

Assessing the attractiveness of hive volatiles to small hive beetles(*Aethina tumida*)

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Introduction

- Small hive beetle

Coleoptera

Nitidulidae

Aethina tumida



- Native to sub-Saharan, parasitic to honeybee hive
- In hive pest of honeybee *Apis mellifera*
- Adult and larvae both damage honeybee and hive materials
- Recent invasion into Korea 2016. Sep. 20

Global distribution

Invasion pathway of the honeybee pest, small hive beetle, *Aethina tumida* in the Republic of Korea inferred by mitochondrial DNA sequence analysis. Saeed Mohamadzade Namin et al., Journal of Asia-Pacific Entomology (2019) 22:963–968

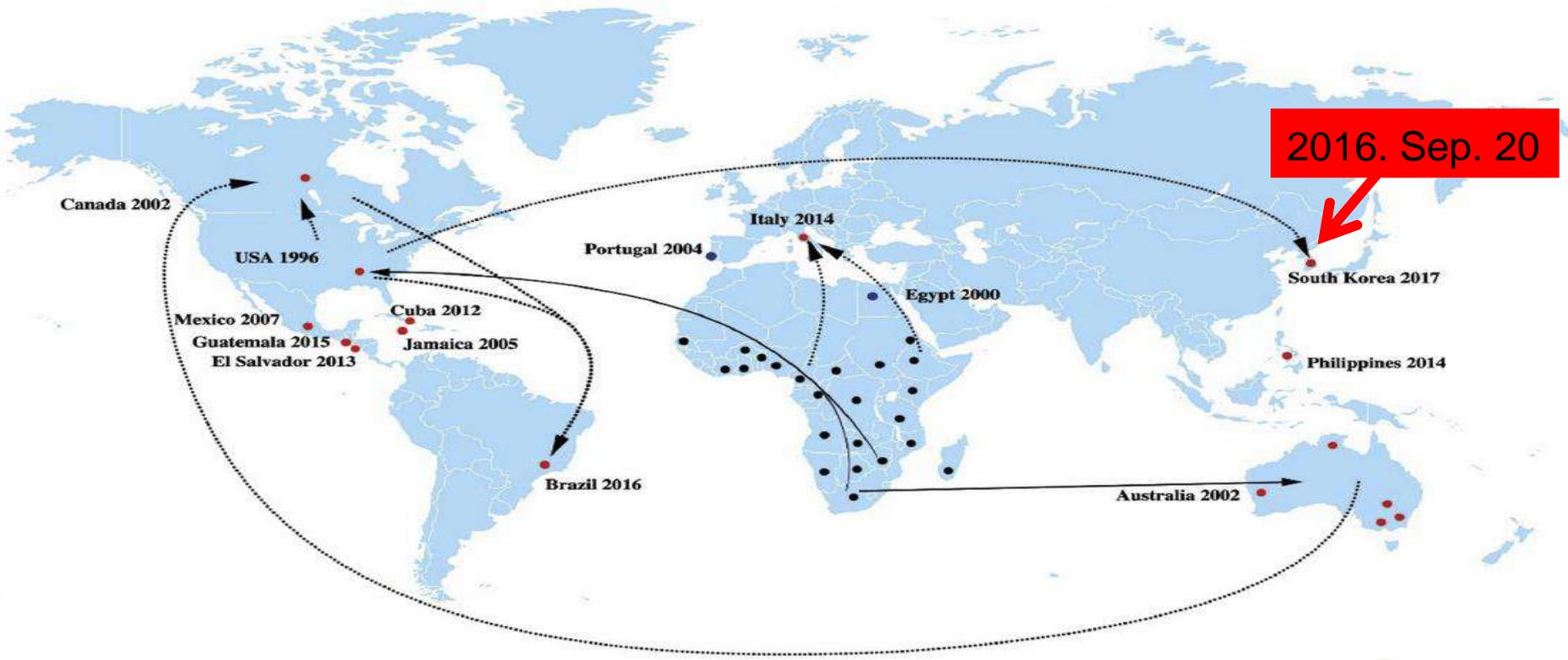


Fig. 1. Global distribution of small hive beetles up to June 2019. Red circle : distribution of SHB, black circle : native range; red circle : well-established populations; blue circle : unestablished areas (for further information, Neumann et al., 2016). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Invasion into Korea



<1면용>경남 밀양 양봉농가서 '작은벌집막정벌레' 국내 첫 발견

밀양에서 40여년간 양봉을 해온 이모씨는 9월23일 벌통 대부분에서 작은벌집막정벌레와 애벌레가 증식한 것을 발견하고 인근 검역기관에 신고했다.



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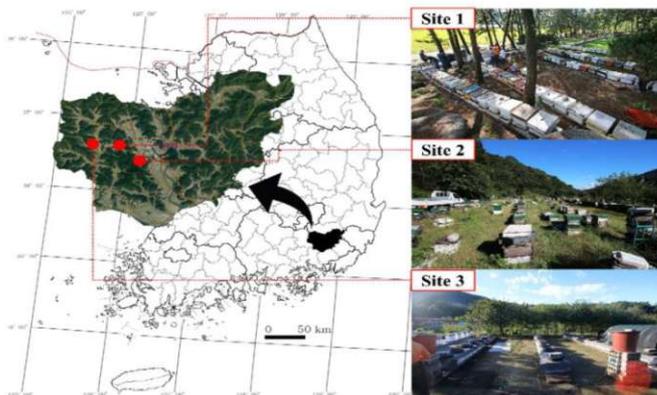
journal homepage: www.elsevier.com/locate/jape



Review of the subgenus *Aethina* Erichson s. str. (Coleoptera: Nitidulidae: Nitidulinae) in Korea, reporting recent invasion of small hive beetle, *Aethina tumida*



Seunghyun Lee ^{a,b}, Ki-Jeong Hong ^c, Yun Sang Cho ^d, Yong Soo Choi ^e, Mi-Sun Yoo ^d, Seunghwan Lee ^{a,b,*}



Left: invasion site of *Aethina tumida*, Miryang city

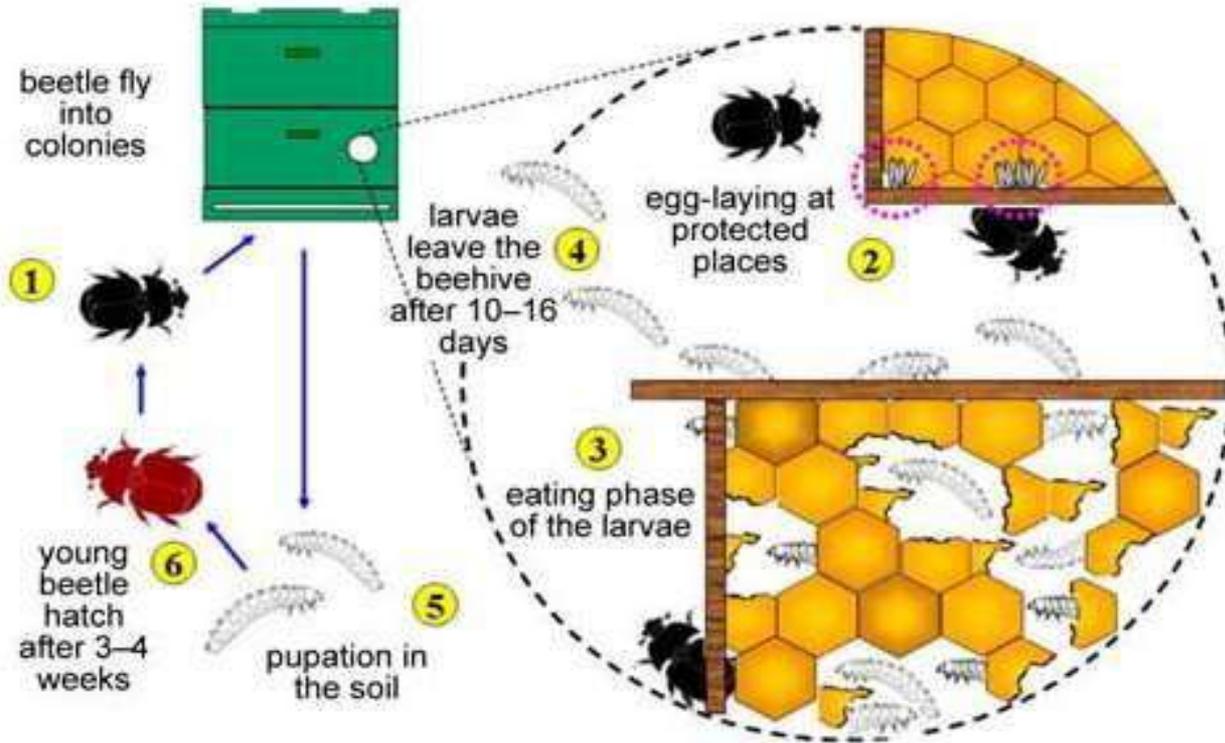
Site 1: 12–3, Gabok 4n-gil, Muan-myeon, Miryang-si, Gyeongsangnam-do, Korea;

Site 2: 823, Hwabong-ri, Muan-myeon, Miryang-si, Gyeongsangnam-do, Korea;

Site 3: 196–54, Songpo-ro, Bubuk-myeon, Miryang-si, Gyeongsangnam-do, Korea

Life cycle and pest potential

The lifecycle of the small hive beetle *Aethina tumida* (Murray 1867)



© Dr Otto Boecking LAVES Institut für Bienenkunde Celle, Germany – 2005

Honeybee brood : direct feeding and competition

Honeybee adult : interference and expelling

Honey : contamination + fermentation

Combs : feeding and borrowing

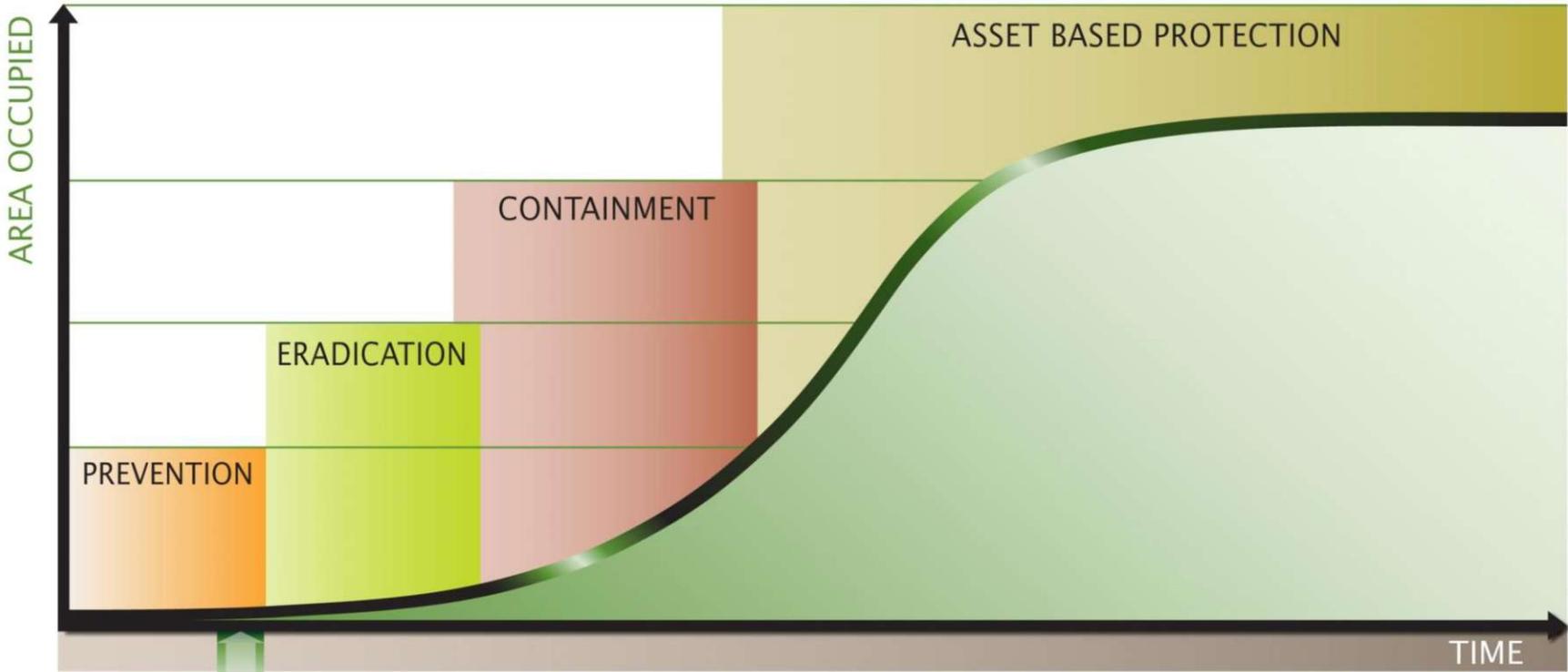
Life cycle and pest potential

- Egg on the crevices of hive 3-7d → Larva feed on honey and comb, larvae 10-16d → wandering larvae move out from hive into soil for pupation 1-3d → pupa 3-6wk → adult
- Adults can fly 8 km per night to locate a hive by odor: pollen/honey
- Once in the hive the beetle mix pollen, honey with the symbiotic yeast (*Kodamaea ohmeri*) and produce alarm pheromone to attract a mate.
- high environmental resistance: Survive on comb for 50 days, honey for 6 months and survive 14 days without food or water.

Control of invaded pest

GENERALISED INVASION CURVE SHOWING ACTIONS APPROPRIATE TO EACH STAGE

Version 1.0: 30 APR 2009



Species absent	Entry of invasive species	Small number of localised populations	Rapid increase in distribution and abundance, many populations	Invasive species widespread and abundant throughout its potential range
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ECONOMIC RETURNS (INDICATIVE ONLY)



Concerns on early stage of invasion

Q1. Location of beetle ? Monitoring method / efficiency

Q2. Control or Eradication : target, agent

Hong and Jung, 2018 medicine screening

Q3. Containment :

Environmental adaptability (Temperature, humidity)

Dispersal pathway

Host distribution or movement

Hong and Jung, 2017 distribution

Hong and Jung, 2017 temperature growth

Q1. Location of beetle ? Monitoring

➤ Mechanical traps

- the Hood Trap,
- the West trap,
- the Freeman Beetle trap,
- disposable plastic trap

Use vegetable oils / pollen for bait

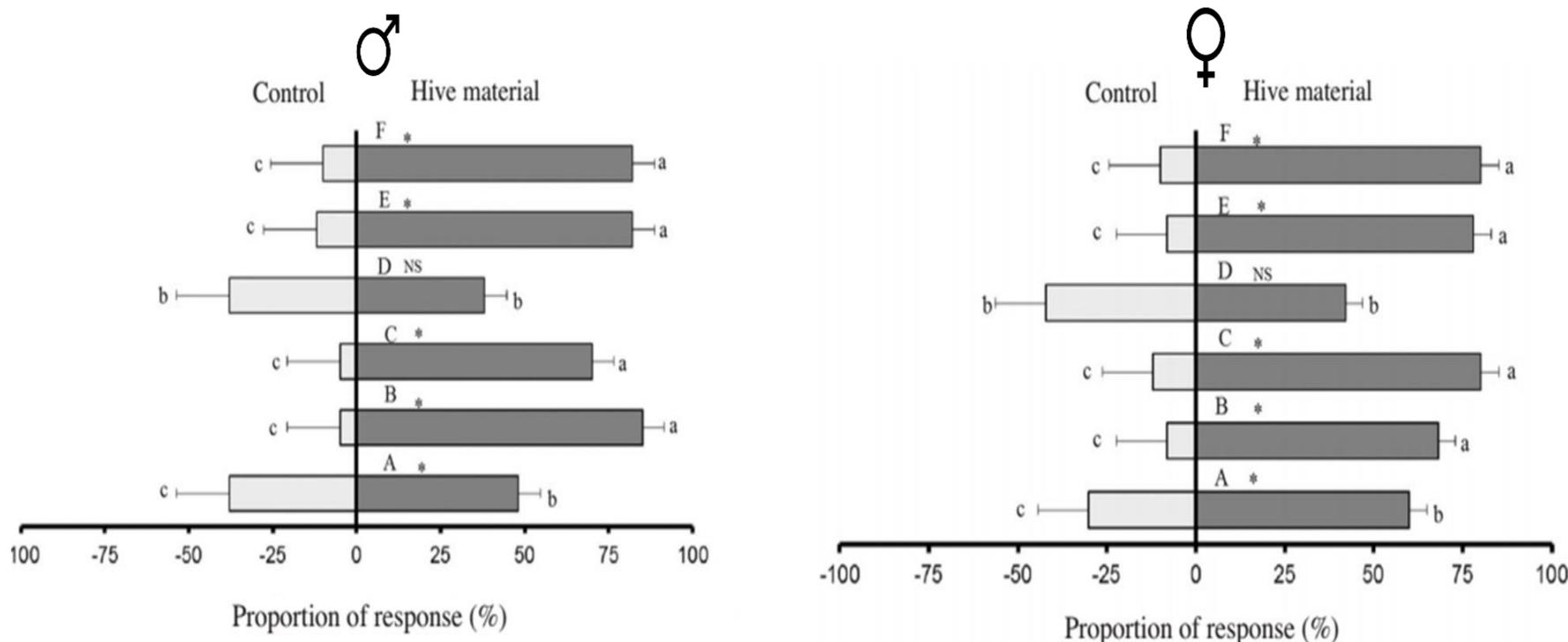


Fig. 2. Disposable plastic trap

- Plastic trap which is designed to be placed between two top bars in the bottom brood chamber or supers. It should be half-filled with vegetable oil and checked at every inspection .
- The outer two compartments should be filled a third to half full with vegetable oil while the middle section can be filled with cider vinegar in order to attract the beetles and larvae.

Preliminary result

- Y-tube olfactometer bioassay: 22 d –fermented pollen dough



- F: Worker bees
- E: 20 day-fermented honey
- D: Fresh honey;
- C: 22 day-fermented pollen dough
- B: 13 days fermented pollen dough;
- A: Fresh pollen patty;

Dekebo et al. , 2017. Attractiveness of the Small Hive Beetle (*Aethina tumida*) to Volatiles from Honey bee (*Apis mellifera*) and Beehive Materials. J. Apic.. 32(4) : 315~326.

Objective of the study

- To test olfactory responses of SHB to volatiles we identified from essential oils of beehive materials
- Specially from 22-d old fermented pollen dough.

Materials and Methods

Small Hive Beetles

The beetles were reared in acrylic cages (38 x 38 x 34 cm) at room temperature ($25 \pm 2^\circ\text{C}$, $60 \pm 5\%$ RH) with 12L: 12D photoperiod. Beetles were fed on a mixture of pollen and honey.



Pupation sand
(7cm)

SHB food
(pollen patty + honey)

Water on
tissue paper

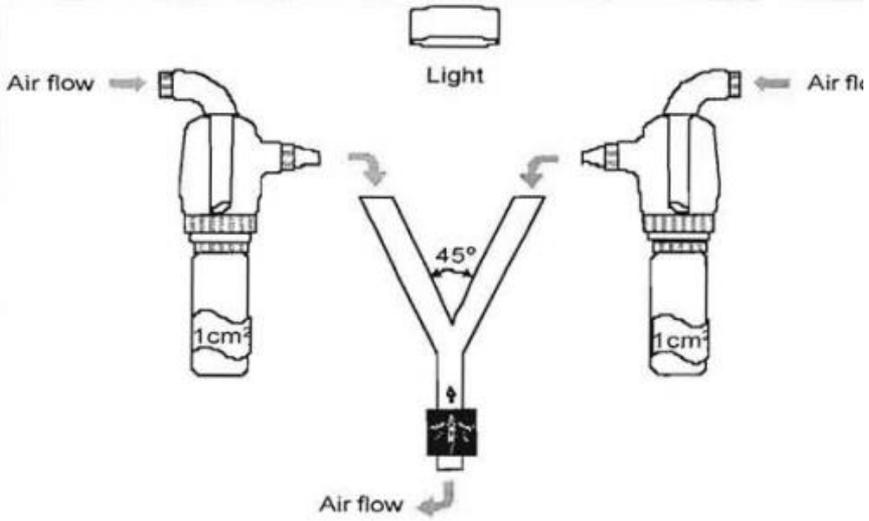
Materials and Methods

Chemicals

All the individual chemicals were purchased from Sigma Aldrich, South Korea.

Each compound was dissolved in concentration of 0.01, 0.1, 1 and 10 mg/ml.

- Ethyl palmitate ($\geq 98.5\%$) + [n-hexane](#),
- Ethyl linolenate ($\geq 98\%$) + [n-hexane](#),
- Eetracosane ($\geq 99\%$) + [Chloroform](#),
- Oleamide ($\geq 99\%$) + [Chloroform](#),
- 5-methyl-2-phenyl-1H-indole + [Methanol](#).



A. Y-tube



GC-MS Instrument



B. Two arm olfactometer

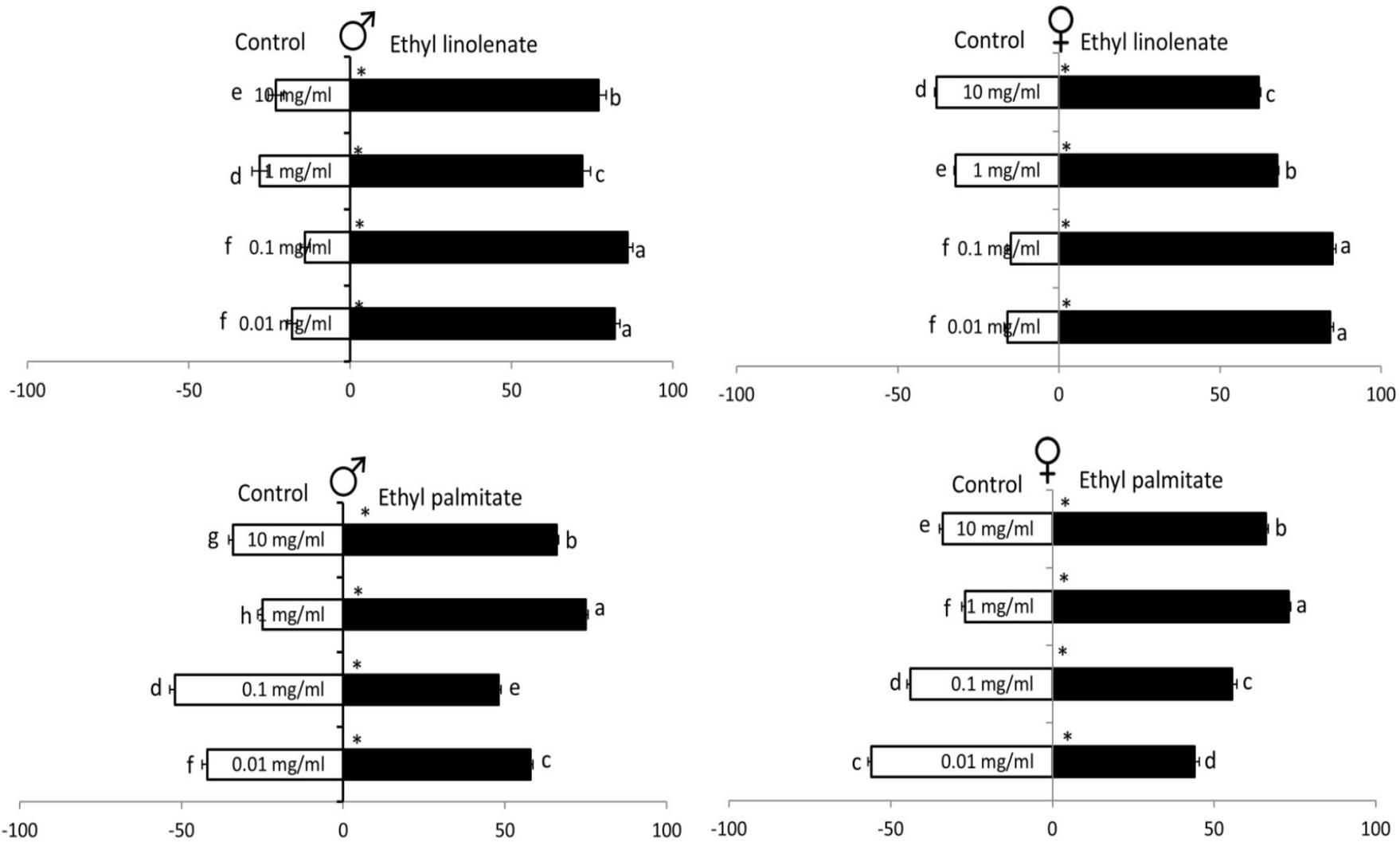
Olfactometers for recording responses of SHBs to volatiles



HPLC instrument

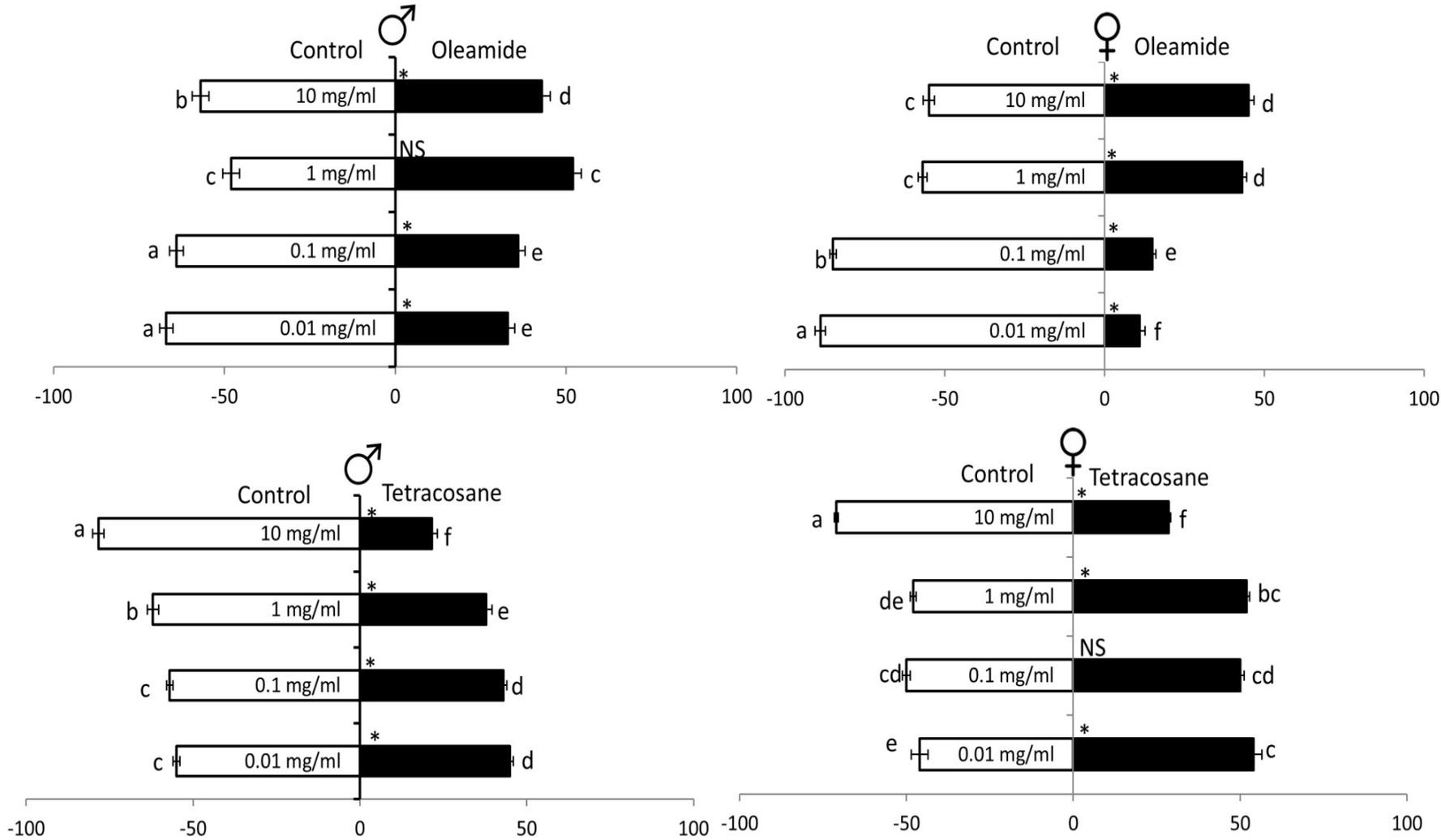
Results

- Ethyl linolenate & Ethyl palmitate (ANOVA : $P < 0.05$)



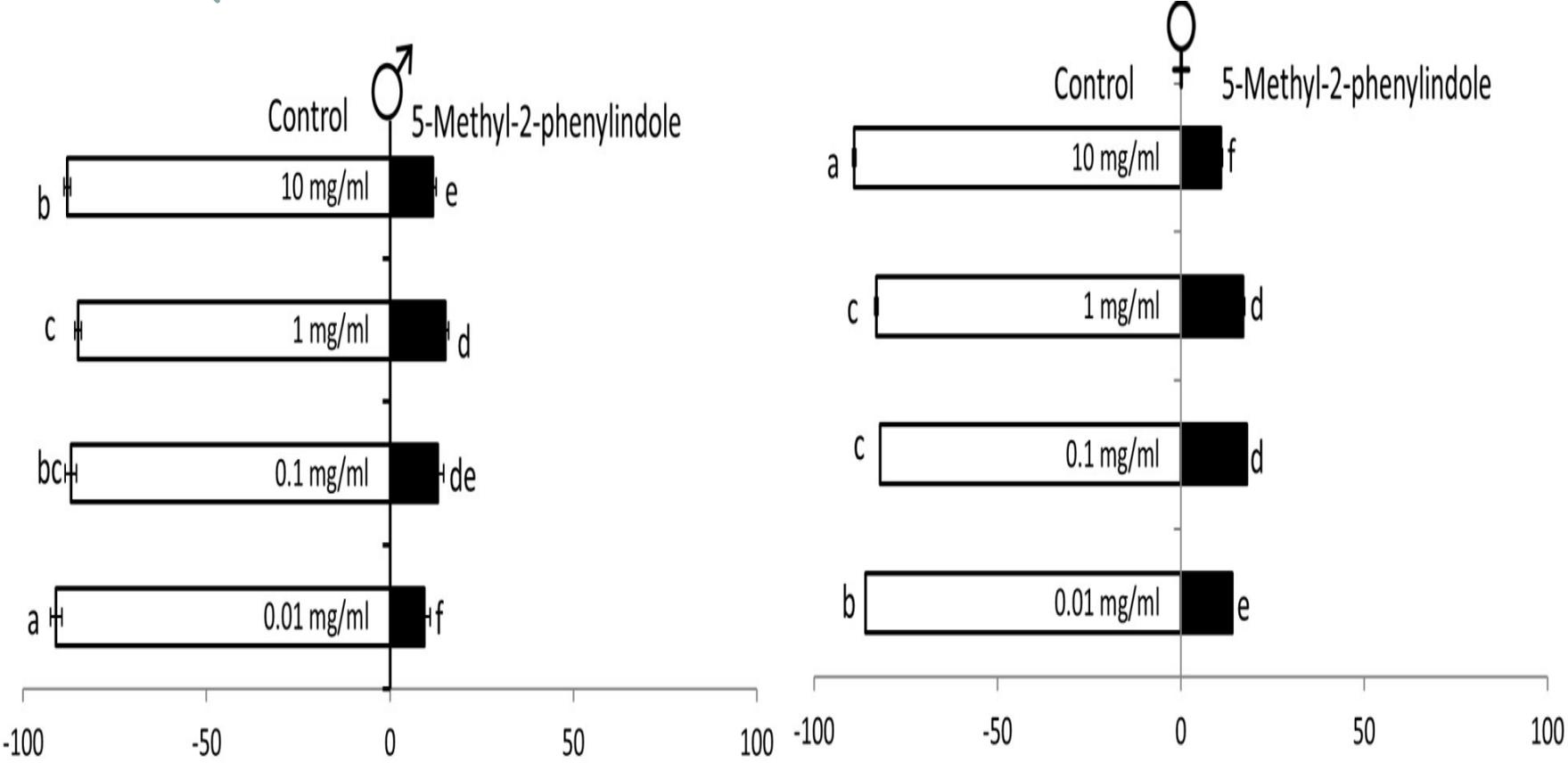
Results

- Oleamide & Tetracosane (ANOVA : $P < 0.05$)



Results

- 5-Methyl-2-phenylindole (ANOVA : $P < 0.05$)



Summary

- Ethyl palmitate and Ethyl linolenate are attractive to SHB.
- Oleamide have a deterrent effect at lower concentration.
- Tetracosane is repulsive at higher concentration.
- 5-methyl-2-phenyl-1H-indole is repulsive at all concentration.

Discussion

- It is interesting to note the presence of ethyl palmitate and ethyl linolenate as principal compounds in fermented pollen dough produced by the action of SHB(Dekebo, et al., 2017) in common with the brood pheromone of honey bees, which can be a possible explanation for attraction of SHB to honey bee hives.
- SHB might utilize the chemical cues from possible food source of pollen dough or indicatives of reproductive sites of honey bee hive from honey bee brood pheromone.

Discussion

- Our previous study indicated 20 **day-fermented honey** contains oleamide and tetracosane (Dekebo, et al., 2017).
- The repellent effects of both oleamide and tetracosane that we documented in the present study indicated that adult SHB may interpret contaminated honey as a repulsive cue.
- Because the resource was already taken by earlier conspecific intruders of the hive, leaving less chance for the new intruder to successfully reproduce in the hive.

Discussion

- Oleamide and 5-methyl-2-phenyl-1H-indole were previously reported as major components of worker bees (*Apis mellifera*) (Dekebo, et al., 2017).
- These were repulsive to SHB, indicating SHB might more attractive to brood or hive materials but not for adult worker bees which are responsible for colony defense.

Based on the previous reports and our findings we believe compounds with ester functional groups might have the potential to lure SHB to hives.