RESEARCH NOTE

REVISED Electroantennogram response of the parasitoid, *Microplitis croceipes* to host-related odors: The discrepancy between relative abundance and level of antennal responses to volatile compound [version 2; referees: 4 approved]

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Abstract

Herbivores emit volatile organic compounds (VOCs) after feeding on plants. Parasitoids exploit these VOCs as odor cues to locate their hosts. In nature, host-related odors are emitted as blends of various compounds occurring in different proportions, and minor blend components can sometimes have profound effects on parasitoid responses. In a previous related study, we identified and quantified VOCs emitted by cotton plant-fed *Heliothis virescens* (Lepidoptera: Noctuidae) larvae, an herbivore host of the parasitoid *Microplitis croceipes* (Hymenoptera: Braconidae). In the present study, the olfactory response of female *M. croceipes* to synthetic versions of 15 previously identified compounds was tested in electroantennogram (EAG) bioassays. Using *M. croceipes* as a model species, we further asked the question: does the relative abundance of a volatile compound match the level of antennal response in parasitoids? Female *M. croceipes* showed varying EAG responses to test compounds, indicating different levels of bioactivity in the insect antenna. Eight compounds, including decanal, 1-octen-3-ol, 3-octanone, 2-ethylhexanol, tridecane, tetradecane, α-farnesene and bisabolene, elicited EAG responses above or equal to the 50th percentile rank of all responses. Interestingly, decanal, which represented only 1% of the total amount of odors emitted by cotton-fed hosts, elicited the highest (0.82 mV) EAG response in parasitoids. On the other hand, (*E*)-β-caryophyllene, the most abundant (29%) blend component, elicited a relatively low (0.17 mV) EAG response. The results suggest that EAG response to host-related volatiles in parasitoids is probably more influenced by the ecological relevance or functional role of the compound in the blend, rather than its relative abundance.

Open Peer Review

Referee Status: ✔ ✔ ✔ ✔

	Invited Referees 1	2	3	4
REVISED version 2 published 09 Mar 2017			✔ report	
version 1 published 21 Nov 2016	✔ report	✔ report	? report	✔ report

1 **Amanuel Tamiru**, International Centre of Insect Physiology and Ecology - ICIPE Kenya

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REVISED **Amendments from Version 1**

General comment: We thank all the referees for the important comments they provided while reviewing this manuscript. In several instances, we made appropriate changes to the new version according to the suggestions of the referees. In a few other areas, we have avoided the inclusion of information that is beyond the scope of the study so as to keep the article focused and concise. As clearly stated, the present study builds on the results of a previous study by the authors.

Introduction: We added a few sentences to better introduce the concept of plant-associated odors emitted by cotton-fed larvae. We clarified that odors emitted from plants play a key role in the location of host patch by parasitoids, and that odors emitted by plant-fed herbivores may be more useful in short-range host location. Finally, we added some more details about the results of the previous study conducted by the authors to create a better background for the present study.

Materials and methods: We have included justification for the mass/volume concentration used to formulate the synthetic compounds and the single dose used in EAG recordings. Furthermore, we clarified the arrangement used to present compounds to each insect replicate. We also clarified that parasitoids used in EAG recordings were reared on host larvae that fed on artificial diet so that their sensitivity to plant odors will not be biased.

Results: EAG response to solvent control was not shown because the data represent absolute EAG, in which the response to control has been deducted from responses to treatment compounds. The dataset is available for review.

Discussion: The suggested citation by one of the referees has been added to the discussion. Also, we added a statement to note that parasitoids can learn to respond to diverse odor cues.

See referee reports

Introduction

Infested plants emit volatile organic compounds (VOCs) as an indirect defense against herbivore damage[1,2]. Informative volatile cues used by parasitoids for host location can be emitted by plants infested with herbivores[1,2] or emitted by herbivores that fed on plants[3,4]. Although plant volatiles may initially lead parasitoids to the host patch, herbivore host-specific odors are important short-range cues used in the later stages of host location[5]. The specific mechanism by which plant-fed host larvae emit these volatiles is not fully understood. However, it is evident that parasitoids use these plant-associated VOCs in the host location process[5]. Such odor cues are usually released as a blend of various compounds in nature. Consequently, differentiating useful cues from ecologically irrelevant odors can be challenging for foraging parasitoids. Therefore, it is expected that antennal sensitivity of parasitoids will vary in response to different compounds. Antenna sensitivity in insects can be measured with electroantennogram (EAG) recording. EAG measures the summed activity of olfactory receptor neurons in the antenna and indicates the level of biological activity elicited by various compounds.

Microplitis croceipes (Hymenoptera: Braconidae) is an endoparasitoid of *Heliothis virescens* (Lepidoptera: Noctuidae), which is an important pest of cotton plant. In a previous related study[5], female *M. croceipes* showed attraction to the odor blend emitted by cotton-fed *H. virescens* larvae in Y-tube olfactometer bioassays[5]. The blend components were identified and quantified using gas chromatography-mass spectrometry (GC/MS). Furthermore, the compounds in the attractive blend occurred in varying proportions (Table 1). However, the relative abundance of a blend component does not necessarily indicate its relevance to resource location in insects[6]. In the present study, olfactory response of *M. croceipes* to synthetic versions of 15 previously identified compounds was tested in EAG bioassays. Comparing EAG results in the present study and GC/MS analyses in a previous study[5], we indicated the discrepancy between relative abundance of a volatile blend component and the level of antennal response in parasitoids.

Methods and materials

Insects

Microplitis croceipes was reared on 2nd–3rd instar larvae of *Heliothis virescens.* The larvae of *H. virescens* used to rear *M. croceipes* were fed pinto bean artificial diet. Thus, parasitoids had no experience with plant odors. Adult wasps were supplied with 10% sugar water upon emergence in our laboratory at Entomology & Plant Pathology Department, Auburn University. For more details about rearing protocol, see Lewis and Burton[7]. Female parasitoids used for EAG bioassays were 2–3 days-old, presumed mated (after at least 24 h of interaction with males), and inexperienced with oviposition or plant material. The general rearing conditions for all insects were 25±1 °C, 75±5 % relative humidity and 14:10 h (light:dark) photoperiod.

Table 1. Composition of headspace volatile organic compounds emitted by cotton-fed *Heliothis virescens* larvae. This table was modified from Morawo and Fadamiro (doi: 10.1007/s10886-016-0779-7)[5], with permission from the authors.

ID[1]	Compound	Relative abundance (%)	Chemical category
1	α-Pinene	15.1	Monoterpene
2	β-Pinene	1.6	Monoterpene
3	1-Octen-3-ol	1.4	Alcohol
4	3-Octanone	0.8	Ketone
5	Myrcene	2.7	Monoterpene
6	*Unknown*[2]	1.2	-
7	Limonene	9.1	Monoterpene
8	2-Ethylhexanol	2.2	Alcohol
9	Decanal	1.0	Aldehyde
10	Tridecane	6.2	Alkane
11	Tetradecane	2.4	Alkane
12	(*E*)-β-Caryophyllene	29.2	Sesquiterpene
13	α-Bergamotene[2]	0.7	Sesquiterpene
14	α-Humulene	6.5	Sesquiterpene
15	α-Farnesene	0.8	Sesquiterpene
16	Bisabolene	8.6	Sesquiterpene
17	α-Bisabolol	7.9	Sesquiterpene

[1]In order of elution during gas chromatography.
[2]Compounds that were not tested in the present study.

EAG recording

EAG responses of *M. croceipes* to 15 synthetic compounds (Table 1), previously identified in the headspace of cotton-fed *H. virescens* larvae[5], were recorded according to the method described by Ngumbi *et al.*[8] with modifications. Two compounds, α-bergamotene (not commercially available) and an unidentified compound reported in the previous study[5] were not tested in the present study. α-Pinene, β-pinene, myrcene, limonene, 2-ethylhexanol, tridecane, (*E*)-β-caryophyllene, α-humulene, α-farnesene and α-bisabolol with purity 95–99% were purchased from Sigma-Aldrich® (St. Louis, MO, USA). 1-Octen-3-ol, 3-octanone, decanal, tetradecane and bisabolene with purity 96–99% were purchased from Alfa Aesar® (Ward Hill, MA, USA). Test compounds were formulated in hexane at 0.1 µg/µl and delivered onto Whatman®No.1 filter paper strips at an optimum dose of 1 µg. Mass/volume concentration was used to correct for differences in purity of synthetic compounds. The dose was selected as ecologically relevant based on GC/MS analyses results of total amount of volatiles emitted by cotton-fed *H. virescens* larvae[5]. Impregnated filter papers were placed inside glass Pasteur pipettes and stimulus was introduced as 0.2 s odor puffs. A glass capillary reference electrode filled with 0.1 M KCl was attached to the back of the wasp head, and a similar recording electrode was connected to the excised tip of the wasp antenna. The analog signal was detected through a probe and processed with a data acquisition controller (IDAC-4, Syntech, The Netherlands). Data was assessed using EAG 2000 software (Syntech, The Netherlands). EAG responses to the 15 compounds and control (hexane) were sequentially recorded for each of 15 insect replicates. Each compound was presented at positions 1 through 15 across replicates to minimize positional bias. For instance, 1-octen-3-ol and 3-octanone were introduced to the first insect as the 3rd and 4th compounds, respectively, but introduced to the second insect as the 4th and 5th compounds, respectively.

Data analyses

Differences in absolute EAG values (EAG response to compound minus response to solvent control) of synthetic compounds were analyzed using the *Kruskal-Wallis* test, followed by *Sidak's* multiple comparison test. The relationship between EAG response and relative abundance was analyzed with *Proc Corr* (correlation) procedure in SAS. All analyses were performed in SAS v9.2 (SAS Institute Inc., Cary, NC, USA) with P=0.05 level of significance.

Results

Female *M. croceipes* showed varying EAG responses to test compounds (range: 0.05–0.82 mV; Figure 1). Decanal elicited the highest EAG response (0.82 mV; $\chi 2$ = 134.13; *df* = 14; $P<0.0001$), while β-pinene elicited the lowest response (0.05 mV) in parasitoids. Decanal, tridecane, 3-octanone, 2-ethylhexanol, 1-octen-3-ol, bisabolene, tetradecane and α-farnesene elicited

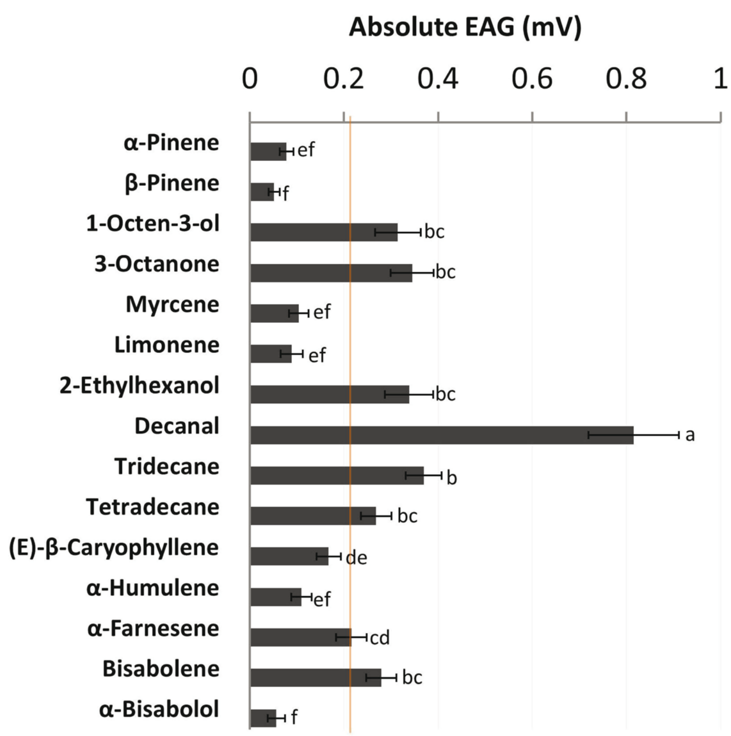

Figure 1. EAG responses of *Microplitis croceipes* to synthetic compounds. Mean absolute Electroantennogram (EAG) responses (mV ± SEM; *N* = 15) of female *Microplitis croceipes* to 15 volatile compounds identified in the headspace of cotton-fed *Heliothis virescens* larvae[5]. Synthetic compounds were formulated in hexane (solvent control) and tested at an optimum dose of 1 µg. Orange line indicates the arbitrary response threshold of 0.22 mV (50th percentile rank). Bars with no letters in common are significantly different ($P<0.05$; *Kruskal-Wallis* test followed by *Sidak's* multiple comparison test).

EAG responses ≥0.22 mV (50th percentile rank). Four of the top bioactive compounds: decanal, 3-octanone, 1-octen-3-ol and 2-ethylhexanol were emitted in quantities ≤2.2% of the total blend (Table 1). On the other hand, (*E*)-β-caryophyllene, the most abundant (29.2% of total blend) component, elicited a relatively low EAG response (0.17 mV) in parasitoids (Figure 1). However, the negative correlation between EAG response and relative abundance of compounds was not statistically significant ($r = -0.33$; $N = 15$; $P=0.23$).

Dataset 1. EAG responses of *Microplitis croceipes* to synthetic compounds and correlation with relative abundance of compounds

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Electroantennogram (EAG) data shows actual EAG response readouts to different compounds for 15 insect replicates. Absolute EAG value for each compound in a replicate can be obtained by deducting the average of two controls (Control 1 and Control 2) from the actual EAG values. Correlation data shows relative abundance of 15 blend components and their corresponding mean absolute EAG values. Details of data analyses were indicated in the main text and Figure 1 legend. Raw data behind the representation shown in Figure 1 and analyses referred to in the Results section are included.

Discussion

EAG responses of *Micropiltis croceipes* in the present study indicated variation in biological activity elicited by test compounds at the peripheral level, and revealed a discrepancy between relative abundance and level of antennal responses in parasitoids. High EAG response elicited by decanal in *M. croceipes* agrees with previous reports on olfactory responses of the parasitoids, *Microplitis mediator*[9] and *Bracon hebetor*[10]. Furthermore, decanal is a key attractant for host-seeking *M. croceipes*[5]. Although compounds are emitted in different quantities in natural blends, minor components can have a profound effect on resource location in parasitoids[6,11]. Interestingly, decanal constituted only 1% of the total blend emitted by cotton-fed *H. virescens*[5], but elicited the highest EAG response in *M. croceipes*, supporting the "little peaks-big effects" concept[6]. On the other hand, (*E*)-β-caryophyllene, the most abundant blend component, elicited a relatively low EAG response in parasitoids.

Therefore, it is more likely that the ecological relevance of a compound, rather than its relative abundance determines the level of olfactory response in foraging insects. For instance, small amounts of isothiocyanates in the volatile blend of brassica plants serve as host location cues for parasitoids of brassica herbivores[12,13]. More importantly, blend components act in concert to provide parasitoids with complete information[14]. Consequently, certain compounds function as background odors to enhance detectability (olfactory contrast) of other attractive components in a blend[12,15]. It is possible that (*E*)-β-caryophyllene serves as a background odor in the blend emitted by cotton-fed *H. virescens*. Finally, it should be noted that while EAG measures the level of bioactivity, behavioral bioassays are usually needed to establish the functional role of various compounds[5,16]. In addition, several species of parasitoids can be conditioned to respond to diverse odor cues, regardless of the relevance of such odor cues to their ecology.

Data availability

Dataset 1. EAG responses of *Microplitis croceipes* to synthetic compounds and correlation with relative abundance of compounds. Electroantennogram (EAG) data shows actual EAG response readouts to different compounds for 15 insect replicates. Absolute EAG value for each compound in a replicate can be obtained by deducting the average of two controls (Control 1 and Control 2) from the actual EAG values. Correlation data shows relative abundance of 15 blend components and their corresponding mean absolute EAG values. Details of data analyses were indicated in the main text and Figure 1 legend. Raw data behind the representation shown in Figure 1 and analyses referred to in the Results section are included. DOI: 10.5256/f1000research.10104.d143446[17].

Author contributions

TM and HF conceived the study. TM designed the experiment. TM and MB carried out the research. All authors contributed to writing and revision of the manuscript.

Competing interests

No competing interests were disclosed.

Grant information

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References

1. De Moraes CM, Lewis WJ, Paré PW, *et al.*: **Herbivore-infested plants selectively attract parasitoids.** *Nature.* 1998; **393**: 570–573.
Publisher Full Text

2. Turlings TC, Wäckers F: **Recruitment of predators and parasitoids by herbivore-injured plants.** *Adv Insect Chem Ecol.* 2004; **2**: 21–75.
Reference Source

3. de Rijk M, Krijn M, Jenniskens W, *et al.*: **Flexible parasitoid behaviour overcomes constraint resulting from position of host and nonhost herbivores.** *Anim Behav.* 2016; **113**: 125–135.
Publisher Full Text

4. Elzen GW, Williams HJ, Vinson SB: **Role of diet in host selection of *Heliothis virescens* by parasitoid *Campoletis sonorensis* (Hymenoptera: Ichneumonidae).** *J Chem Ecol.* 1984; **10**: 1535–1541.
Publisher Full Text

5. Morawo T, Fadamiro H: **Identification of key plant-associated volatiles emitted by *Heliothis virescens* larvae that attract the parasitoid, *Microplitis croceipes*: implications for parasitoid perception of odor blends.** *J Chem Ecol.* 2016; 1–10.
PubMed Abstract | Publisher Full Text

6. Clavijo McCormick A, Gershenzon J, Unsicker SB: **Little peaks with big effects: establishing the role of minor plant volatiles in plant-insect interactions.** *Plant Cell Environ.* 2014; **37**(8): 1836–1844.
PubMed Abstract | Publisher Full Text

7. Lewis WJ, Burton RL: **Rearing *Microplitis croceipes* in the laboratory with *Heliothis zea* as host.** *J Econ Entomol.* 1970; **63**(2): 656–658.
Publisher Full Text

8. Ngumbi E, Chen L, Fadamiro H: **Electroantennogram (EAG) responses of *Microplitis croceipes* and *Cotesia marginiventris* and their lepidopteran hosts to a wide array of odor stimuli: correlation between EAG response and degree of host specificity?** *J Insect Physiol.* 2010; **56**(9): 1260–1268.
PubMed Abstract | Publisher Full Text

9. Yu H, Zhang Y, Wyckhuys KA, *et al.*: **Electrophysiological and behavioral responses of *Microplitis mediator* (Hymenoptera: Braconidae) to caterpillar-induced volatiles from cotton.** *Environ Entomol.* 2010; **39**(2): 600–609.
PubMed Abstract | Publisher Full Text

10. Dweck HK, Svensson GP, Gündüz EA, *et al.*: **Kairomonal response of the parasitoid, *Bracon hebetor* Say, to the male-produced sex pheromone of its host, the greater waxmoth, *Galleria mellonella* (L.).** *J Chem Ecol.* 2010; **36**(2): 171–178.
PubMed Abstract | Publisher Full Text

11. Beyaert I, Wäschke N, Scholz A, *et al.*: **Relevance of resource-indicating key volatiles and habitat odour for insect orientation.** *Anim Behav.* 2010; **79**(5): 1077–1086.
Publisher Full Text

12. Wajnberg É, Bernstein C, van Alphen J: **Behavioral ecology of insect parasitoids: from theoretical approaches to field applications.** Wiley-Blackwell, Malden, MA, 2008.
Publisher Full Text

13. Najar-Rodriguez AJ, Friedli M, Klaiber J, *et al.*: **Aphid-deprivation from *Brassica* plants results in increased isothiocyanate release and parasitoid attraction.** *Chemoecology.* 2015; **25**(6): 303–311.
Publisher Full Text

14. van Wijk M, de Bruijn PJ, Sabelis MW: **Complex odor from plants under attack: herbivore's enemies react to the whole, not its parts.** *PLoS One.* 2011; **6**(7): e21742.
PubMed Abstract | Publisher Full Text | Free Full Text

15. Mumm R, Hilker M: **The significance of background odour for an egg parasitoid to detect plants with host eggs.** *Chem Senses.* 2005; **30**(4): 337–343.
PubMed Abstract | Publisher Full Text

16. Tamiru A, Bruce T, Woodcock C, *et al.*: **Chemical cues modulating electrophysiological and behavioural responses in the parasitic wasp *Cotesia sesamiae*.** *Can J Zool.* 2015; **93**(4): 281–287.
Publisher Full Text

17. Morawo T, Burrows M, Fadamiro H: **Dataset 1 in: Electroantennogram response of the parasitoid, *Microplitis croceipes* to host-related odors: The discrepancy between relative abundance and level of antennal responses to volatile compound.** *F1000research.* 2016.
Data Source

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Current Referee Status: ✓ ✓ ✓ ✓

Version 2

Referee Report 11 April 2017

doi:10.5256/f1000research.11957.r20837

Torsten Meiners
Federal Research Centre for Cultivated Plants Ecological Chemistry, Plant Analysis and Stored Product Protection, Julius Kühn-Institut (JKI), Berlin, Germany

The authors provided more information on a previous study and on the methods in the revision. They furthermore introduced the research topic and discussed their results more comprehensively.

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Referee Report 13 February 2017

doi:10.5256/f1000research.10885.r19829

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General comments

This study investigated the EAG response of a parasitoid *Microplitis croceipes* to the volatiles emitted from its host, the cotton plant-fed *Heliothis virescens* larvae. 15 compounds were tested at the dose of 1 µg on the female antennae of *M. croceipes*. Different level of bioactivity of *M. croceipes* to these compounds were presented and discrepancy was observed between the relative abundance and level of antennal responses in parasitoids. In the end, the authors suggested that ecological relevance but not the relative abundance of these compounds weighted more on the EAG responses of *M. croceipes*. However, since there is no behavior bioassay showing that these host-released compounds were truly importantly in the host-seeking process, it is hard to tell the ecological relevance of those compounds (like decanal) with strong EAG responses. A more cautious conclusion would be appropriate.

Specific comments

Introduction: Please give more information about why the authors specifically stated that the *Heliothis virescens* larvae were cotten-fed in the lab. Will different food sources affect the compounds released from the insect bodies?

Method: Please justify why mass concentration was used in preparing the compounds. Different chemicals possess various molecular weight and vapor pressure. Therefore, the number of molecules delivered onto the antenna may be dramatically different. In addition, only female wasps were used in the experiments. Apparently females need to find host to lay eggs. Just curious to know what the male's EAG responses to these compounds or if there are any related studies.

Results: Since 50% of the EAG responses from blend volatiles were used as a standard to make comparison, it would be better to add the EAG response to the blend volatiles in the bar figure. In addition, please specify the EAG response to the control solvent (hexane).

Discussion: The authors initiated a good start to discuss some compounds in the blend may function as background odors to enhance olfactory contract. There are many excellent reviews about the possible mechanisms behind this pheromone, such as Riffell and Hildebrand (2016).The authors may discuss a little bit about the mechanisms.

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Referee Report 10 February 2017

doi:10.5256/f1000research.10885.r19230

?

Torsten Meiners

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The research note of Tolulope et al. reports EAG responses of the parasitic wasp *Microplitis croceipes* and relates the antennal responses to the relative abundance of the compounds in the odour bouquet of the larvae.

When looking at the relative abundance of a compound you might need to consider all environmentally occurring contexts and compounds. *Microplitis* is orientating to the host habitat first, then it locates the host plant in the habitat, and then the host. That means that plant odours play an important role (see Li et al.[1]) and not only larval odours. In my opinion you have to rank the importance of odours according to all environmental contexts and consider the abundance of compounds in cotton or also in other relevant host (and habitat) plants. *Heliothis* feeds on more than 100 plant species, thus *M. croceipes* is confronted with the odour of this plants and with the odour of larvae having fed on these plants. Li et al.[1] have performed antennal studies with *M. croceipes* and cotton plant compounds and found similar responses to similar compounds as in your study. Heptanal was the most stimulating tested compound while caryophyllene was less stimulating. The the discrepancy you indicate in the title might be easily explained when including habitat and host plant volatiles.

The wasps in your study had the experience of living and hatching from larvae having fed on cotton. Thus they might have experience with the compounds you present in Table 1. It has been shown that *M. croceipes* can learn almost any compund (e.g. Olson et al.[2]).

In your discussion you point out that it might be the ecological relevance of a compound that determines the antennal response – however, Park et al.[3] showed in electroantennogram studies that the antenna of *M. croceipes* is also responding to anthropogenic compounds with high sensitivity. Thus, the antennal response might not reflect the ecological relevance. This might be more reflected in the behavioural response, as you indicate in your discussion. And this might be fine-tuned by leaning in case of a parasitoid with a polyphagous host.

Minor points:

Methods: Why is 1 µg an optimal dose?

Data analyses: Differences …were analysed

References

1. Li Y, Dickens JC, Steiner WW: Antennal olfactory responsiveness ofMicroplitis croceipes (Hymenoptera: Braconidae) to cotton plant volatiles.*J Chem Ecol*. 1992; **18** (10): 1761-73 PubMed Abstract | Publisher Full Text
2. Olson DM, Rains GC, Meiners T, Takasu K, Tertuliano M, Tumlinson JH, Wäckers FL, Lewis WJ: Parasitic wasps learn and report diverse chemicals with unique conditionable behaviors.*Chem Senses*. 2003; **28** (6): 545-9 PubMed Abstract
3. Park K, Zhu J, Harris J, Ochieng S, Baker T: Electroantennogram responses of a parasitic wasp, Microplitis croceipes, to host-related volatile and anthropogenic compounds. *Physiological Entomology*. 2008; **26** (1): 69-77 Publisher Full Text

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Referee Report 06 February 2017

doi:10.5256/f1000research.10885.r19925

Yonggen Lou
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The manuscript reported the the relationship between the relative abundance of volatile chemical emitted from *Heliothis virescens* larvae and the antennal response by the larval parasitoid of the herbivore, *Microplitis croceipes*. By electroantennogram bioassays, the authors found that the level of parasitoid's EAG response is not related to relative abundance of the volatile components in the blend. Since a chemical that elicits an EAG response in an insect does not mean to elicit a behavioral response, and a chemical eliciting a bigger EAG response does not mean to elicit a stronger behavioral response, these experiments seem little and the novelty and significance of this study seem limited.

Introduction: In a previous study, the authors have investigated the attractiveness of these individual volatile compounds to the parasitoid, thus it would be better to introduce these results briefly in the section of Introduction.

Methods: It has been well documented that an insect has different responsive ranges to different chemicals. Thus, only using one concentration of chemicals is not enough.

Discussion: Based on the previous results reported by the authors[1], 8 chemicals in the blend, including decanal and (*E*)-β-Caryophyllene, had a role in attraction of the parasitoid. The authors should give a discussion based on above results.

References

1. Morawo T, Fadamiro H: Identification of Key Plant-Associated Volatiles Emitted by Heliothis virescens Larvae that Attract the Parasitoid, Microplitis croceipes: Implications for Parasitoid Perception of Odor Blends.*J Chem Ecol*. 2016; **42** (11): 1112-1121 PubMed Abstract | Publisher Full Text

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Referee Report 16 January 2017

doi:10.5256/f1000research.10885.r19376

Amanuel Tamiru

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General comments:

This study examined the relationship between the relative abundance of a volatile organic compounds (VOCs) emitted by *Heliothis virescens* (Lepidoptera: Noctuidae) and level of antennal response by the larval parasitoid, *Microplitis croceipes* (Hymenoptera: Braconidae). The study builds on the previous work by Morawo & Fadamiro (2016) which identified key plant-associated volatiles emitted by cotton-fed *H. virescens* larvae that attract the parasitoid, *M. croceipes.* Here, the synthetic versions of 15 previously identified plant-associated volatiles emitted by *H. virescens* larvae were tested in electroantennogram (EAG) bioassays. The authors conclude that the level of parasitoid's antennal response is not directly related to relative abundance of the volatile components rather influenced by the ecological relevance or functional role of the compound in the blend. It should be noted that identifying volatile compounds that insects detect through EAG is an initial step in understanding olfactory stimuli responsible for modulating insect behavior. Hence, behavioral studies with the identified EAG active compounds should be carried out, individually and as blends, to determine responses of parasitoids to the volatile compounds and explore their ecological or functional role. Though the authors intended to examine relationship between relative abundance of the VOCs and level of antennal responses, different levels of the volatile compounds used in the study and their corresponding EAG response is not shown. Rather, only one dose (1 μg) is used for all volatile compounds tested. The study would have been very informative if different levels of the test compounds are tested and their corresponding EAG response recorded.

Specific comments

Title: The title is appropriate for the content of the article; however, it can be made more concise. E.g. the first part of the existing title would adequate, i.e. 'Electroantennogram response of the parasitoid, *Microplitis croceipes* to host-related odors'.

Introduction: The introduction clearly states the objective of the study. However, adequate background is missing in the area of herbivore emitted plant associated VOCs. Are these plant derived volatile compounds emitted by herbivore itself (after feeding) or are they adsorbed into the hervivore body during feeding process (e.g. from frass)

Methods and materials: The authors followed standard insect rearing (Lewis & Burton, 1970) and electroantennogram recording (Ngumbi *et al.*, 2010) and data analysis procedures. However, the number of insect replicates used in the study is not clear. Was a single insect antennal preparation used to record EAG responses to the 15 compounds and control (hexane)? Was the abundance of volatile components varied based on the corresponding quantities in the natural headspace samples? The latter has not been specified in the methodology except mentioning '*Test compounds were formulated in hexane at 0.1 µg/µl and delivered onto Whatman®No.1 filter paper strips at an optimum dose of 1 µg*'.

Results: The results show that female *M. croceipes* showed varying EAG responses to test compounds. Notably, decanal which constituted only 1% of total blend emitted by cotton-fed *H. virescens* elicited the highest EAG response (0.82 mV); while (*E*)-β-caryophyllene, the most abundant component (29.2% of total blend), elicited a relatively low EAG response (0.17 mV) in the parasitoid antenna. This is possible as earlier reports also indicated compounds with highest EAG response may not necessarily be those emitted in largest quantity (Tamiru *et al.* 2015[1]). Given the fact that the main research question of this study is examine the relationship between relative abundance and level of antennal responses, it would have been more informative to test different concentrations/amounts of the test compounds and their corresponding EAG response to reliably measure statistical significance of correlations between relative abundance of the compounds and level of EAG response.

Discussion: The authors discuss about the ecological relevance of a compounds. However, electrophysiological responses do not necessarily mean that a behavioral response will occur; rather it elucidates potential behaviorally relevant compounds. To fully explore the significance of the results, bioassays need to be carried out with identified compounds, both individually and as a blend, to determine the kind of behavioral response the volatile compounds trigger in the parasitoid and their ecological/functional role. I suggested authors to refer and perhaps include in their discussion the work by Tamiru et al. (2015)[1] which demonstrated combined use of electrophysiological and behavioral studies for better understanding of odor mediated behavior in insects.

References

1. Tamiru A, Bruce T, Woodcock C, Birkett M, Midega C, Pickett J, Khan Z: Chemical cues modulating electrophysiological and behavioural responses in the parasitic waspCotesia sesamiae. *Canadian Journal of Zoology*. 2015; **93** (4): 281-287 Publisher Full Text

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.