How background odor affects the host searching behavior of the ectoparasitoid *Holepyris sylvanidis*

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INTRODUCTION

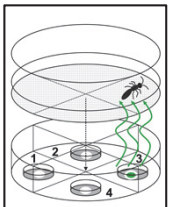
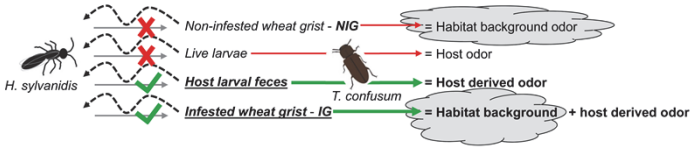


Fig. 1 Static 4-field olfactometer for biotests



Holepyris sylvanidis females parasitize the larvae of the confused flour beetle, *Tribolium confusum,* a major pest of stored products. But how does the parasitoid locate its host living inside the storage? Our previous results showed that odors of host larval feces and host infested wheat grist are highly attractive to *H. sylvanidis*.

The present study aims to elucidate the composition of the volatile blend responsible for attraction. Special emphasis was placed on the question whether background odor from the host´s habitat (*per se* not attractive) affects the olfactory orientation of the parasitoid to the attractive volatile cues.

METHODS

Attractive host derived odor was collected by dynamic headspace sampling from fresh larval feces and from infested wheat grist (= *IG*). Non-attractive habitat background odor was collected from non-infested wheat grist (= *NIG*). The volatiles were trapped on Tenax TA, eluted with hexane and analyzed by **GC-MS.** The parasitoid´s antennal responses to the identified compounds were measured by **GC-EAD** and **EAG**. We used a **static 4-field olfactometer** to investigate the behavioral responses of female *H. sylvanidis* to ***a)*** electrophysiologically active host and habitat odors. We further tested whether the parasitoids discriminate between ***b)*** behaviorally active unique host odors ***vs*** larval feces and ***c)*** behaviorally active unique host odors + *NIG* ***vs*** larval feces.

RESULTS

Volatile composition / EAG-responses

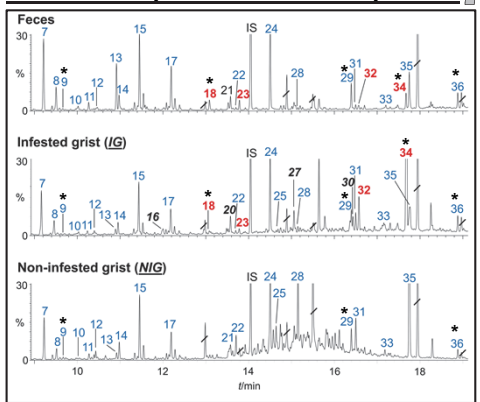


Fig. 2 Partial GC-MS profiles of headspace volatiles collected from fresh feces, *IG* and *NIG*. Numbers above peaks represent identified compounds; red = **unique host odors**, black = **unique *IG* odors**, blue = **common habitat/host odors**. Dashed compounds are contaminations.

(*) = Electrophysiologically active compounds

Odor blends

Unique host odors

18)*	**(*E*)-2-nonenal** ((*E*)-2-al)
19)*	**octanoic acid** (8-ac)
23)	decanal
32)	6,10-dimethyl-2-undecanone
34)*	**1-pentadecene** (1-C15)

Unique IG odors

16)	2-nonanone
20)	2-decanone
27)	2-undecanone
30)	2-dodecanone

Common habitat/host odors
(→ **habitat background odor** = grey cloud)

as for example

9)*	**benzaldehyde**
29)*	**1-tetradecene** (1-C14)
36)*	**1-hexadecene** (1-C16)

(*) = Electrophysiologically active compounds

[Hence, the habitat background odor did not contain unique GC-EAD active compounds, but several ones that were also detected in the attractive odors.]

Qualitative and quantitative differences between host and habitat odor blends

Six EAG-active unique host and common habitat odors

Behavioral activity

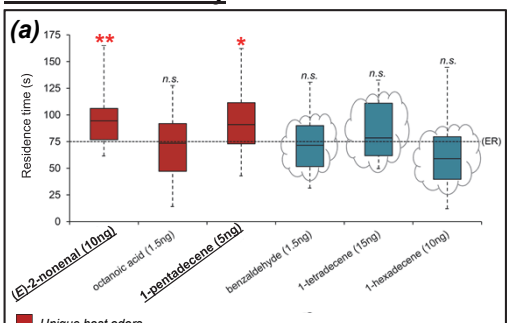


Fig. 3 Fig. 3 Behavioral responses of parasitoids in a static 4-field olfactometer to **one test stimulus** (N=15 each test, *t*=300s): Applied amounts of the electrophysiologically active compounds are equivalent to amounts detected in the headspace of 500ng fresh feces. Median values with interquartile range for responses to test stimuli are displayed (empty control fields: data not shown). The residence time in each field was compared with the expected residence time for equal distribution in all four fields (ER = 75s). One-sample Wilcoxon Signed Rank test (*$P \leq 0.05$, **$P \leq 0.01$, *n.s.* = not significant).

Two behaviorally active unique host odors (= possible key components)

Impact of habitat background odor

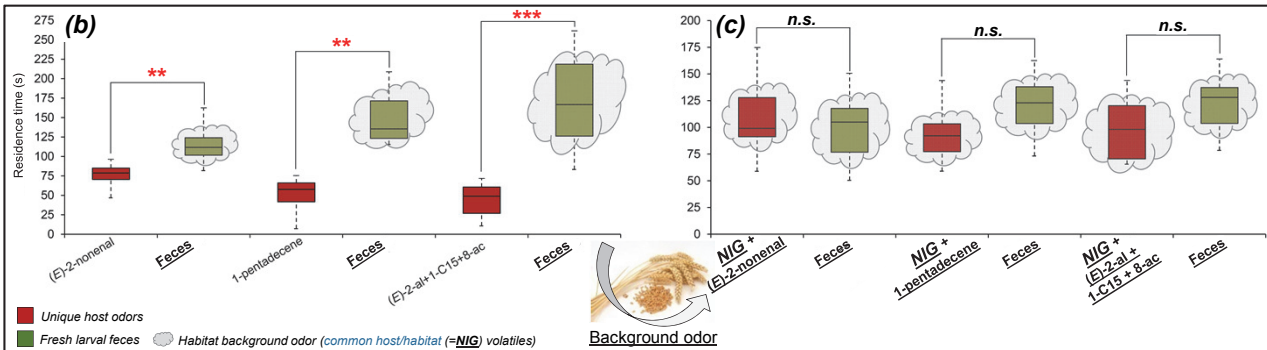


Fig. 4 Behavioral responses of parasitoids in a static 4-field olfactometer to **two test stimuli** (N=15 each test, *t*=300s). ***(b)*** Active **unique host odors *vs*** feces (500ng) containing **common habitat/host odors** = grey cloud; ***(c)*** Background habitat odor from *NIG* (500ng = grey cloud + **unique host odors** *vs* feces (500ng). Applied amounts are equivalent to amounts detected in the headspace of 500ng fresh feces. Residence time of parasitoids in test fields was analyzed for differences by repeated measures ANOVA for dependent data followed by Bonferroni corrected multiple comparisons (**$P \leq 0.01$, ***$P \leq 0.001$; *n.s.* = not significant).

Larval feces are more attractive than the host-indicating key components

Habitat background odor (*NIG*) enhances behavioral responses to these key components

CONCLUSIONS

The attractive host odor blend and non-attractive habitat volatiles showed qualitative and quantitative differences in their compositions. *H. sylvanidis* females responded electrophysiologically and behaviorally to **(*E*)-2-nonenal** and **1-pentadecene** which are released from host larval feces and appear to be **key components** for host location. **Habitat background odor** from non-infested wheat grist **enhanced** the parasitoid's response to these compounds. We hypothesize that the background odor is used by *H. sylvanidis* as a frame of reference for the host-indicating cues and thus, most likely improves the parasitoid´s host location success by providing a ´sharper view´. These findings may promote the development of a sustainable management of *T. confusum* by improving the attraction of *H. sylvanidis* as biological control agent.