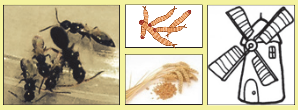
How a parasitoid finds host larvae in a mill:

The larval ectoparasitoid *Holepyris sylvanidis* smells host + habitat volatiles at long range and tracks trails of contact kairomones at short range to locate its host *Tribolium*



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INTRODUCTION

H*olepyris sylvanidis* parasitizes larvae of the confused flour beetle *Tribolium confusum,* a major pest of stored food products. Female parasitoids are highly attracted to host larval feces, in particular to two host-associated components of the fecal odor, 1-pentadecene and (*E*)-2-nonenal. Volatiles ubiquitously present in the host´s habitat have been shown to enhance the parasitoid´s response to these key compounds (Fürstenau *et al.* 2016). Thus, a blend of host-specific compounds and habitat odor may serve as long-range attractant.

The present study aims to elucidate how *H. sylvanidis* locates its host larvae at short range. We tested the hypotheses that female wasps *i)* use cuticular hydrocarbons of their host larvae for host recognition and *ii)* track a trail of these compounds left by host larvae on the substrate.

Fürstenau et al. (2016) *Chemical Senses*. **DOI:10.1093/chemse/bjw065**

CHC = cuticular hydrocarbon, **L.E.** = larval equivalents, ***NIG*** = noninfested wheat grist

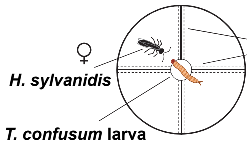
METHODS

I) Identification of host larval CHCs by GC-MS

II) Contact bioassay (*n*=30; *t*=5 min)

(A) number of located host larvae (first contacts)

(B) time until first contact (parasitization attempt) with host larvae



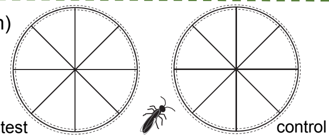
III) Trail following bioassay (*n*=20; *t*=5min)

1) CHC extract ***vs.*** **hexane**

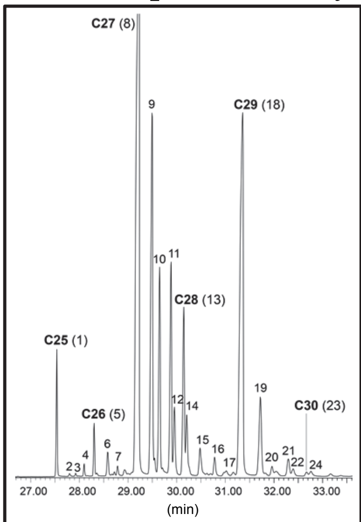
100 dead 4th instar larvae/500 µL hexane (10 min) → 25 µL/trail = **5 L.E.**

2) CHC infested grist ***vs. NIG***

100 dead 4th instar larvae/5 g *NIG* (2h) → 250 mg/trail = **5 L.E.**



I) CHC profile of *T. confusum* host larvae

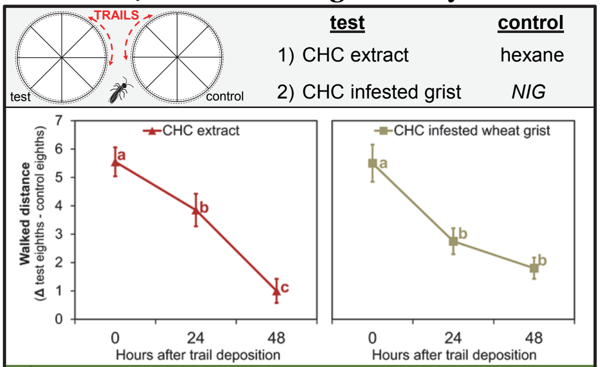


1)	**C25**
2)	11-Me C25
3)	5-Me C25
4)	3-Me C25
5)	**C26**
6)	11-/12-/13-Me C26
7)	4-Me C26
8)	**C27**
9)	11-/13-Me C27
10)	5-Me C27
11)	3-Me C27
12)	5,15-diMe C27
13)	**C28**
14)	3,11-/3,13-diMe C27
15)	12-/14-Me C28
16)	4-Me C28
17)	3-Me C28
18)	**C29**
19)	11-Me C29
20)	5-Me C29
21)	3-Me C29
22)	5,13-/5,15-diMe C29
23)	**C30**
24)	3,13-/3,15-diMe C29

31 linear + methyl-branched hydrocarbons

Fig. 1 Partial GC-MS profile of CHC extract from 4th instar *T. confusum* larvae (20 larvae in 150 µL hexane for 10 min). Numbers above peaks (1-24) represent the identified cuticular hydrocarbons listed in the table on the right. The *n*-alkanes (C25-C30) are highlighted in bold.

III) Trail following bioassay



CHC trails left by host larvae on substrate elicit trail following in the parasitoids.

The trail following elicitor activity of CHCs is kept for at least 48 hours after deposition.

Fig. 3 Behavioral responses of female *H. sylvanidis* to larval CHC trails (short-range cues) of its host *T. confusum*. Mean differences (**Δ**) of walked eighths on test and control circles at different time points (0, 24 and 48 h) after trail deposition are displayed; trail following activities at different time points of trail deposition are compared by Bonferroni corrected *t*-test for independent data.

II) Contact bioassay

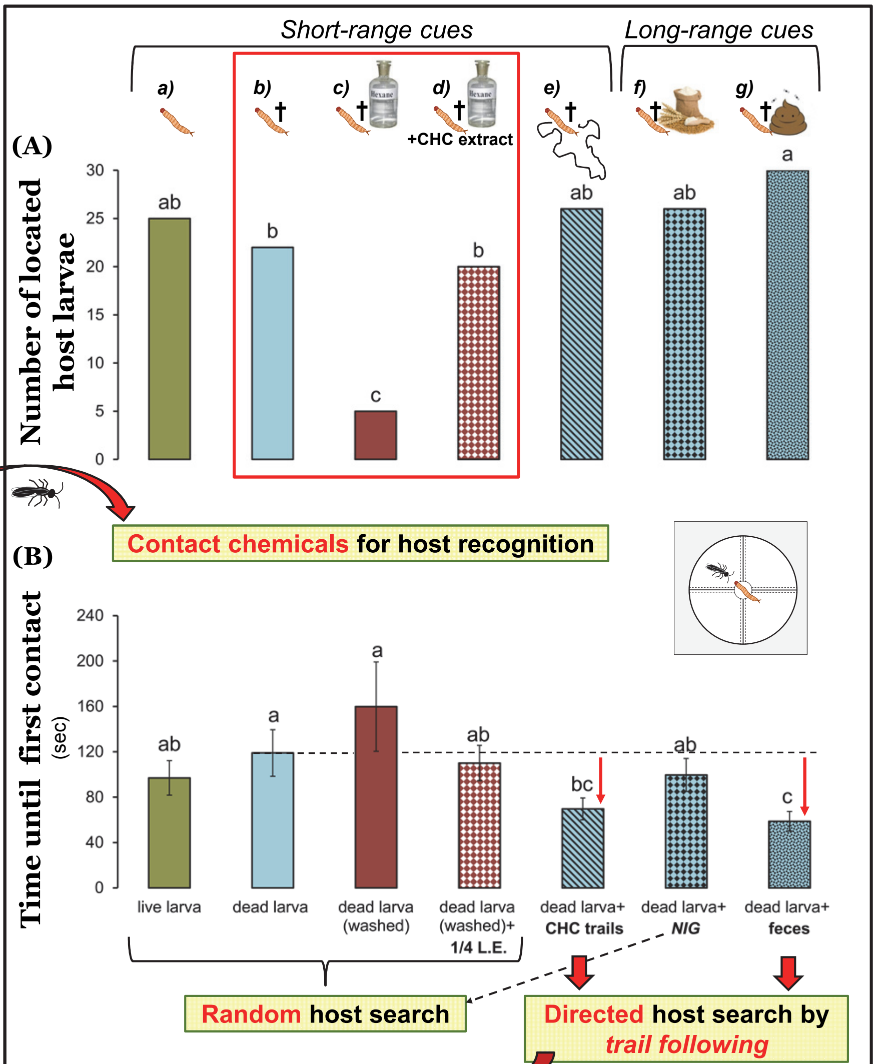


Fig. 2 Behavioral responses of female *H. sylvanidis* to host larvae in combination with short-range cues **(a-e)** and long-range attractants **(f+g)** of its host *T. confusum* and the host habitat (*NIG*). ***a)*** live larva, ***b)*** dead larva, ***c)*** dead larva washed with solvent, ***d)*** dead larva washed with solvent and treated with CHC extract (1/4 L.E.), ***e)*** dead larva plus 4 additional CHC extract trails (2 L.E./track), ***f)*** dead larva plus *NIG* (10 mg) and ***g)*** dead larva plus host larval feces (10 mg). **(A)** number of located host larvae (first contacts); analyzed by pairwise comparison of proportions and **(B)** time until a first contact/parasitization attempt occurred; analyzed by one-way ANOVA followed by Fisher´s *LSD* test.

SUMMARY

GC-MS analyses of the *T. confusum* larval CHC profile revealed 31 long-chain linear and methyl-branched hydrocarbons.

H. sylvanidis **females use larval CHCs for host recognition.** Trails of host larval CHCs and host larval feces significantly reduce the time until the host larva is located. Parasitoids follow fresh CHC trails more intensely than older trails.

CONCLUSIONS

Based on our current study and a previous one (Fürstenau et al. 2016), we suggest the following scenario for host location by *H. sylvanidis: (i)* Long-range attraction by odor of larval feces and *NIG*, *(ii)* random search for contact host kairomones, *(iii)* perception of fresh CHC trails left by host larvae on substrate (wheat grist) elicits trail following to host larvae, *(iv)* successful host location.