortality worldwide
- mite resistance to chemical treatments is growing

Identify the cues that the mite uses to acquire its brood host

Cell invasion behaviors

maternal mite encounters brood of various ages on phoretic host



detection, excitation, and invasion of brood host cell (attraction behavior)

mite moves into the back of the host cell, movement stops (arrestant behavior)

host cell capped by worker bees

mite feeds and produces young on developing bee brood

1-3 daughters/round

brood host emerges as adult bee

mites emerge with the newly emerged host

mites disperse to other adult bees

photos of pupae courtesy of USDA

Varroa cell invasion behavior

- attraction occurs over very short distances (less than 7 mm)

  adult female mite - 2.0 mm wide

  adult worker bee – 14 mm long

  worker brood cell – 11 mm deep

- mites have strong brood caste preferences for larvae

  drone > worker >> queen

- mites only invade during a narrow window of host development

  worker brood – 15-20 hrs before capping through capping

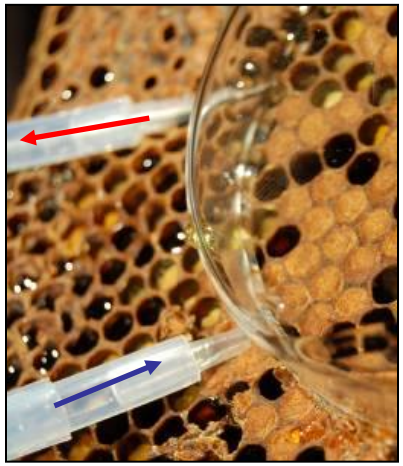
  drone brood – 40-50 hrs before capping through capping

Mites are attracted to volatiles from host brood

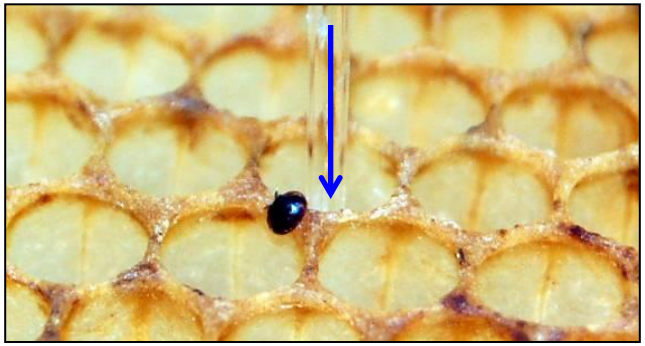
Near contact cue ~ 7 to 10 mm from target cell

Are mite attracted to brood odors alone?

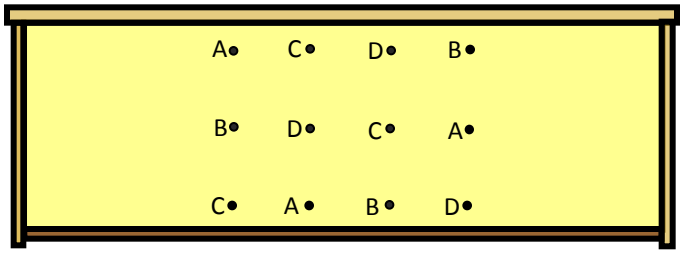
On-comb volatile infusion bioassay



odors collected off enclosed brood



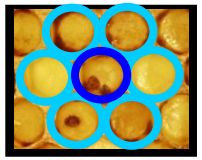
volatiles <u>slowly</u> infused into single cells through capillary tubes (~2mL/min)



choice arena

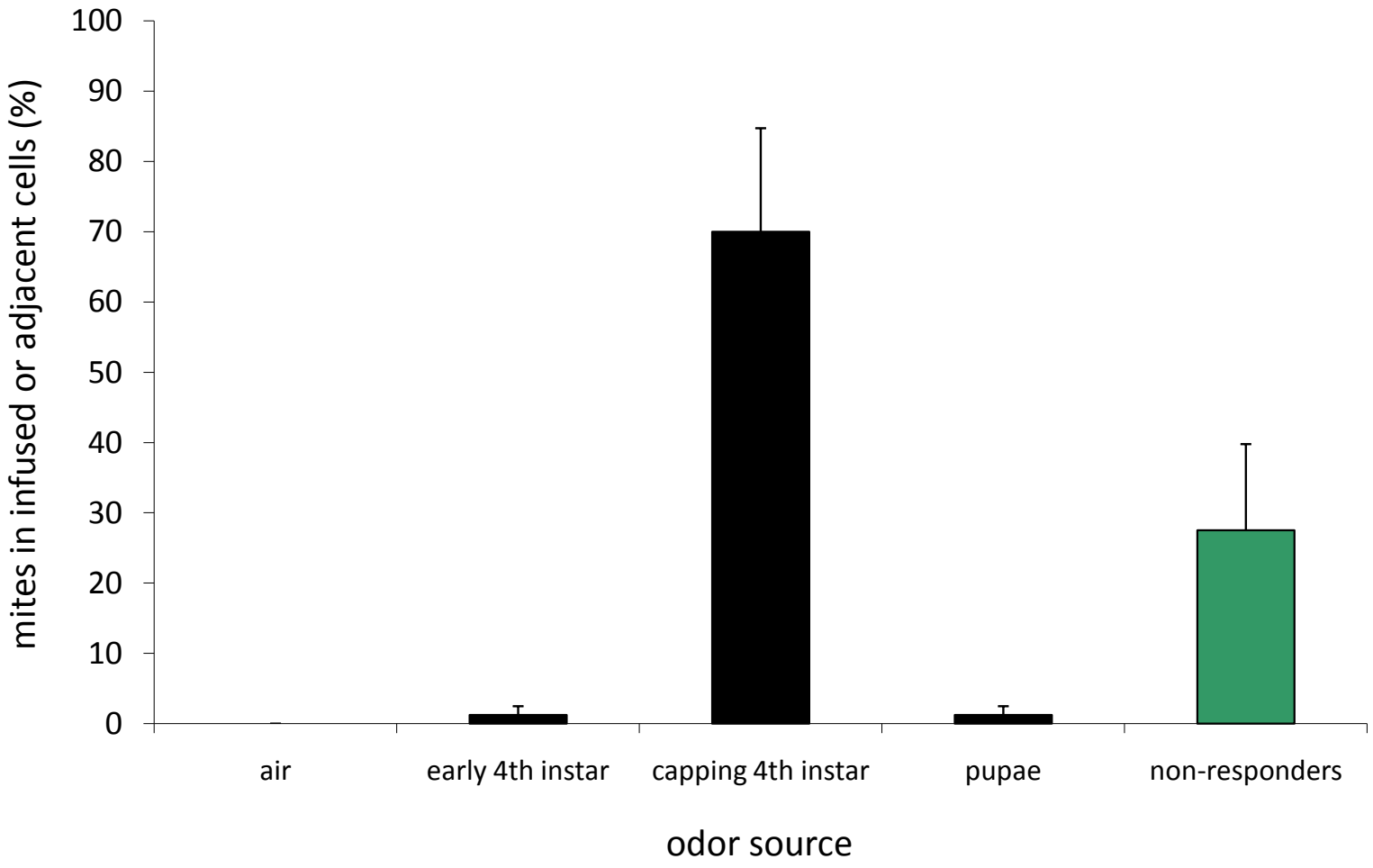
volatiles from 3 brood ages (plus control) <u>infused</u> into single cells across empty comb

- 40 free-roaming mites released
- position of mites in arena noted 30 minutes after release

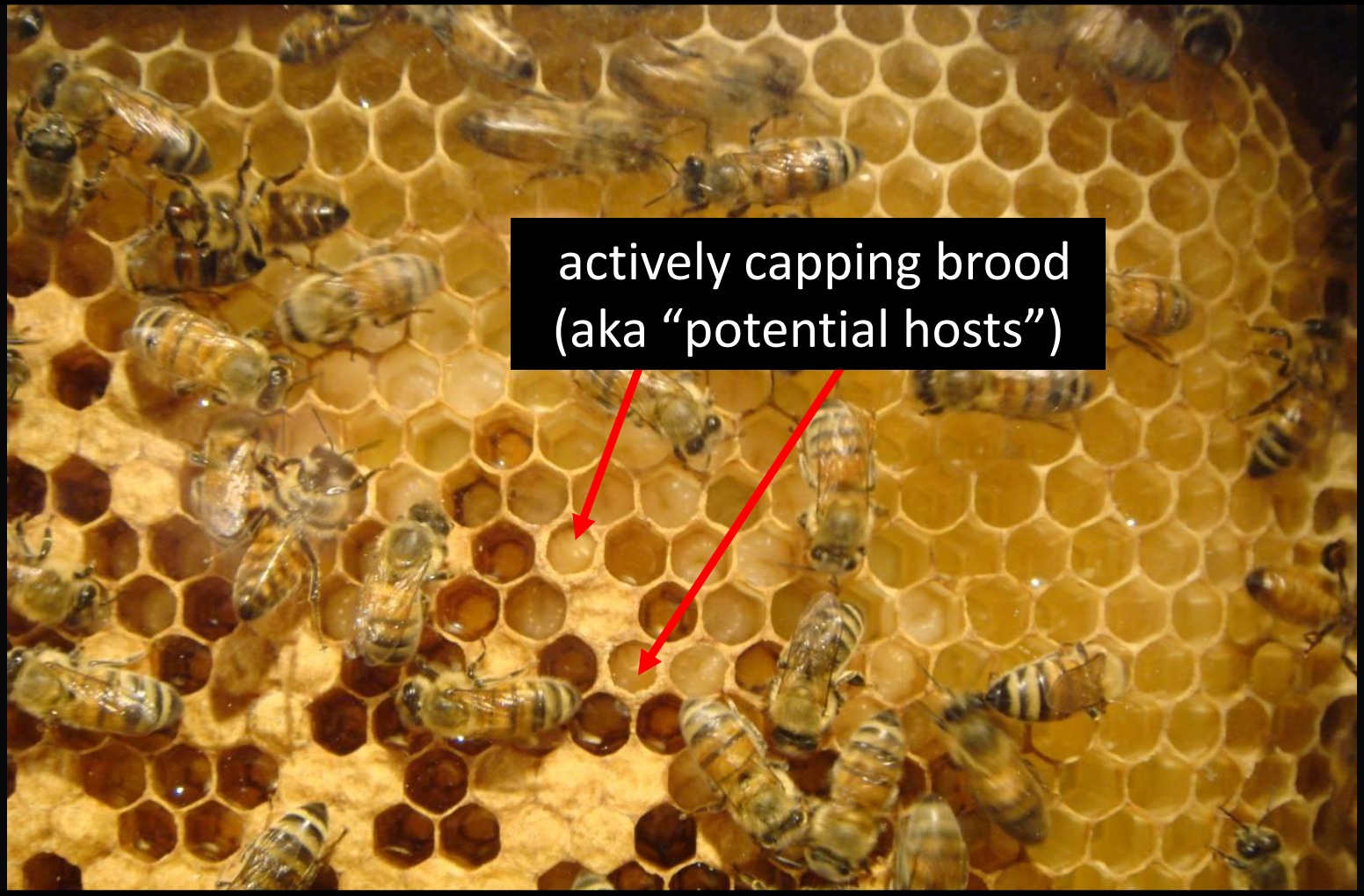


Mites are attracted to odors from capping brood

On-comb volatile infusion bioassay



Mites find capping brood hosts in a chemically confusing environment



“… a sea of competing odors from different-age brood …”

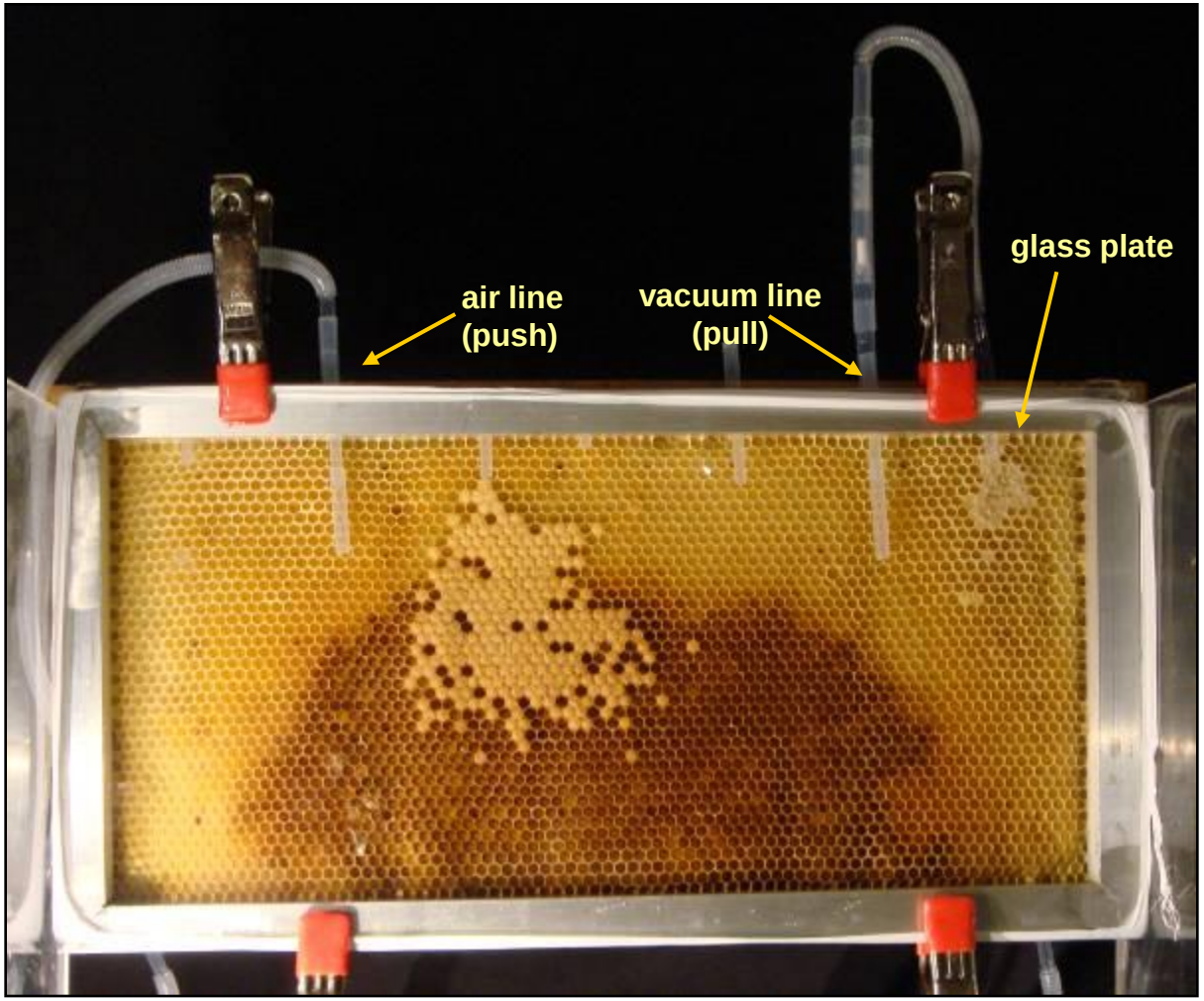
Problems with identifying odor cues from brood comb (from a human perspective)

- few brood of any particular age
- excess background odors from hive materials
- handling? stress? artifacts?

Isolated brood pulled from the comb = stressed larvae!

Aluminum observation frame (AOF)

Collection and manipulation of colony odors



a push-pull airflow system

Aluminum observation frame

Fits inside the perimeter of any deep frame

Multifunctional ports
(air, vacuum, food, waste disposal, odor sampling)

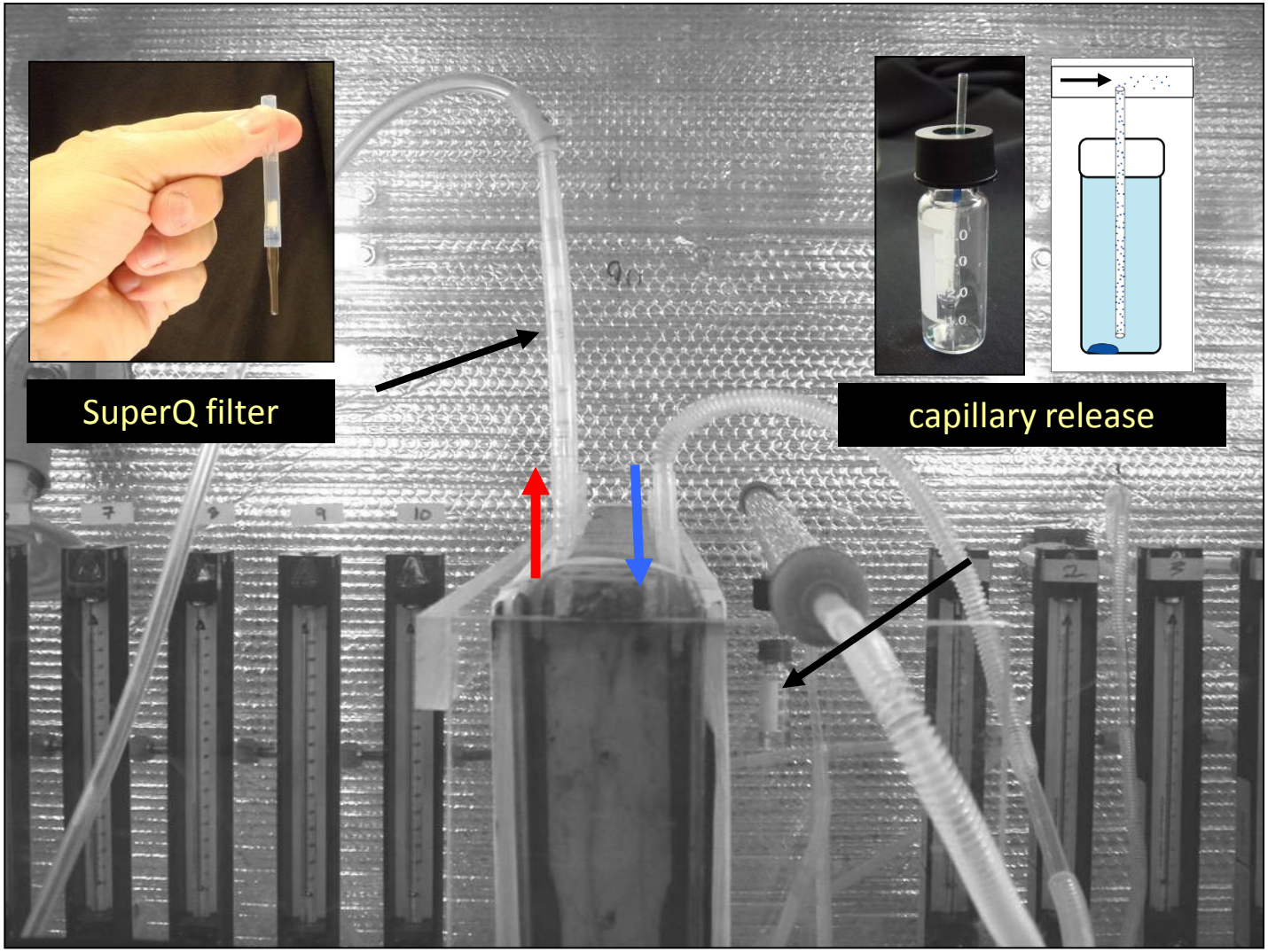
ports run along top edge of AOF

view into open port with bees

air flow ports

Push-pull airflow system

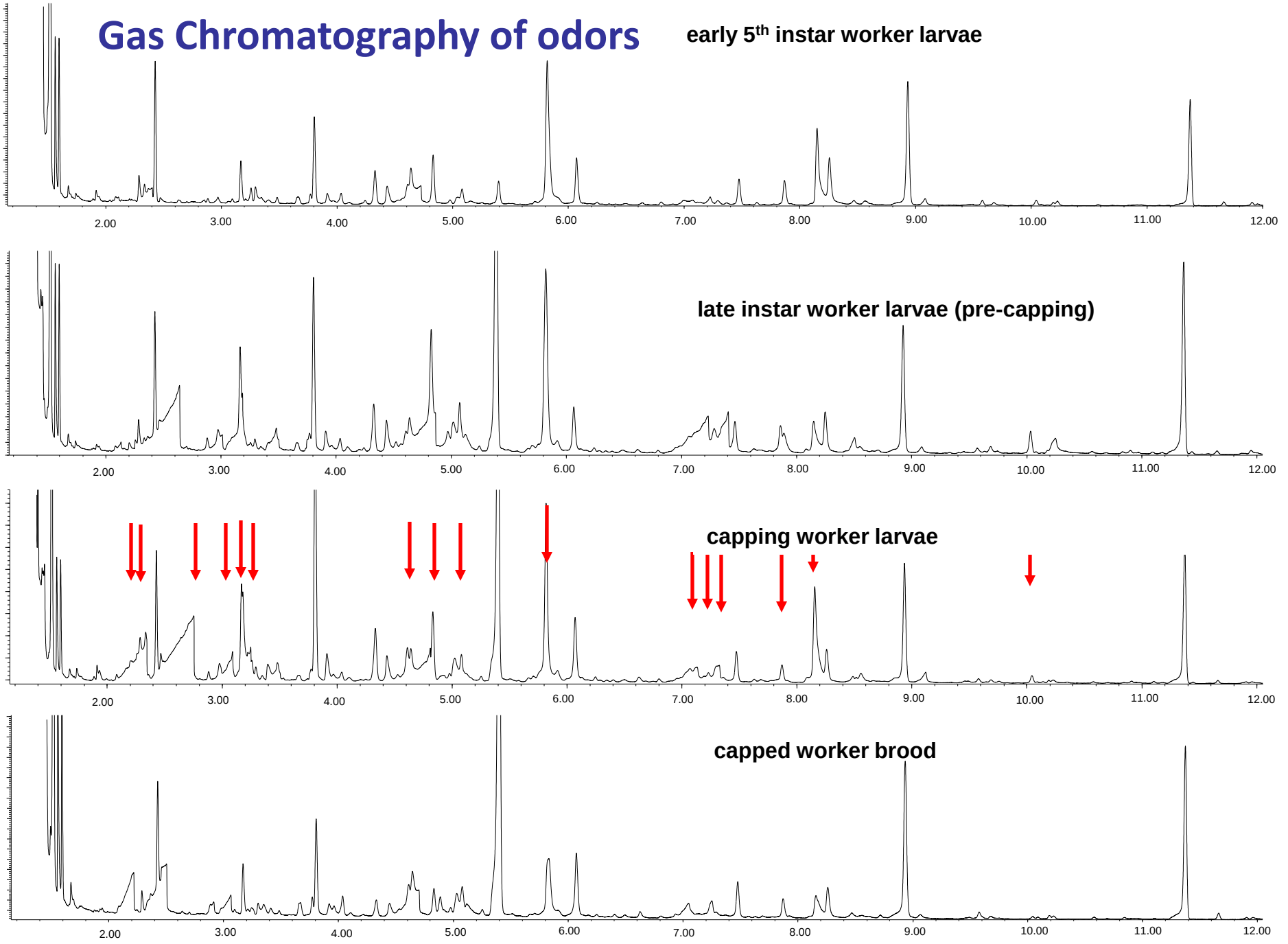
Sampling or adding odors



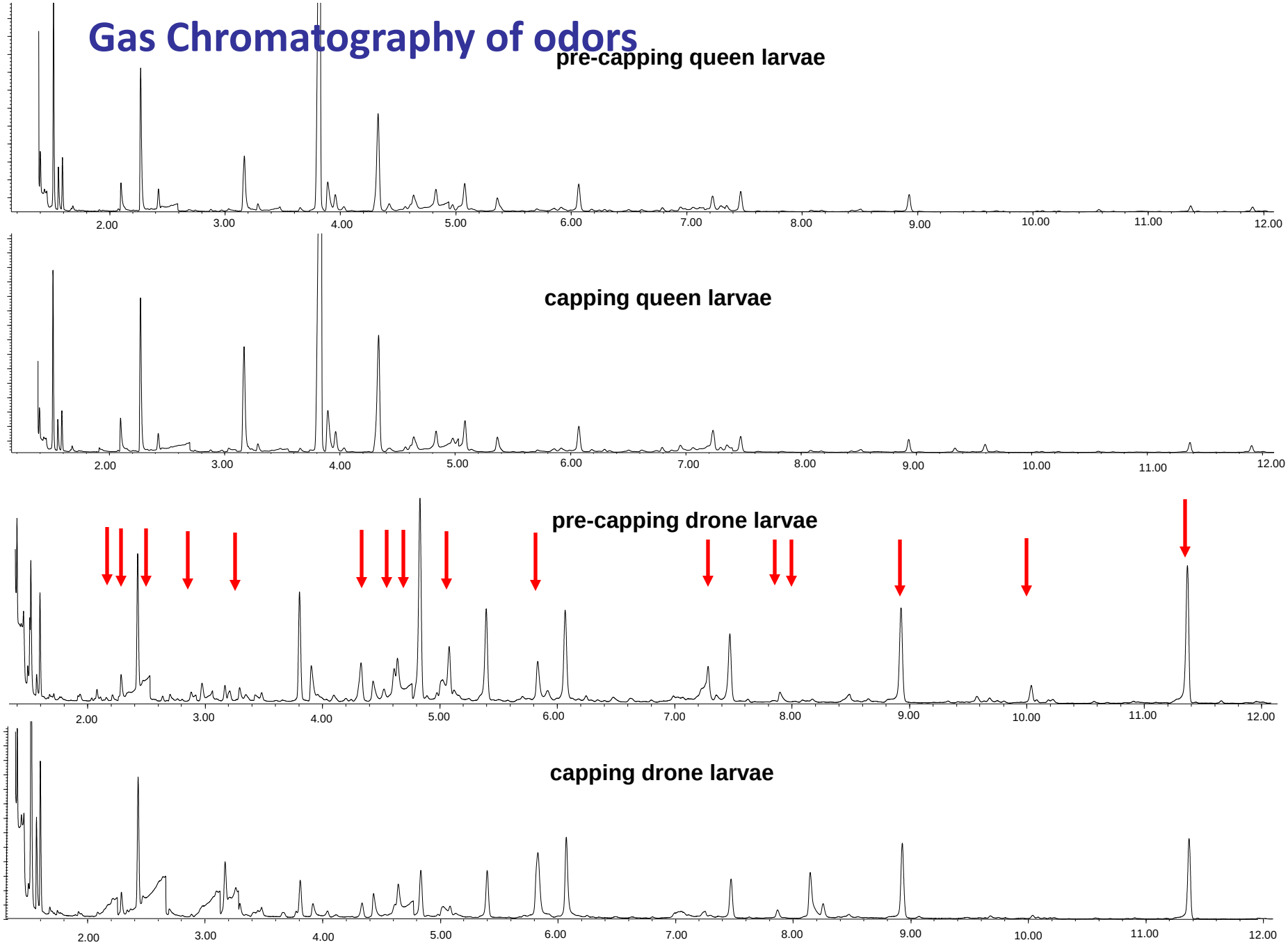
vacuum (sampling odors)

airflow (adding odors)

Gas Chromatography of odors

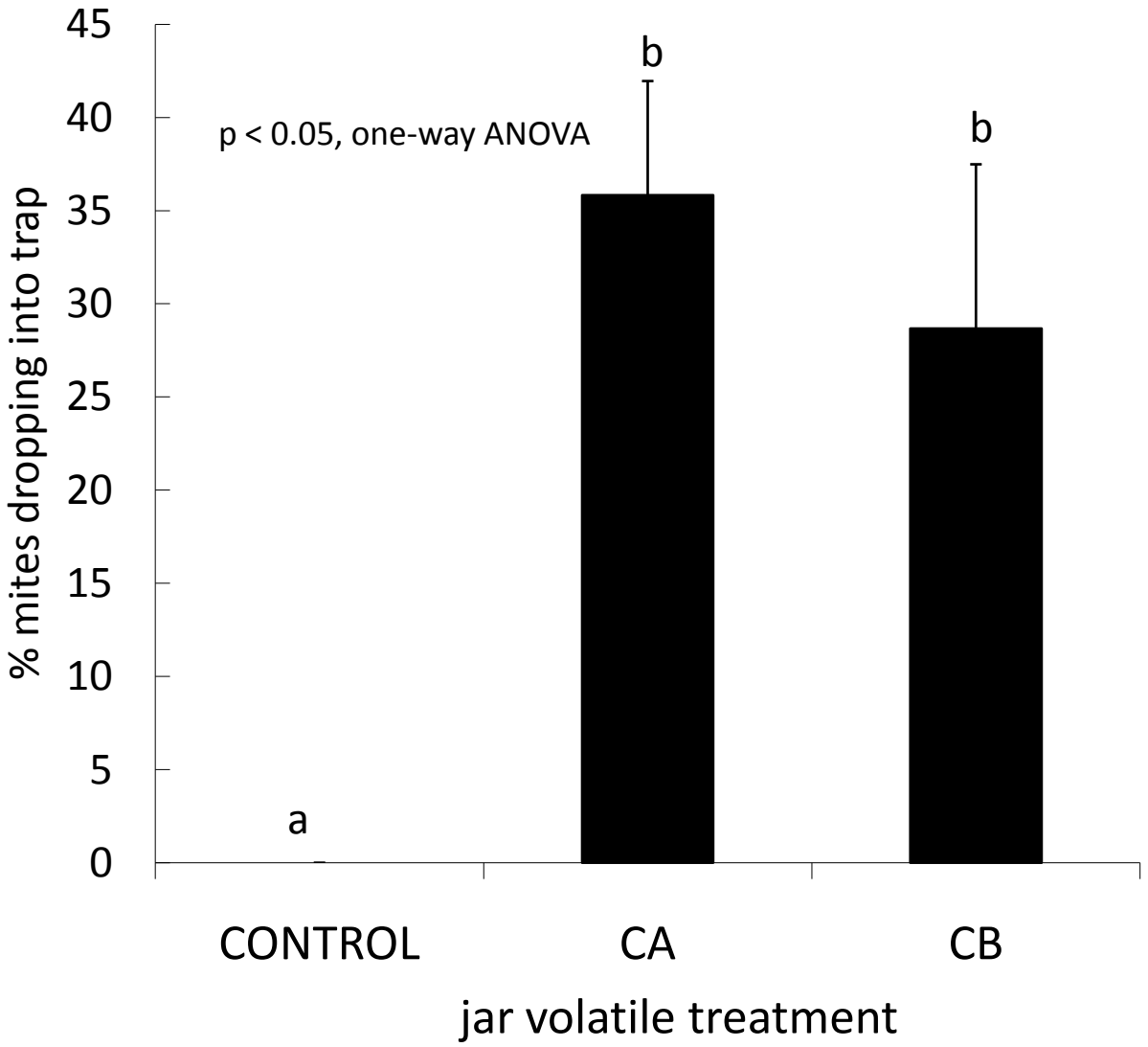


Gas Chromatography of odors

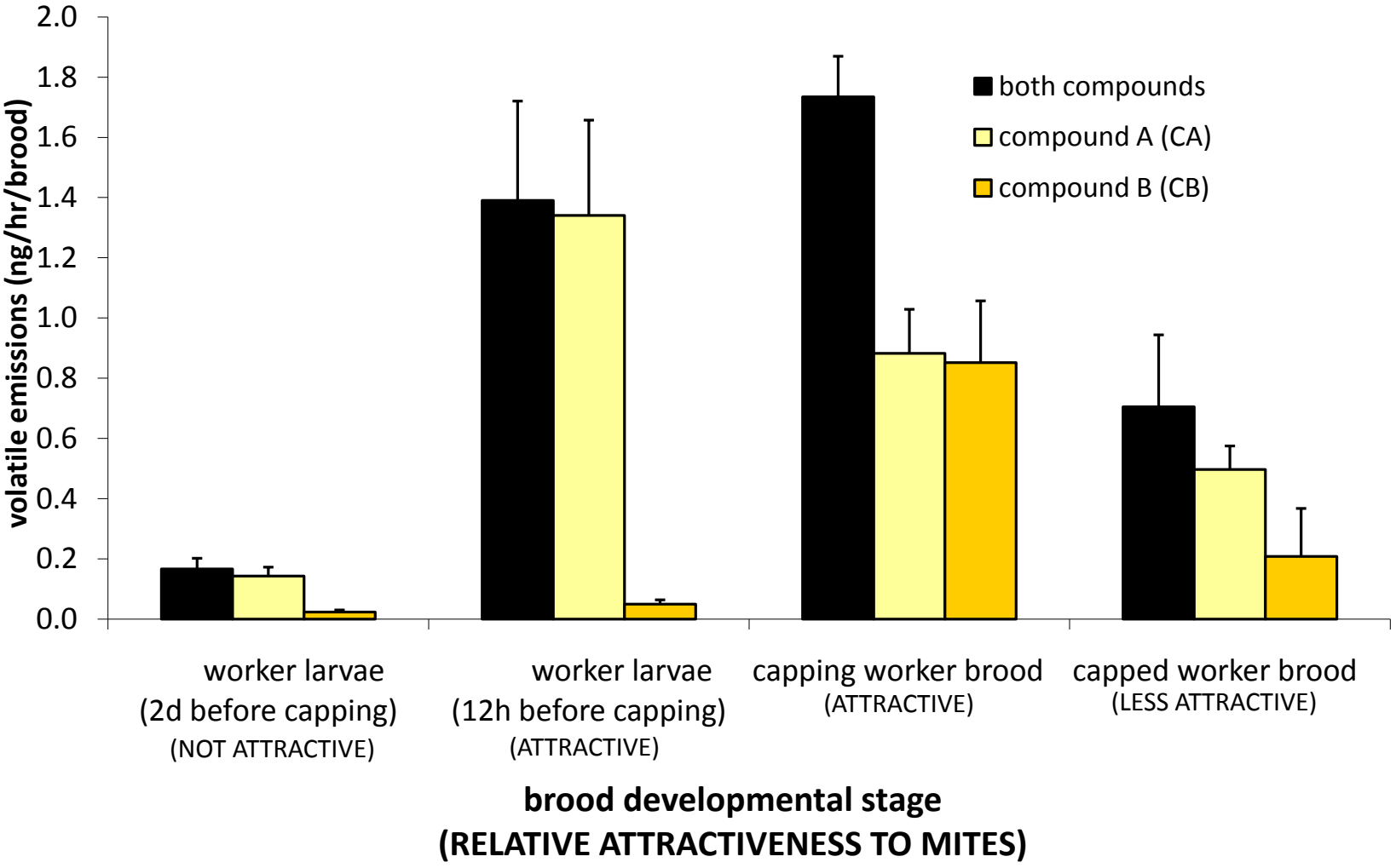


Brood volatiles CA and CB cause phoretic mites to move off adult bees

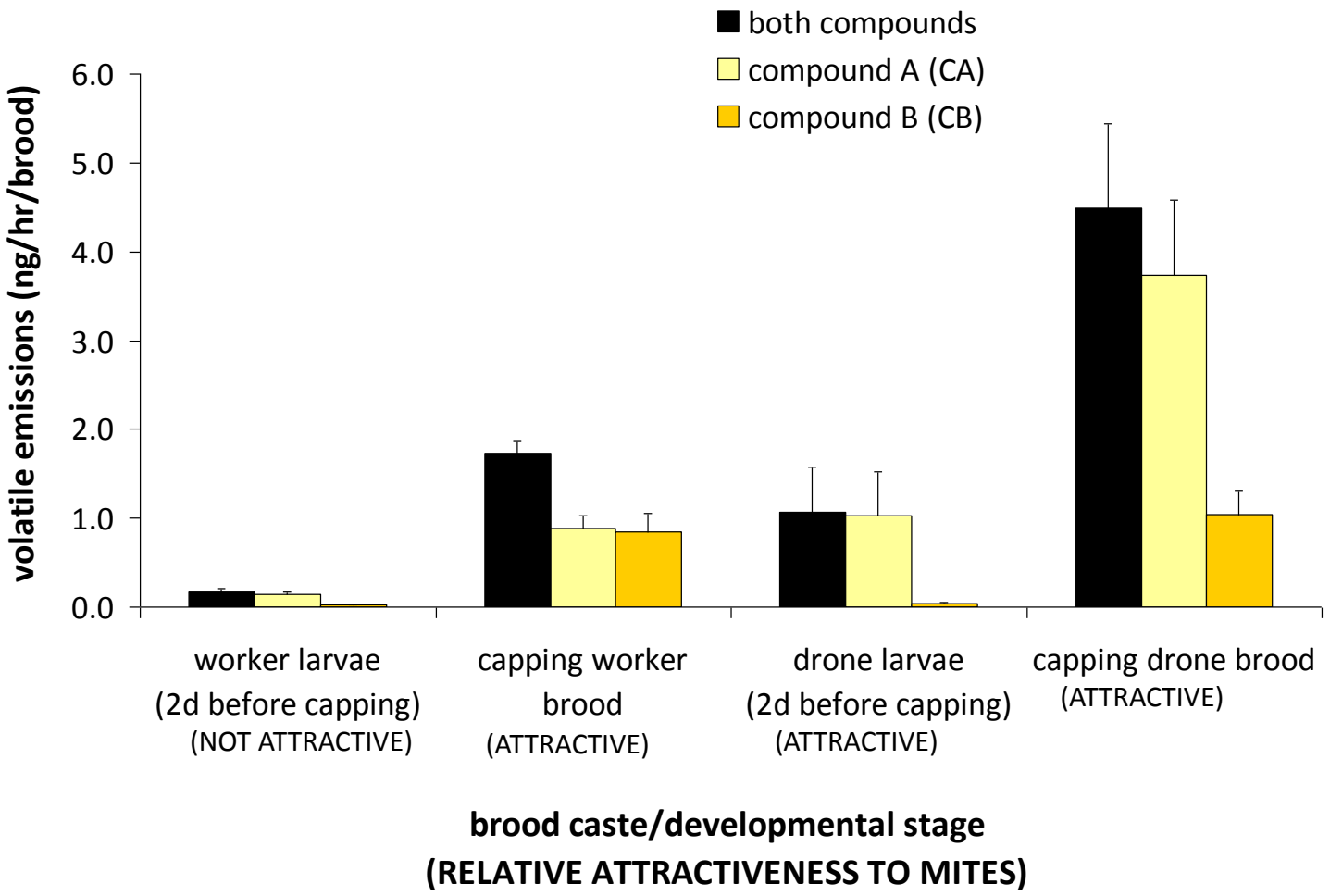
Mite bottom screen (jar) bioassay



Capping brood emit higher amounts of CA and CB than other developmental stages

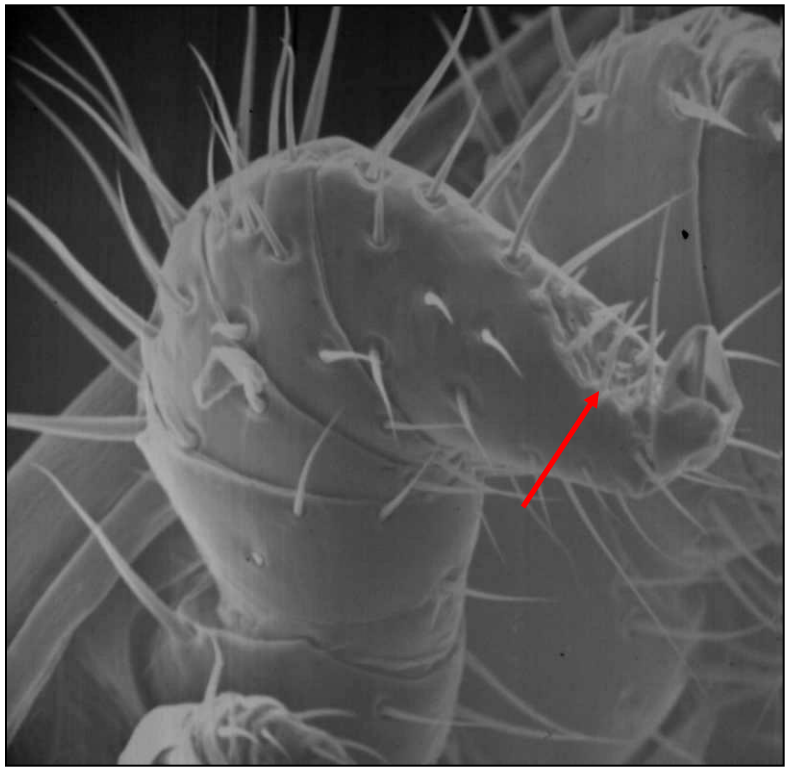


Capping drone larvae emit greater amounts of CA and CB volatiles than worker brood



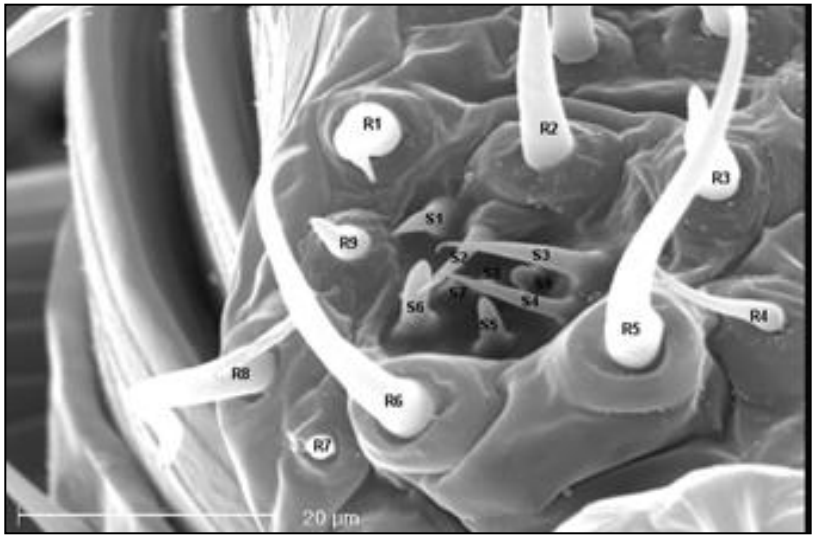
Detecting the sense of smell
Electrophysiological responses to odors

Adrian Duehl - USDA CMAVE



location of pit organ on foreleg

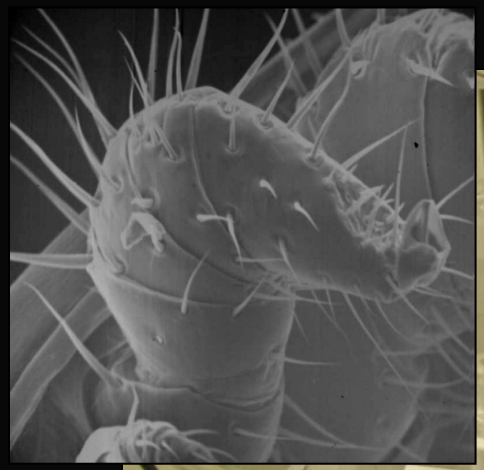
SEM by Adrian Duehl



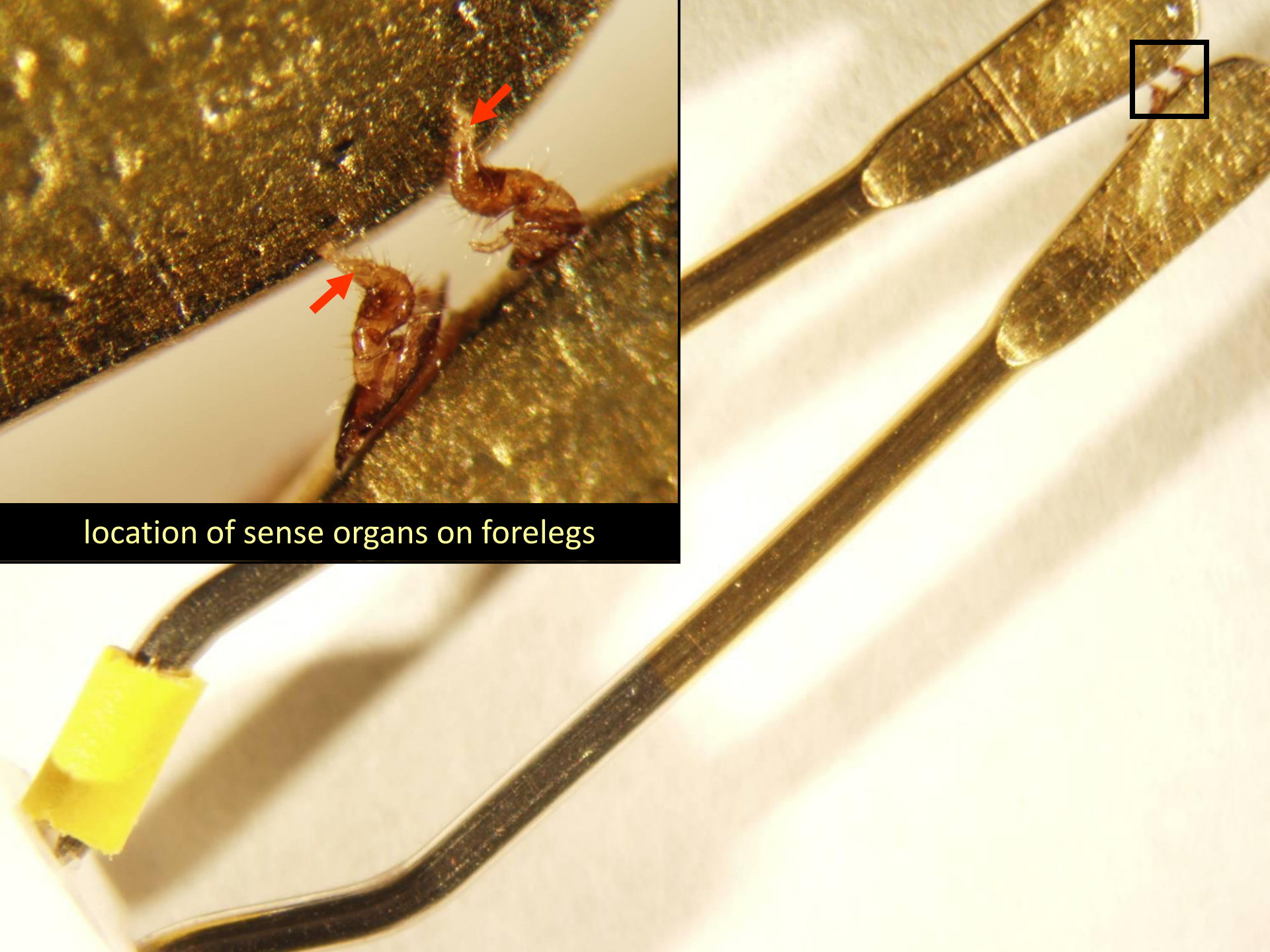
olfactory sensillae in the pit organ

SEM from Dillier et al, 2003, Swiss Bee Research Centre

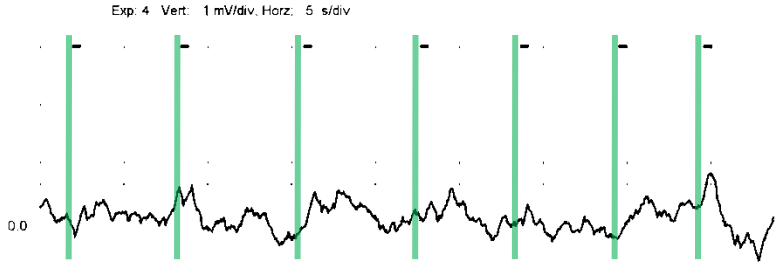
Mite foreleg electrophysiological responses to CA and CB



EAG (EFG) - Responses to synthetic chemical puffs


location of sense organs on forelegs

Control



Mites detect CA and CB with their forelegs

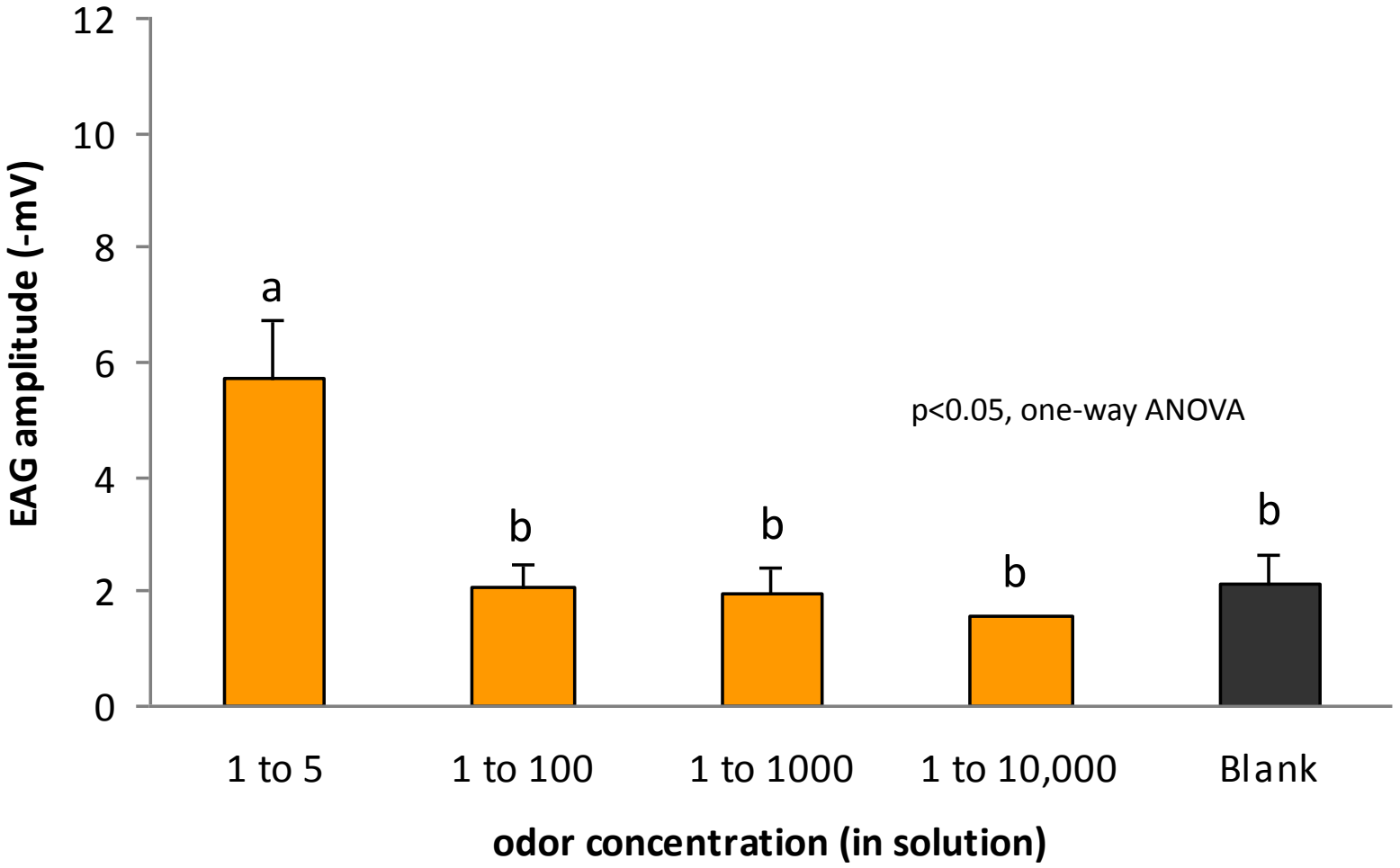
CA

EAG (EFG) responses

green line and black bar indicates odor puff

CB

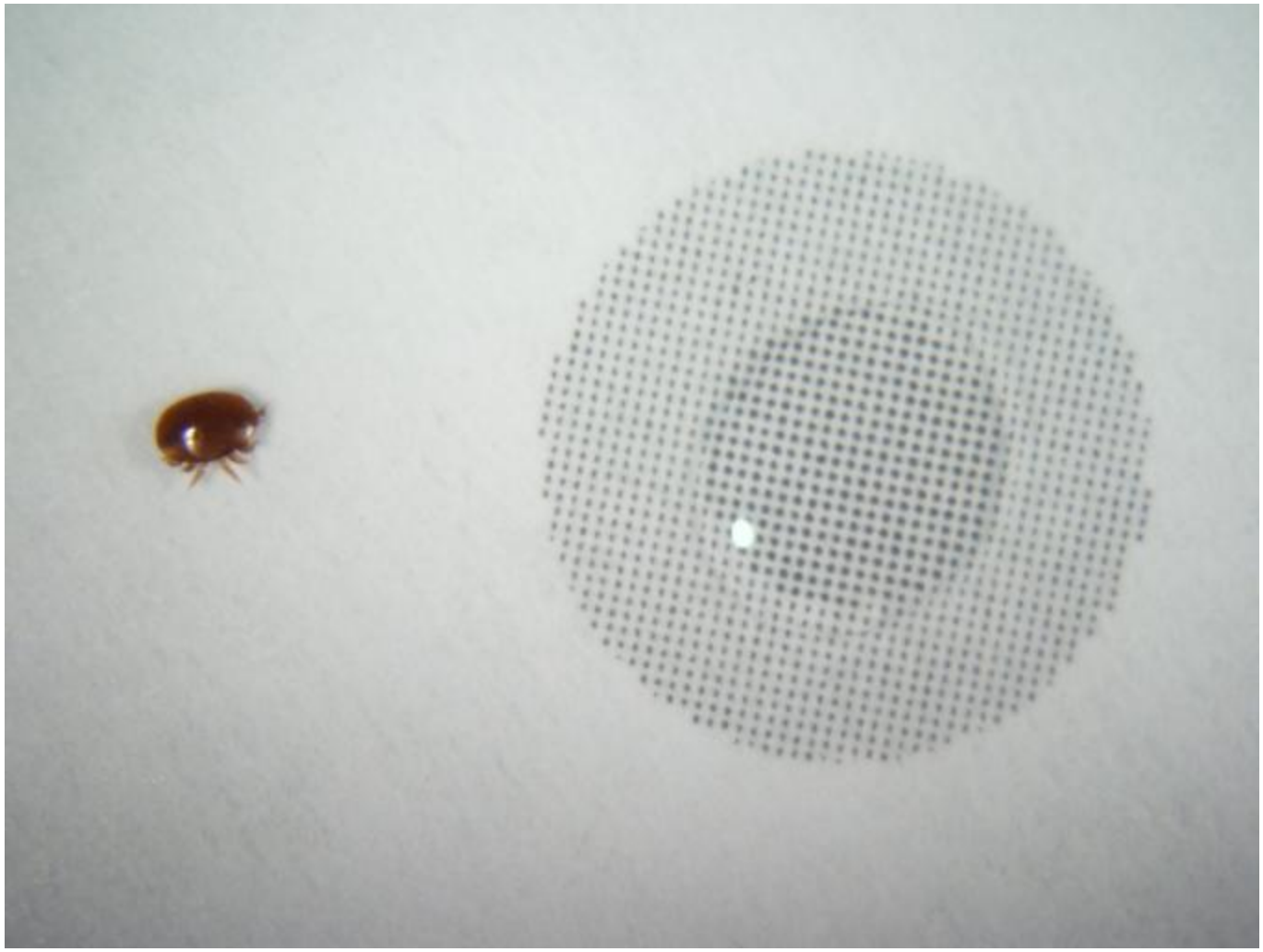
But mites only detect high concentrations of CA



Behavioral responses to synthetic CA and CB

Mites become excited by CA and CB at a close distance
Casting – twitching of forelegs

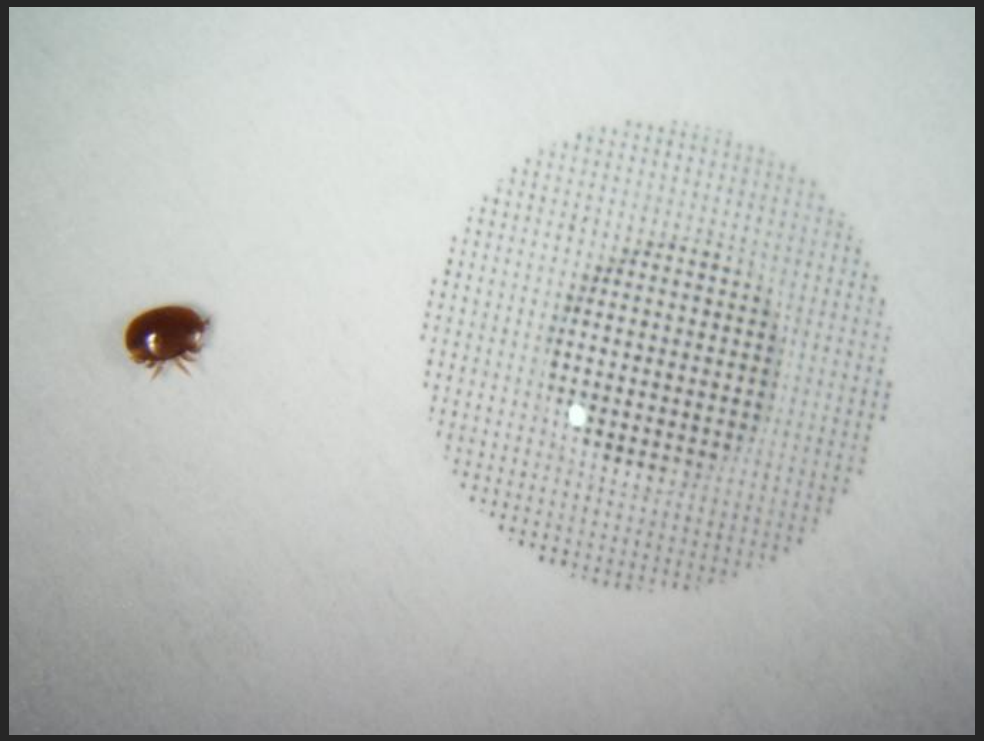
Final approach



Casting path straightens out and velocity increases at the last second

Problems in evaluating a near contact chemical cue

Too fast, too small to easily observe

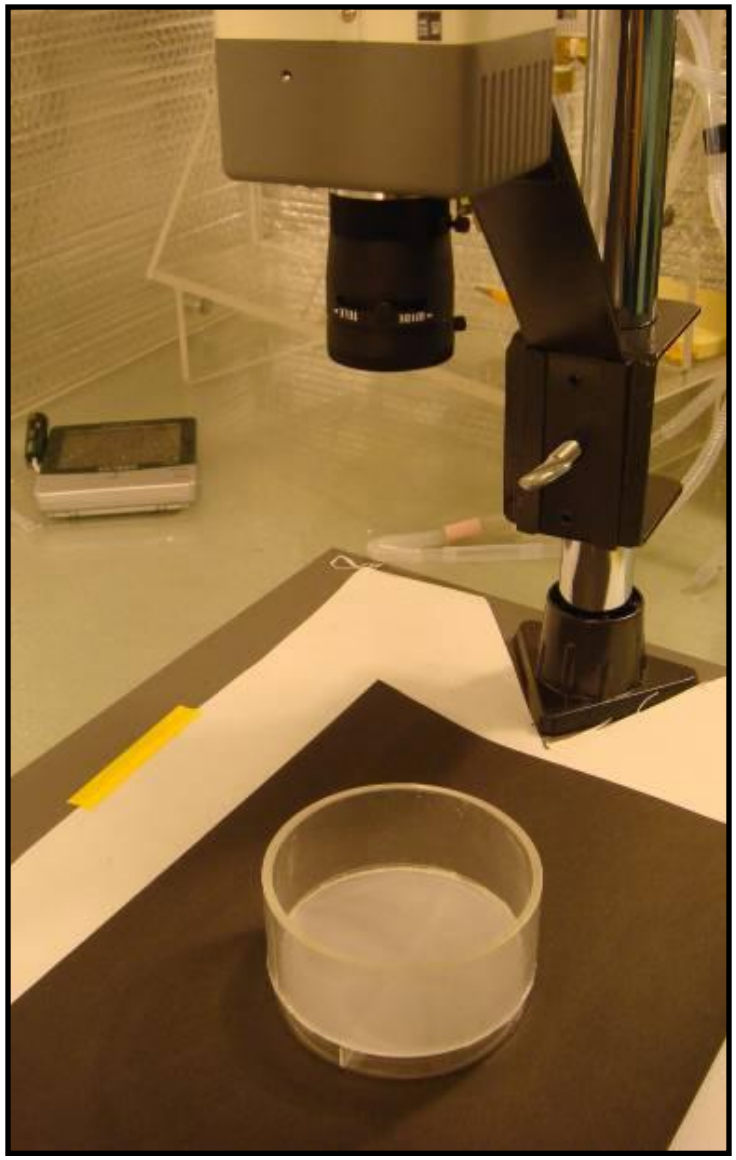


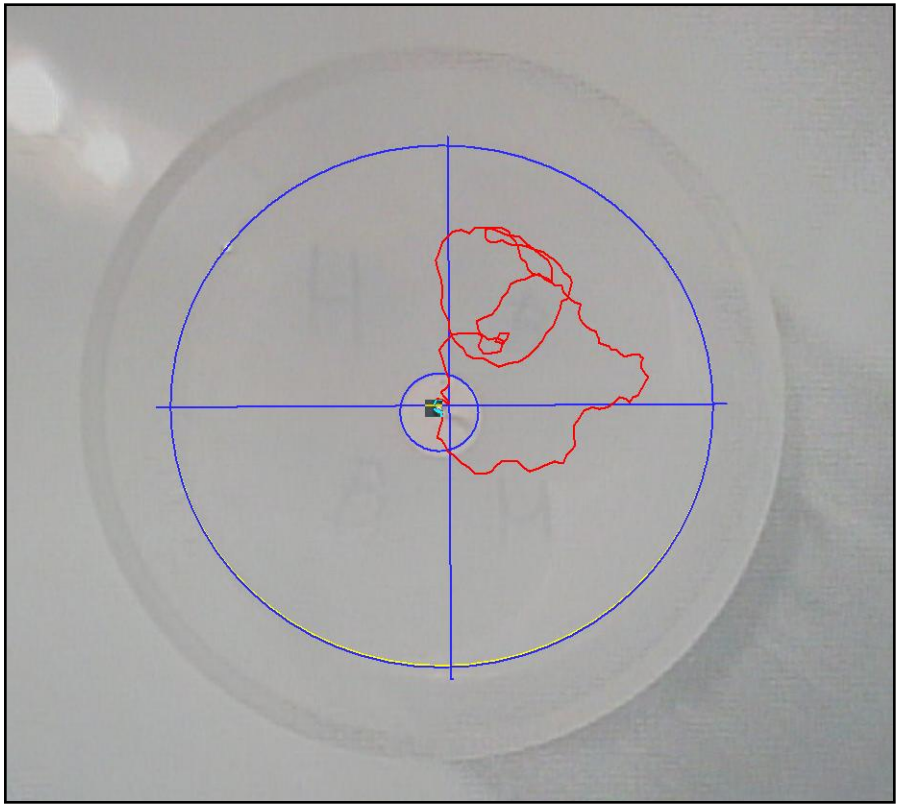
Open odor sources – contact fatal

Point odor sources - too localized

Need to spread out mite response in time and space

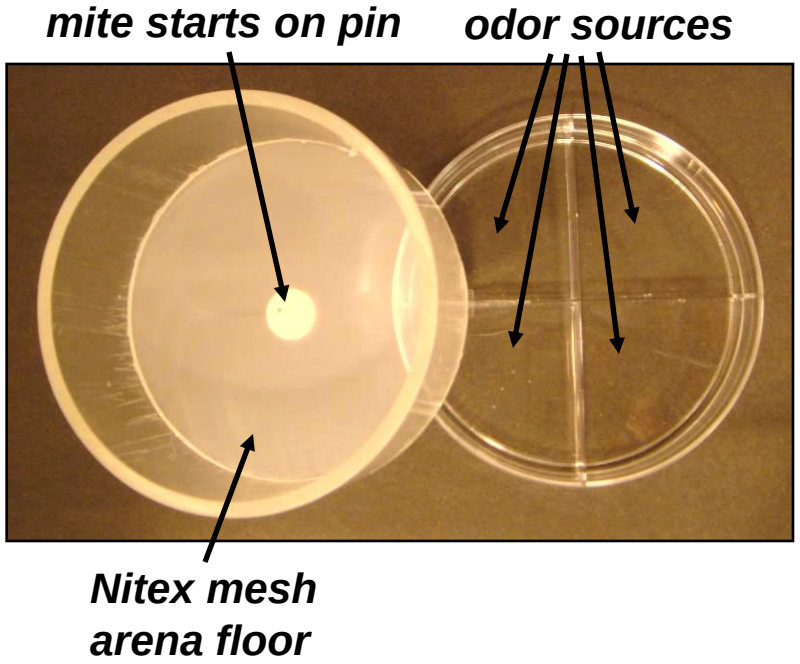
EthoVision – video analysis of mite movements



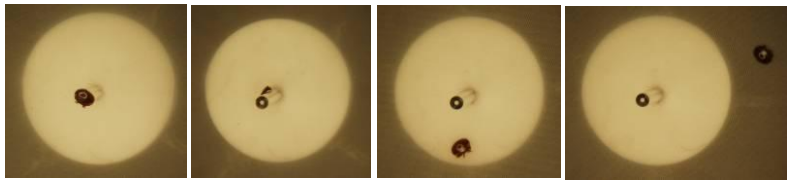


mite tracks in user-defined arena & odor zones

Diffusion bioassays



- odors diffuse up through mesh floor
- relatively even odor presentation
- mite never contacts odor source

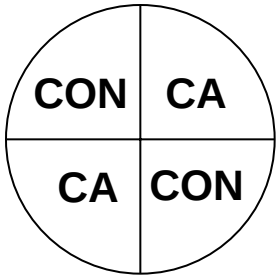


mite moving off central glass pin

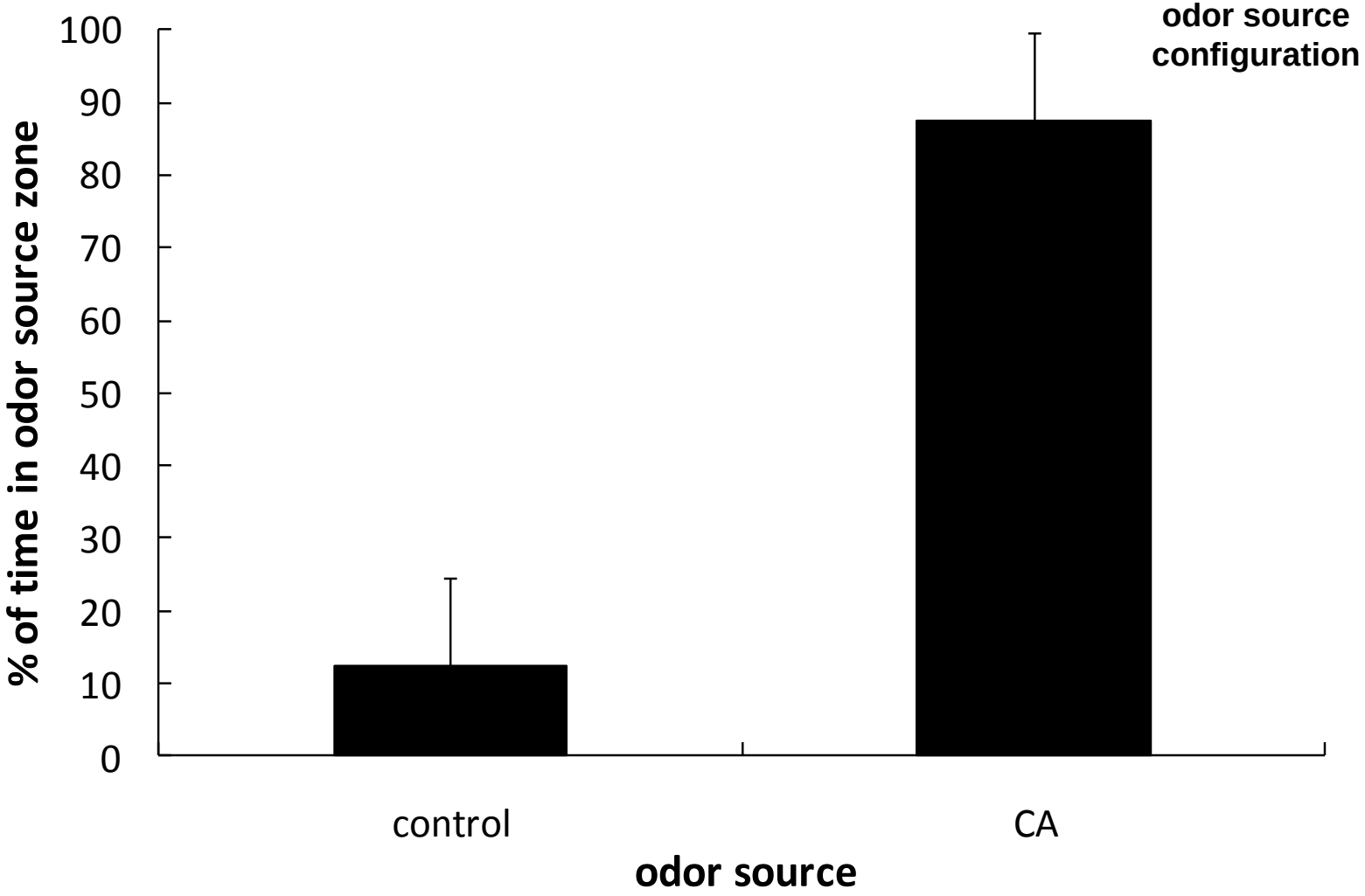
- mite starts on central glass pin, moves off

Mites are attracted to CA volatiles

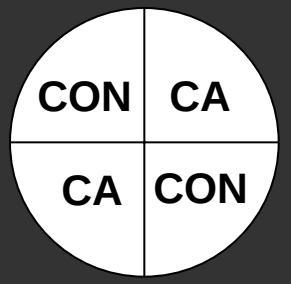
Dual choice diffusion bioassay



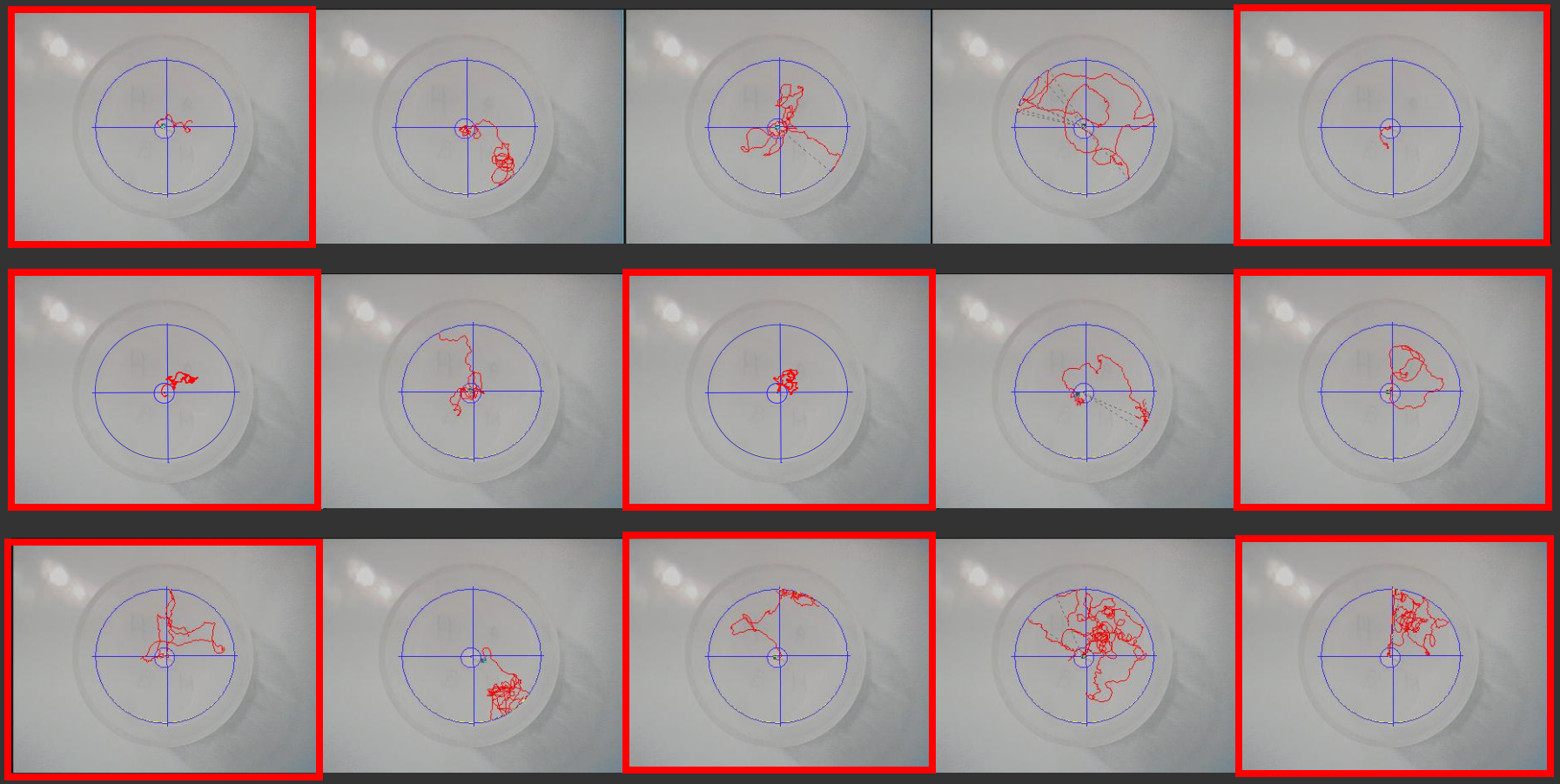
odor source configuration



Most, but not all mites, display arrestant behaviors (stopping) when moving over CA volatiles

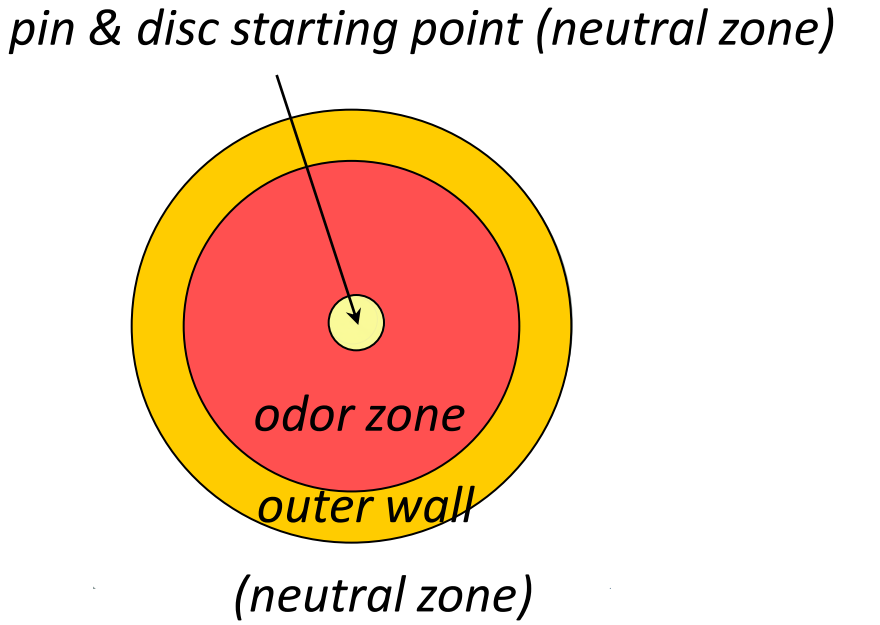


odor source configuration



No choice diffusion bioassay

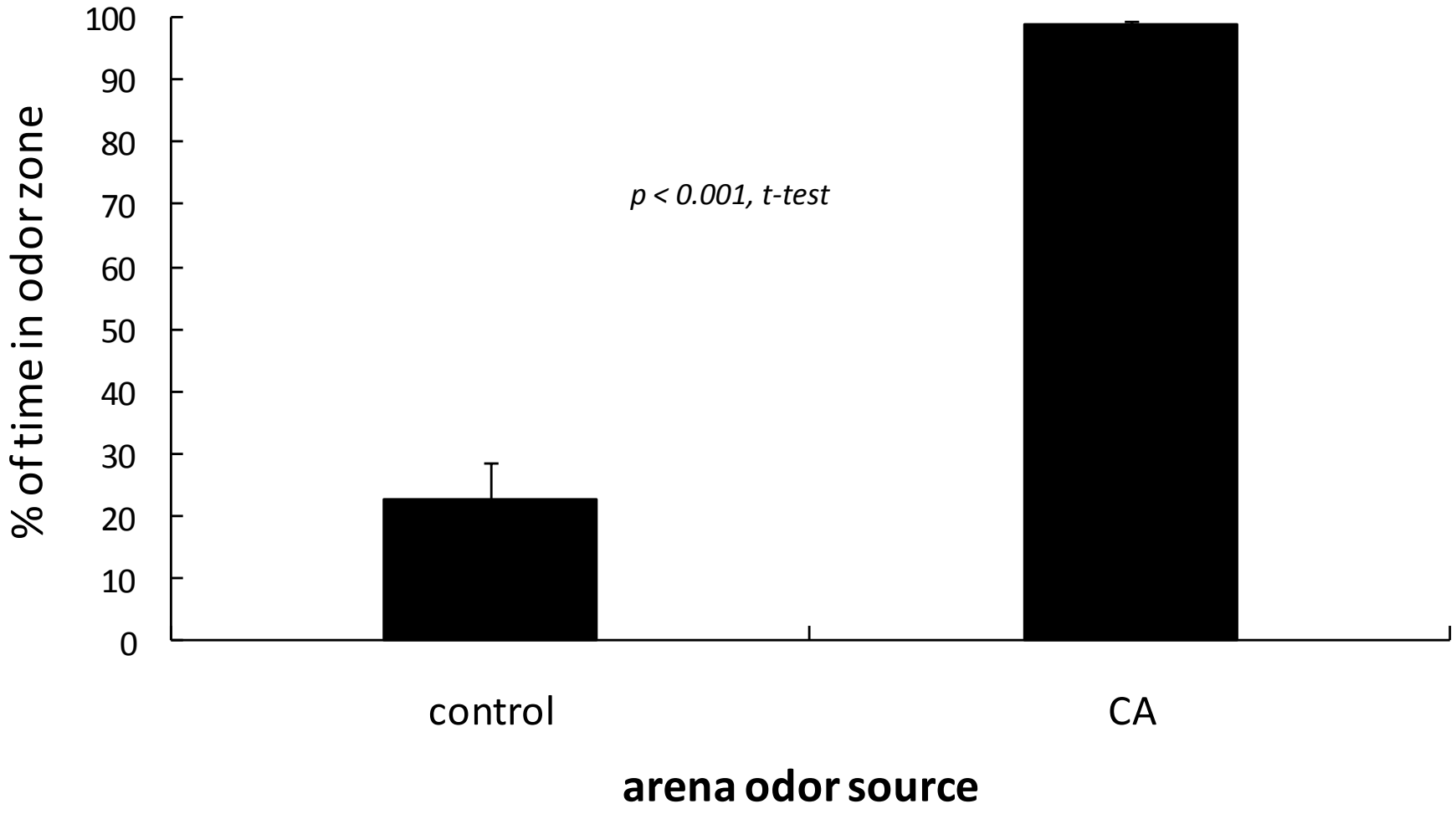
Mite responses to a single odor source



Without attractant or arrestant cues, mites tend to move out of the arena

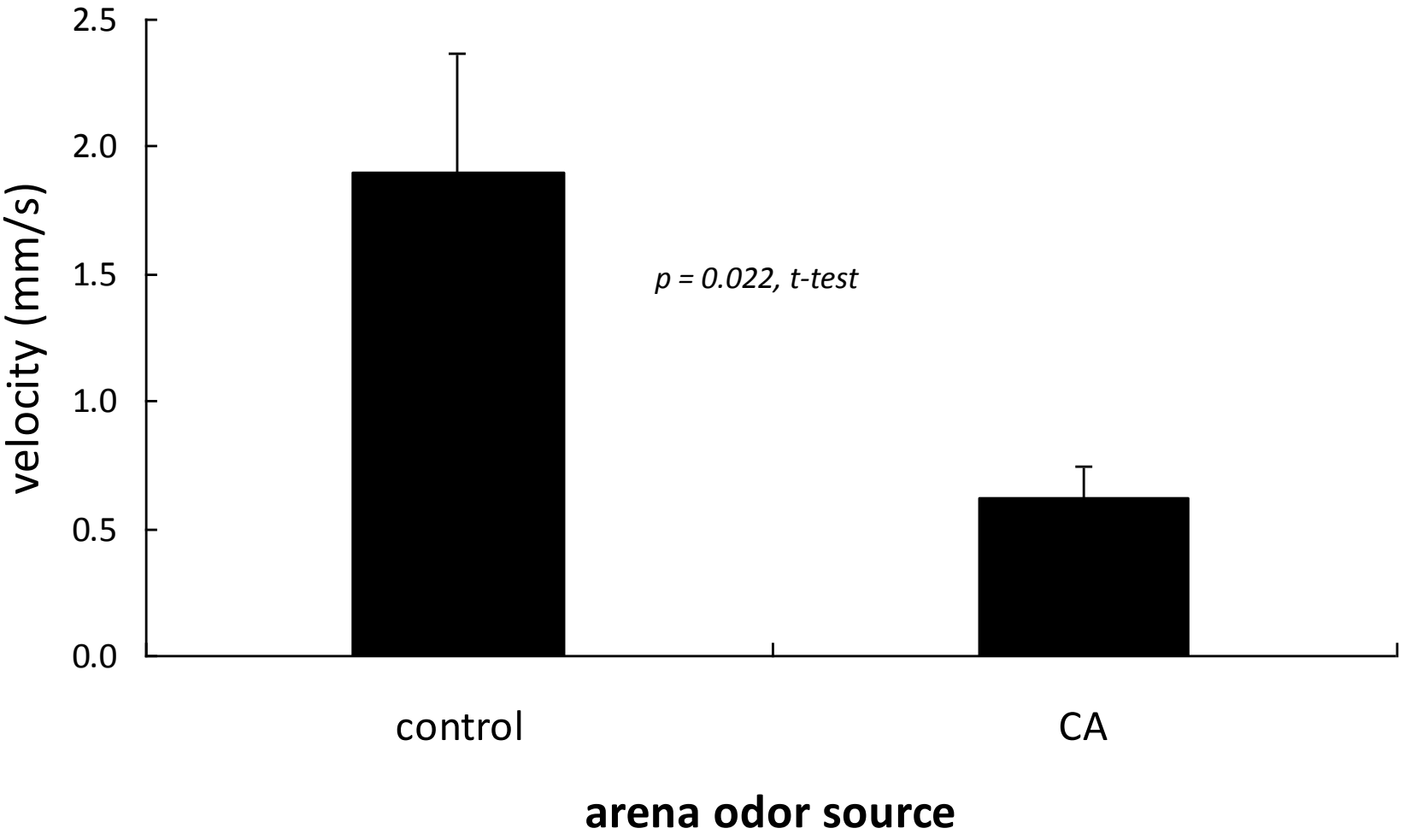
Mites remain in near contact with CA

No choice diffusion bioassay (EthoVision)



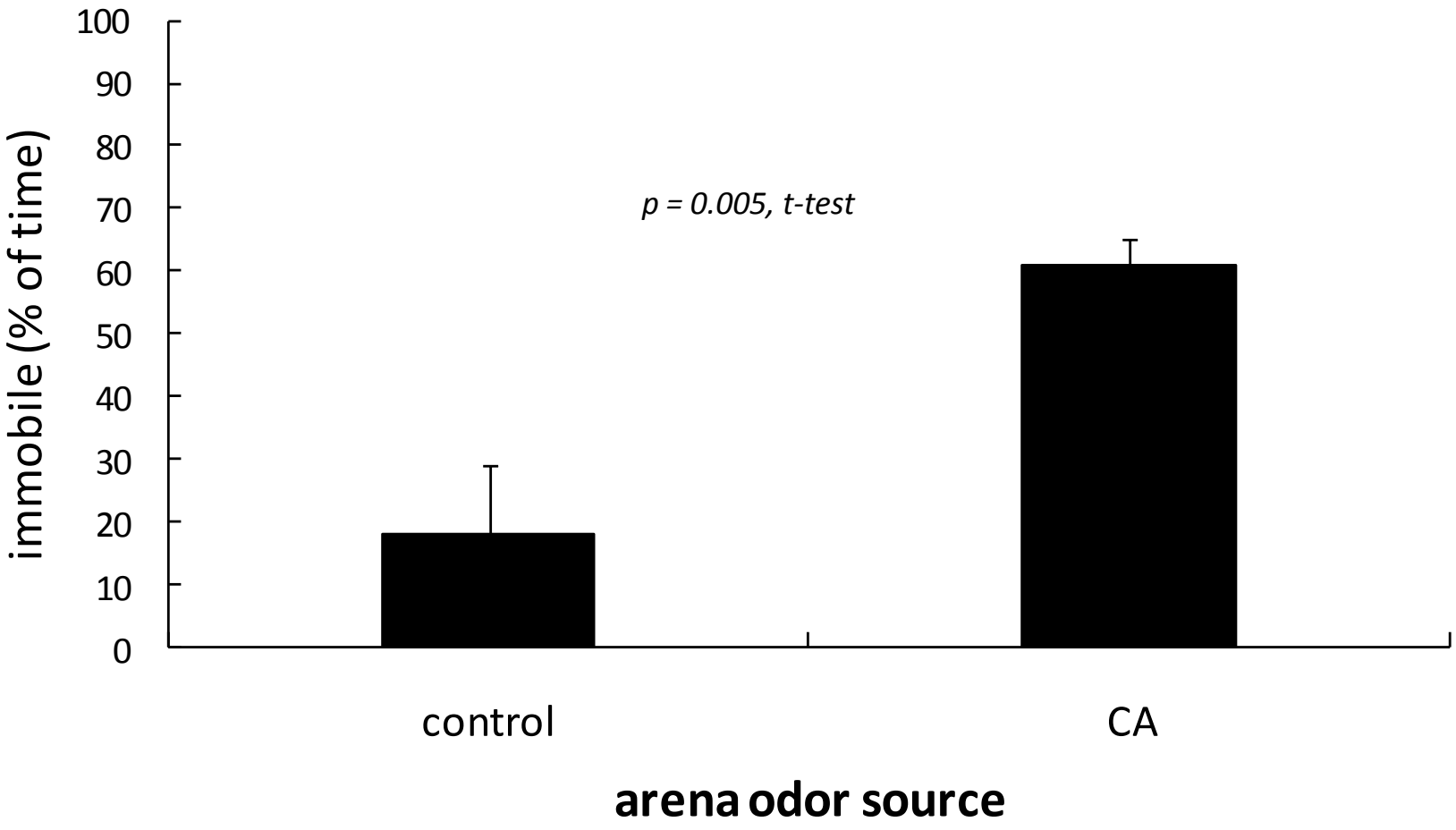
Mites move slower during exposure to CA volatiles

No choice diffusion bioassay (EthoVision)

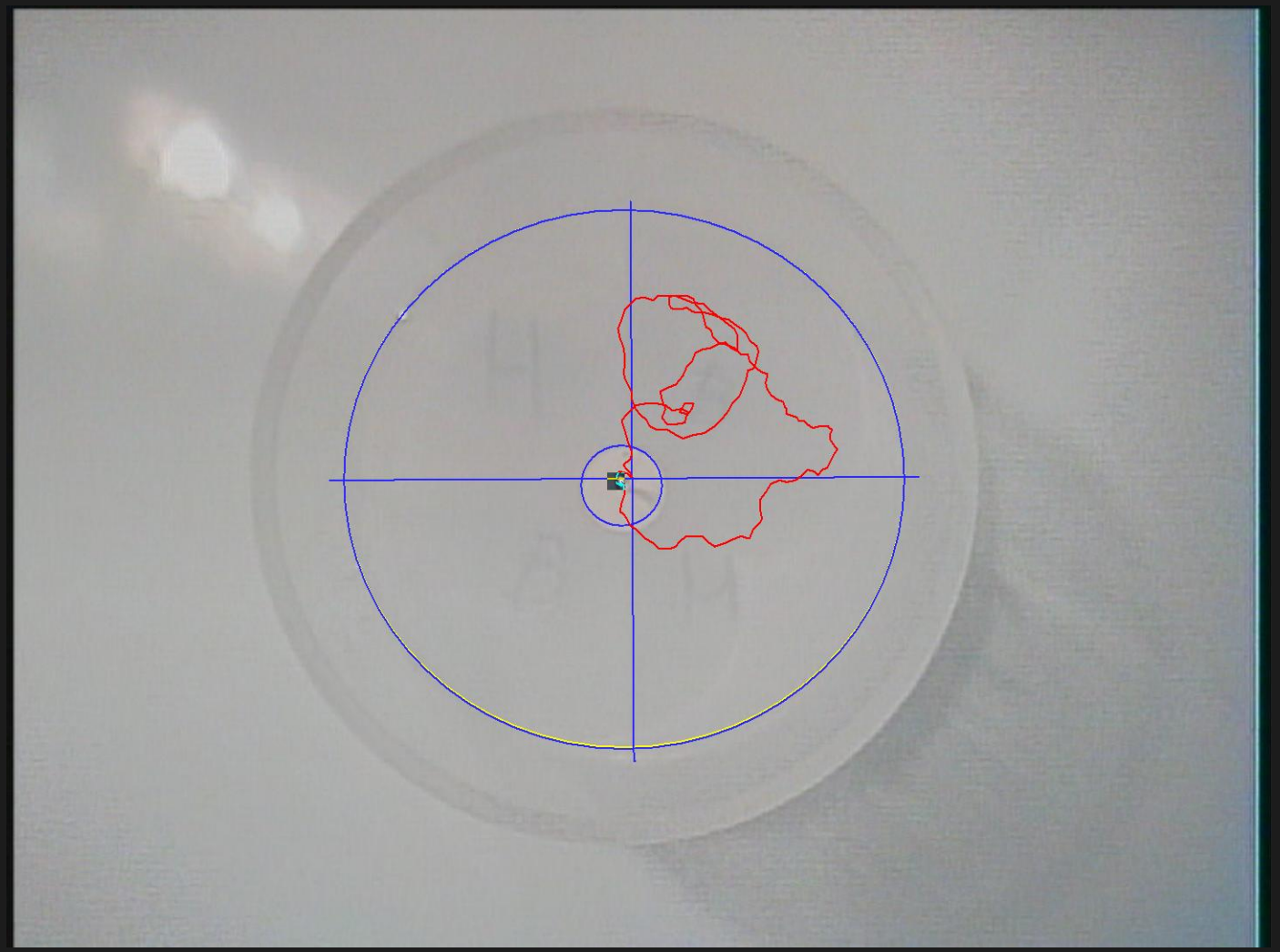


Mites are immobile more often during exposure to CA

No choice diffusion bioassay (EthoVision)



Mite responses to CA – m2p video clip

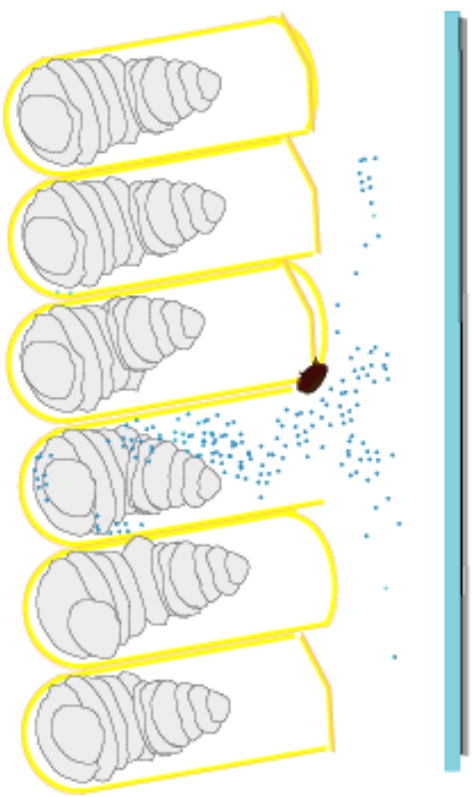


Using CA to control mites in the hive environment

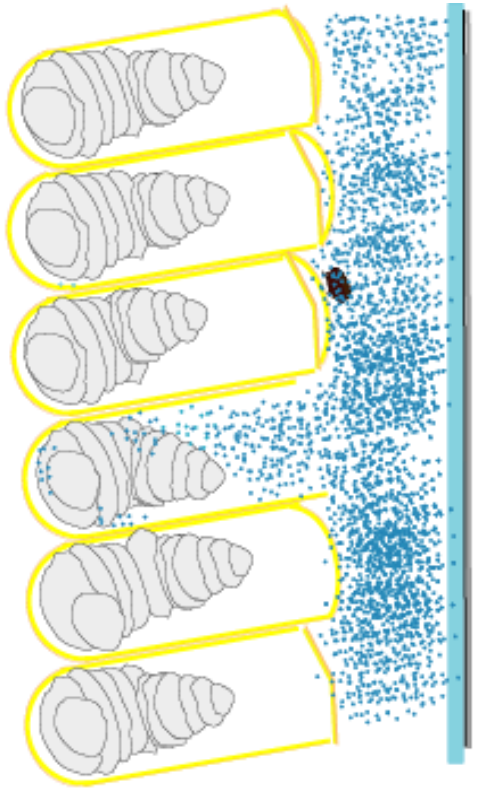
I. Flooding

II. In-hive trap

Flooding – disruption of behaviors by saturating the sense of smell with synthetic chemicals



control airspace



flooded airspace

Flooding bioassay

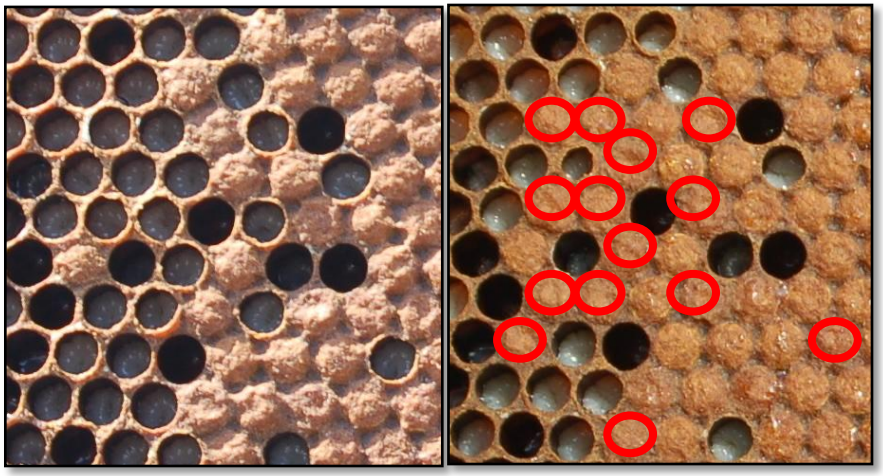
- enclose mite-infested adult bees with capping brood in observation frames
- treat each frame's airspace for 36 hrs

  Control - air only

  Flooded - synthetic CA

- count the mites that invaded brood cells during the experiment

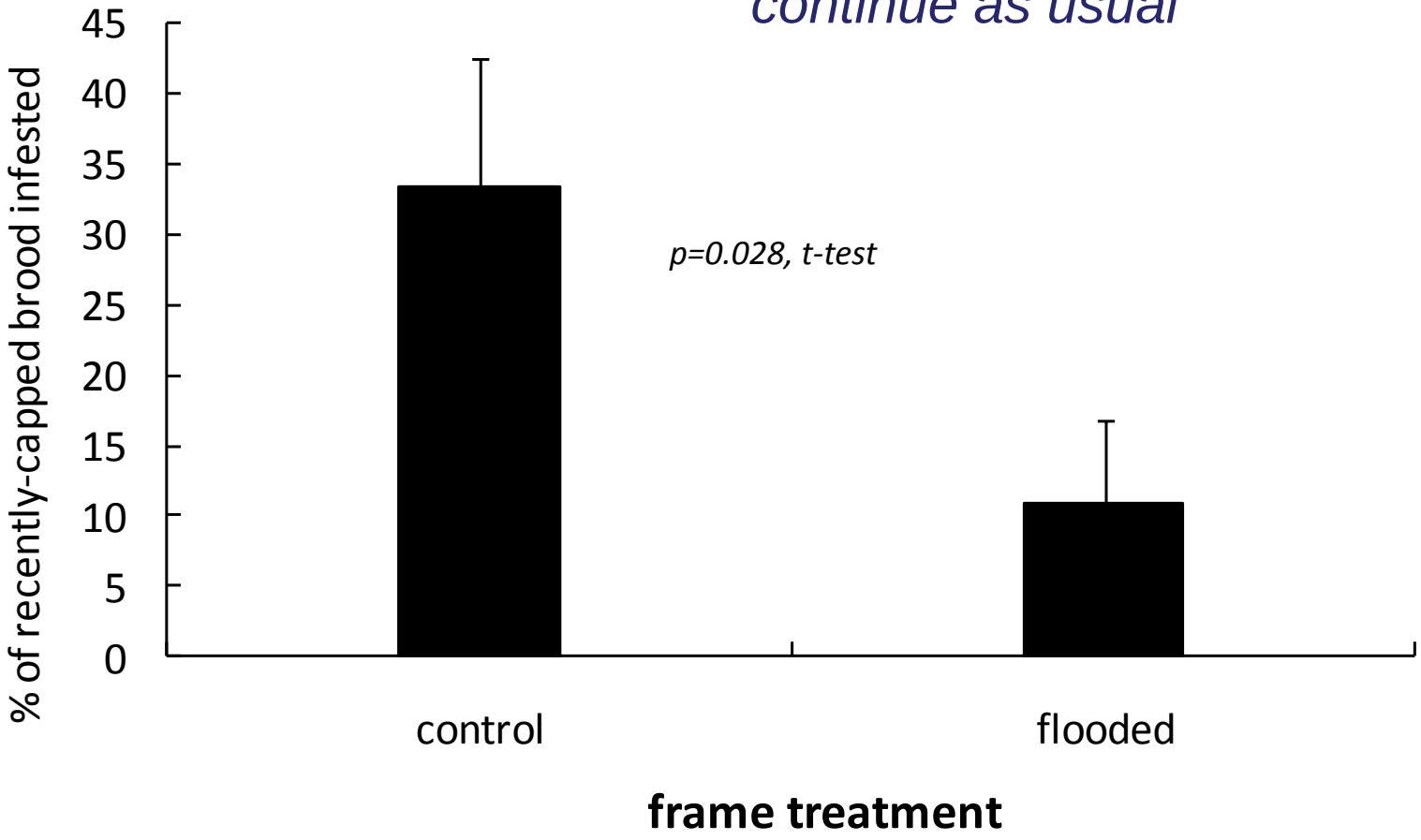
release of CA into airflow of flooded AOF frame



detection of cell capping by comparing photos

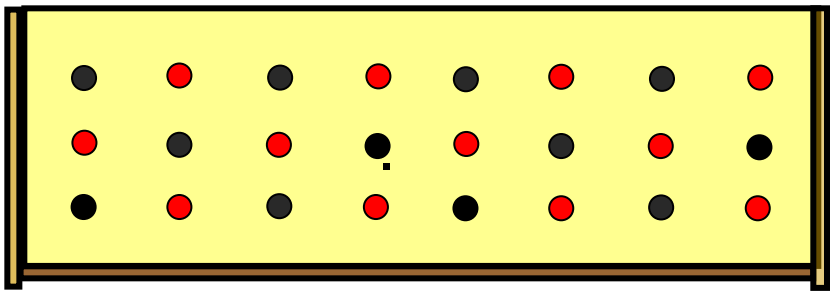
Flooding the brood comb airspace with synthetic CA reduces mite cell invasion

… yet colony functions (larval rearing and capping) continue as usual



Are mites attracted to cells treated with CA?

- One of two treatments <u>added in solvent</u> to the bottom of single cells (12 cells/tmt)

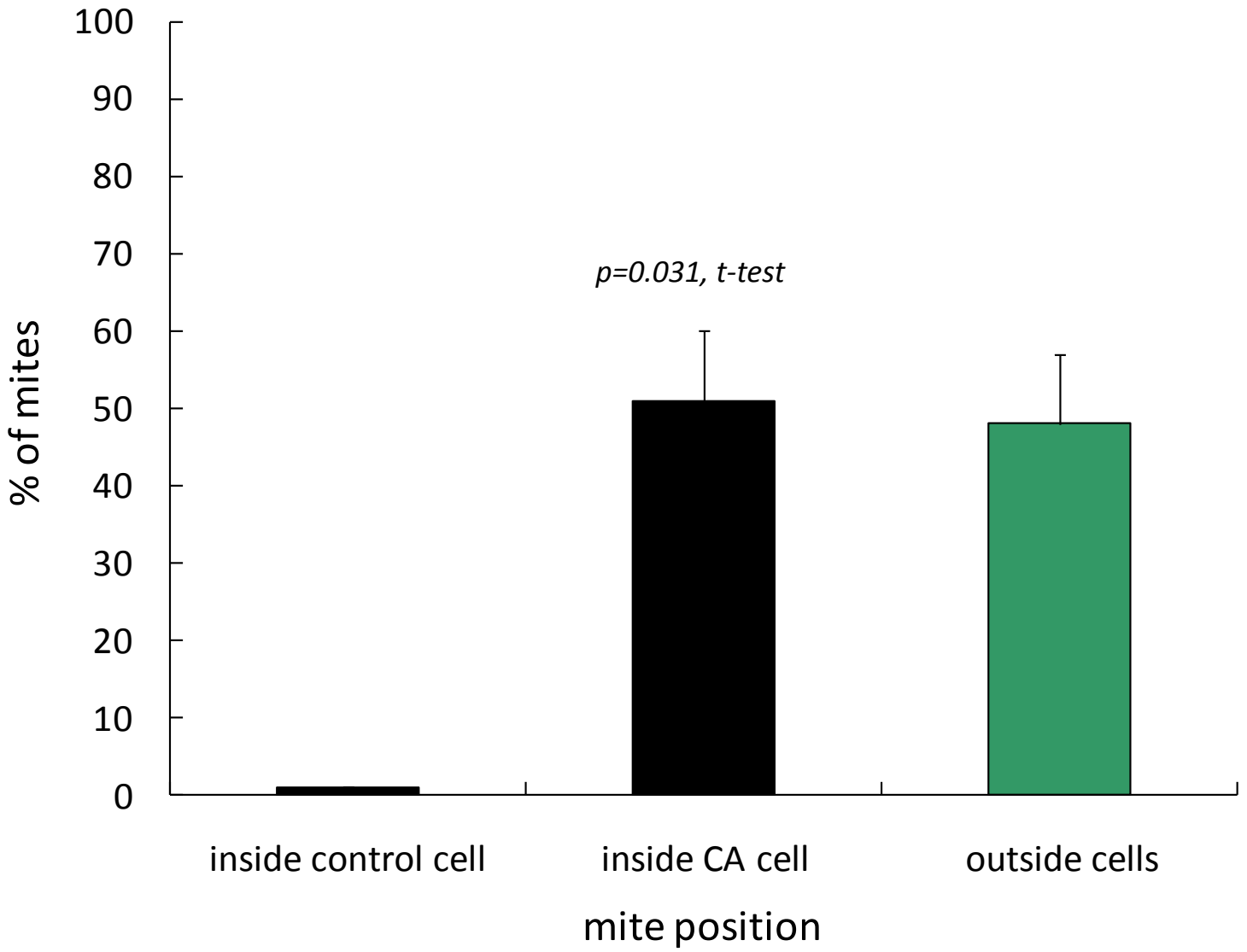


Control – solvent only

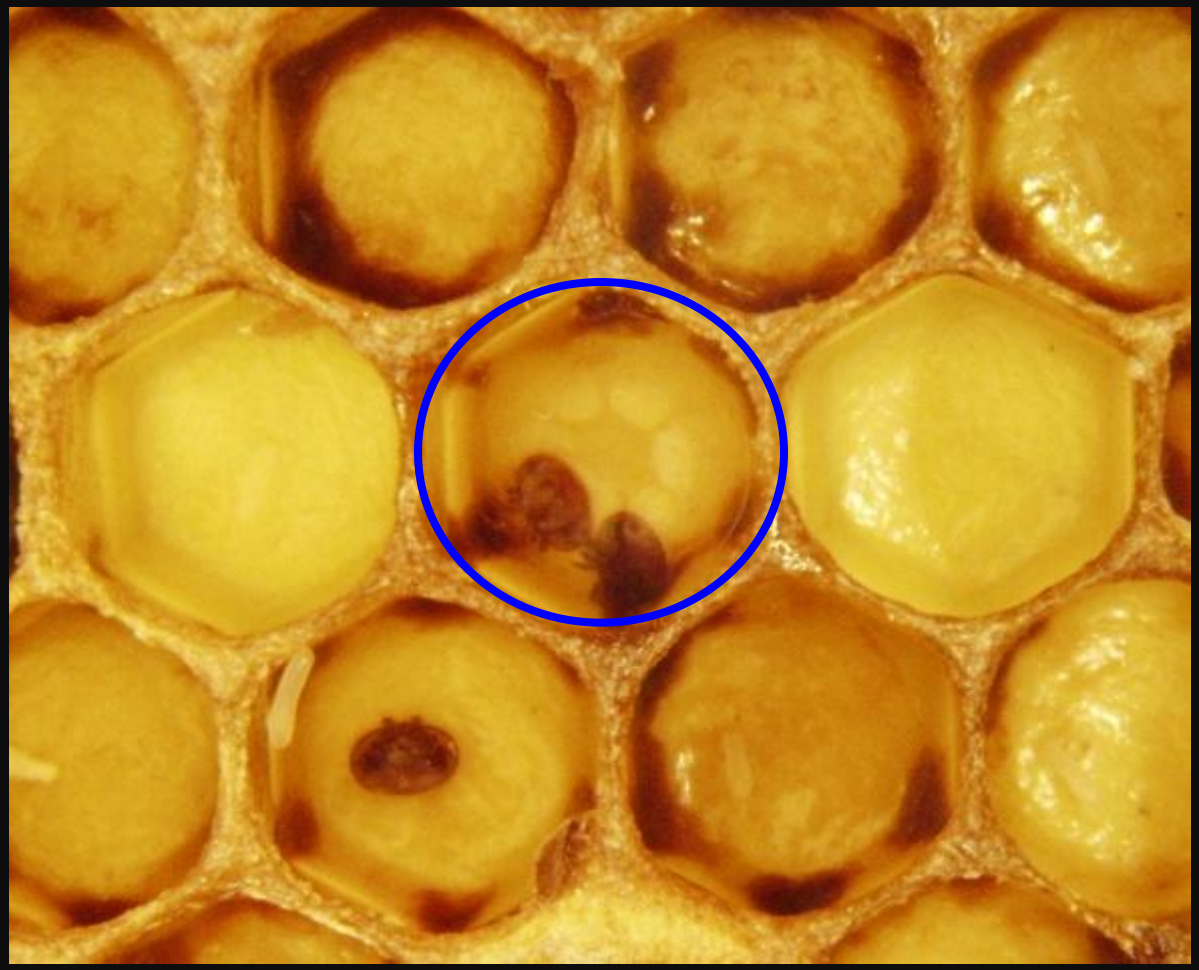
CA - CA in solvent

- 100 free-roaming mites released directly onto frame
- mite positions noted 2 hours after release

Free-roaming mites are attracted to CA-treated cells



Mites in CA-treated cells remain at the bottom (81%) … and often turn upside-down



treated cell outlined in blue

Challenges for in-hive trap development

Building a better bait (formulation)

- Improve activity with minor volatile synergists
- Provide odor release over a full mite reproductive cycle (3 weeks)
- Achieve consistent release under a variety of environmental conditions – <u>stable colony temperatures help</u>

early spring 40 F

mid-summer 105 F

Is “bribery” the answer?

Bees.

The other half of the equation.

- Proximity - must attract the bees near the bait (within ~5 mm) <u>without direct contact</u>
- Bait must not be repellent to bees
- Bait must not disrupt colony function

Varroa volatiles – an ongoing collaborative project

CMAVE Chemistry Unit

Peter Teal

Adrian Duehl

Alex King

Tredina Davis

Shelley Olson

Carl Hayden Bee Research Unit

Tommy Deeby

... and more to follow!