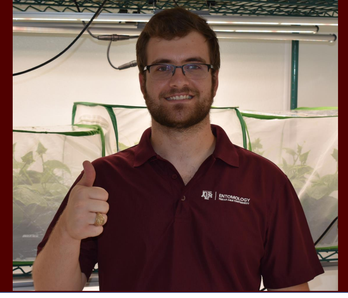
Timing is everything: root-feeding herbivore overcomes biological control by reducing recruitment of entomopathogenic nematodes with sustained herbivory

John Michael Grunseich and Anjel Helms
Department of Entomology, Texas A&M University



Abstract

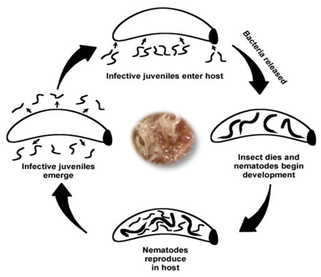
Root-feeding herbivores are among the most devastating crop pests because their belowground infestations are challenging to detect and control. Conventional management strategies often rely on prophylactic pesticide applications, which can be expensive and environmentally damaging, highlighting a need for more sustainable control options. Biological control with entomopathogenic nematodes (EPNs) is a promising strategy for managing root-feeding insects, like striped cucumber beetles (*Acalymma vittatum*), which are devastating pests of cucurbit crops. Here, we examined the potential for EPN biological control of cucumber beetles by comparing recruitment of EPNs (*Heterorhabditis bacteriophora*) to roots of cucumber plants (*Cucumis sativus*) with or without *A. vittatum* herbivory. We also characterized the EPN-attracting volatile cues produced by *C. sativus* roots and investigated how EPN attraction changes over the course of herbivory.

We found that feeding damage by striped cucumber beetle larvae induced a characteristic blend of volatiles, including three key compounds, camphene, alpha pinene and sabinene, which successfully recruited EPNs. However, after 7 days of continuous herbivory, beetle larvae suppressed production of these volatiles and EPNs were no longer attracted to larvae feeding on roots.

This study revealed a potential challenge of using EPNs to control cucumber beetles, as larvae could avoid attracting EPNs during later infestations. However, we also identified volatile chemical attractants for EPNs that could potentially be introduced as synthetic lures to enhance EPN biological control. Overall, our findings suggest that timing of introduction could be important for successful recruitment of EPNs and effective biological control of striped cucumber beetles.

Materials and Methods

Striped cucumber beetles (*Acalymma vittatum*) are important agricultural pests in the USA. They are specialist herbivores on plants in the family Cucurbitaceae. Adult beetles feed on aboveground tissues, while larvae feed on roots.



Entomopathogenic nematodes (EPNs, *Heterorhabditis bacteriophora*) are natural enemies of insects and can be used for biological control of root-feeding insect pests. The role of infected juveniles is to seek out and infect hosts.

1. Root VOC Collections: Damaged vs Undamaged VOCs

- Cucumber root volatiles were collected using dynamic *in situ* sampling
- VOCs were collected after 24 hr and 7 d of continuous feeding by larvae or from undamaged controls and analyzed using GCMS

2. EPN Choice Test: Damaged vs Undamaged Root VOCs

- Cucumber plants were transplanted into glass pots in clean sand
- Plants were paired and one was infested with larvae for 24 hr and 7 days
- EPNs were placed in the center of the olfactometer (Figure 1) and were extracted using an adapted Baermann funnel method after 48 hr and counted.

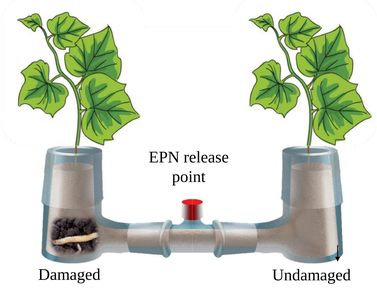


Figure 1. Two-choice belowground olfactometer used to assess larval and EPN choice with damage cues

Objectives

1. Characterize volatiles released by cucumber roots fed on by cucumber beetle larvae at 24 hr and 7 d
2. Measure EPN attraction to *C. sativus* roots with and without cucumber beetle larvae feeding damage
3. Determine how the change of root volatile organic compounds over time influences EPN foraging

Results

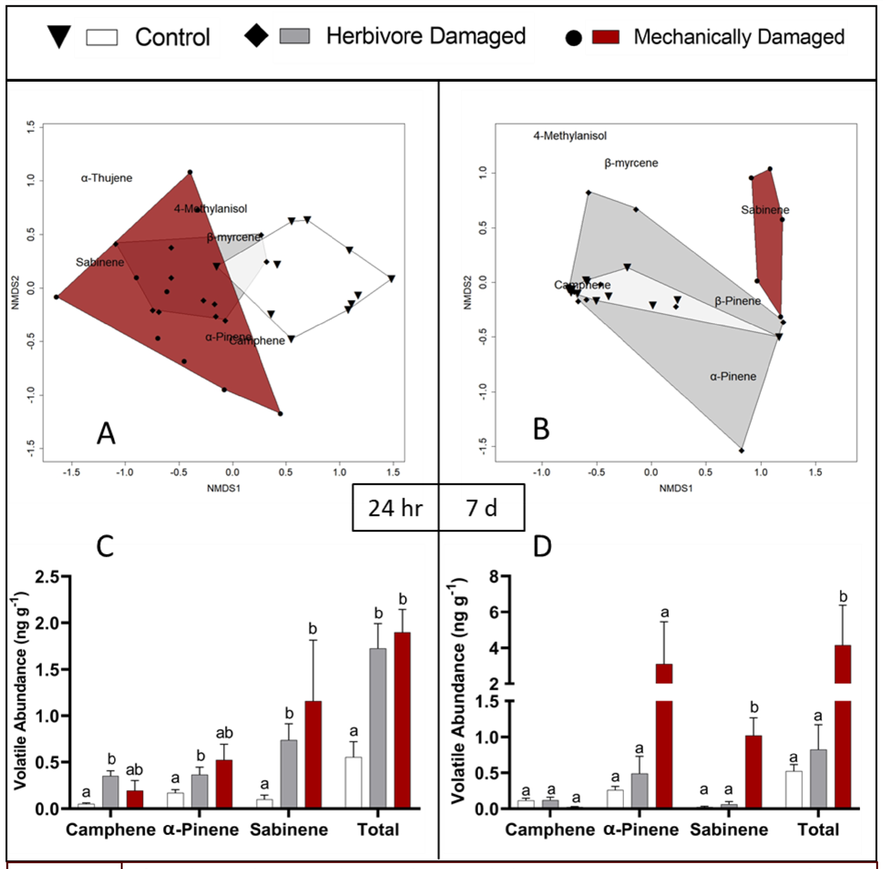


Figure 1 **A)** Herbivory by striped cucumber beetle larvae or mechanical wounding for 24 hr induced similar volatile blends that both differed from undamaged controls. **B)** Herbivory or wounding of cucumber roots (24 hr) increased production of monoterpenes and total volatiles. **C)** After 7 d, volatile blends were not different for larvae damaged and control roots, while mechanically damaged roots remained different. **D)** After 7 d, induced volatile production was suppressed in herbivore-damaged plants but not mechanically wounded plants.

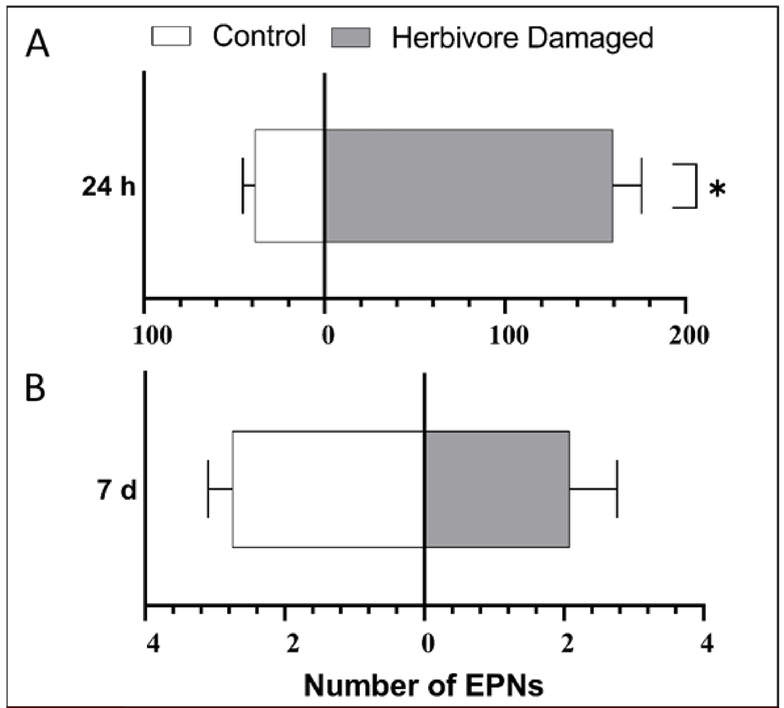


Figure 2 A) EPNS preffered cucumber root volatiles after 24 hr of herbivory. B) After 7 d of herbivory, no difference in attraction was observed. Means ± SE are presented.

Significance

- This study documents the behavior of belowground natural enemies in response HIPV's.
- We identify biologically relevant volatiles that could serve as potential synthetic lures to enhance EPN biological control
- There are important implications for pest management in agroecosystems
  - Continued feeding by larvae suppress root volatiles which potentially reduces EPN foraging success
  - EPN natural enemies directly control cucumber beetle larvae through predation

Conclusion

- We highlight potential challenges for biological control
- Identify potential volatile chemical attractants for synthetic lures to enhance EPN biological control
- We emphasized the overall importance of the timing of introduction of application of EPNs in regard to insect herbivory

Citations