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## Olfactory host location and host preference of *Holepyris sylvanidis* (Hymenoptera: Bethyridae) and *Cephalonomia waterstoni* (Bethyridae), two natural enemies of *Tribolium* and *Cryptolestes* species

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### Abstract

Parasitoids can suppress populations of their host and thus play a primary role in Integrated Pest Management. In the stored product environment, stimuli deriving from plant products, damaged plant products and hosts might be important for host location by the parasitoids. We studied foraging cues in *Holepyris sylvanidis* (Hymenoptera: Bethyridae), a larval parasitoid of *Tribolium* species and *Cephalonomia waterstoni* (Bethyridae), a natural enemy of the rusty grain beetle *Cryptolestes ferrugineus* (Coleoptera: Cucujidae). Our studies in a four-chamber olfactometer revealed that the host complexes of both *Tribolium* species and different living host stages attract naive *H. sylvanidis* females, whereas no reaction was observed to uninfested substrates. The olfactory response of *C. waterstoni* was found to be strongly elicited both by chemicals emitted by the dust, adult *C. ferrugineus* and *C. ferrugineus* third and fourth instar larvae. Our findings may contribute to the development of biological control strategies of *T. castaneum*, *T. confusum* and *C. ferrugineus* with parasitoids.

**Keywords:** natural enemies, Bethyridae, stored product pests, biological control

### Introduction

The bethylid wasp *Cephalonomia waterstoni* Gahan is an external, arrhenotokous idiobiont larval ectoparasitoid. Hosts are *Cryptolestes ferrugineus* (Stephens), *C. pusillus* (Schönherr) and *C. turcicus* (Grouvelle) (Coleoptera: Cucujidae) (Finlayson, 1950a; 1950b). *C. waterstoni* is able to find hosts by recognizing residual kairomonal cues on infested substrates, similar to other parasitoids (Howard & Flinn, 1990). Hagstrum (1987) and Reichmuth et al. (2007) reported the ability of *C. waterstoni* to maintain the population of rusty grain beetles below the economic threshold.

The parasitic wasp *Holepyris sylvanidis* (Brèthes) (Hymenoptera: Bethyridae) is a larval parasitoid of *Tribolium confusum* Jacqueline du Val and *T. castaneum* (Herbst) (Coleoptera: Tenebrionidae), the economically most important stored product pests worldwide (Athanassiou et al., 2005; García et al., 2005). The host-searching behaviour of *H. sylvanidis* is influenced by the presence of host faeces, in which two compounds are thought to be responsible for the attraction: (*E*)-2-nonenal and 1-pentadecene (Fürstenau et al., 2016). The ability of *H. sylvanidis* to penetrate cracks and crevices makes it a promising natural enemy against stored-product pests. Pest larvae are often hidden under thin layers of substrate, in aeration ducts, in machines and in areas that are difficult to clean, but this wasp is able to access these critical environments.

## Materials and Methods

### Olfactory responses of *C. waterstoni*

The experiments were carried out in a static four-chamber olfactometer proposed by Steidle & Schöller (1997). The experiments evaluated the parasitoids response towards the following odour sources: (1) healthy grain (HGR), consisting of undamaged and uninfested wheat harvested in Germany in 2013; (2) grain infested by *Sitophilus granarius* (L.) (Coleoptera, Dryophthoridae) (IGR), which was obtained from the stock rearing of Hohenheim University; (3) a mixture of grain dust from the mass rearings of *Rhyzopertha dominica* (F.) (Coleoptera, Bostrichidae), *Oryzaephilus surinamensis* (L.) (Coleoptera, Silvanidae) and *C. ferrugineus* on durum wheat (DST), which was collected from cultures kept at 30°C and 60 % relative humidity (RH), the insects and the wheat were kept in a 100 l bin for about 6 months; (4) dust which was obtained by sieving infested durum wheat after being fed on by adult *S. granarius* from Hohenheim's University laboratory (DSH); (5) diet without *C. ferrugineus* (DIT), consisting of big oats, small oats and wheat in the ratio 1:1:2, this diet also contained one tea-spoon of yeast and water, respectively; (6) diet plus *C. ferrugineus* (DCR), which was like the diet without *C. ferrugineus*, but in this case 50 randomly chosen adults were added, (7) male and female *C. ferrugineus* adults (ADU), (8) mixed larvae (LRM), i.e. 50 randomly chosen larvae of *C. ferrugineus*.

### Olfactory responses of *H. sylvanidis*

In the experiments we studied the parasitoid females reaction towards the following odour sources: (1) *T. castaneum* host complex (CAH) consisting of 0.5 g whole grains, 0.5 g broken grains, first to second instar larvae (n = 2), fourth instar larvae (n = 2), pupae (n = 2) and adults (n = 2); (2) *T. confusum* host complex (COH) consisting of 0.5 g whole grains, 0.5 g broken grains, first to second instar larvae (n = 2), fourth instar larvae (n = 2), pupae (n = 2) and adults (n = 2); (3) whole grains (WHG) consisting of 1 g whole grains (*Triticum durum*); (4) flour (FLR) consisting of 1 g of flour (*Triticum aestivum*); (5) *T. castaneum* first to second instar larvae (CA1-2) (n = 10); (6) *T. castaneum* fourth instar larvae (CA4) (n = 10); (7) *T. confusum* first to second instar larvae (CO1-2) (n = 10); (8) *T. confusum* fourth instar larvae (CO4) (n = 10); (9) *T. castaneum* pupae (CAP) (n = 5); (10) *T. confusum* pupae (COP) (n = 5); (11) *T. castaneum* adults (CAA) (n = 5); (12) *T. confusum* adults (COA) (n = 5).

## Results

### Olfactory responses of *C. waterstoni*

**Table 1** Substrates tested and mean walking time upon each odour source.

Substrates	Mean Time ± SE	P-value
DCR vs DIT	189.50 (±27.70) – 96.80 (±14.00)	P<0.05
DST vs EMP	202.43 (±28.10) – 94.22 (±15.70)	P<0.05
DSH vs EMP	198.16 (±28.40) – 110.70 (±17.70)	P<0.05
DST vs HGR	272.54 (±24.80) – 77.36 (±13.70)	P<0.05
DST vs IGR	214.53 (±33.03) – 71.30 (±20.50)	P<0.05
DCR vs DST	203.50 (±33.50) – 107.75 (±17.70)	P>0.05
ADU vs 3-4 L	164.16 (±31.40) – 95.39 (±21.80)	P>0.05
LRM vs ADU	121.74 (±28.10) – 102.64 (±22.80)	P>0.05
LRM vs EMP	178.29 (±15.40) – 114.43 (±10.70)	P<0.05
3-4 L vs 1-2 L	166.50 (±25.60) – 103.90 (±22.30)	P<0.05

### Olfactory responses of *H. sylvanidis*

**Table 2** Substrates tested and mean walking time upon each odour source

Substrates	Mean Time ± SE	P-value
CAH vs EMP	250.16 (±81.90) – 81.9 (±61.30)	P<0.05
COH vs EMP	248.22 (±93.20) – 102.74 (±46.55)	P<0.05

CAH vs COH	282.78 (±85.49) – 118.78 (±48.57)	P<0.05
WHG vs EMP	162.00 (±52.00) – 129.00 (±44.00)	P>0.05
FLR vs EMP	131.00 (±81.00) – 163.00 (±48.00)	P>0.05
WHG vs FLR	135.00 (±55.00) – 162.00 (±57.00)	P>0.05
CA1-2 vs CA4	183.36 (±70.78) – 123.54 (±42.06)	P<0.05
CO1-2 vs CO4	228.85 (±83.21) – 97.18 (±56.40)	P<0.05
CA1-2 vs CO1-2	204.77 (±74.37) – 143.92 (±62.77)	P<0.05
CA4 vs CO4	150.79 (±69.59) – 120.35 (±66.03)	P>0.05
CAP vs COP	190.25 (±64.27) – 138.26 (±69.90)	P<0.05
CAA vs EMP	208.00 (±96.00) – 136.00 (±80.00)	P<0.05
COA vs EMP	177.10 (±78.89) – 118.79 (±46.85)	P<0.05
CAA vs COA	164.38 (±72.04) – 152.18 (±59.85)	P<0.05

## Discussion

*C. waterstoni* is an arrhenotokous ecto-parasitoid able to locate hosts recognizing their kairomonal cues left on infested substrates (Amante et al., 2017 b,c; Howard & Flinn, 1990). Among the odours tested in the host habitat experiments, dust was the most attractive. The dust contains host faeces and particles from the host's feeding substrate, i.e. plant materials. Our study showed dust from infested products plus larvae and adults were most attractive to the parasitoid *C. waterstoni*. Consequently our study suggests laboratory reared *C. waterstoni* released for biological control would be arrested in areas infested by *C. ferrugineus*, but would not stay in uninfested stored products.

The present study demonstrates that *H. sylvanidis* primarily relies on volatile chemical cues from all host stages for host location. Our results demonstrate that the host complexes of at least two *Tribolium* species, *T. castaneum* and *T. confusum*, release volatile chemical signals, which are attractive for naive *H. sylvanidis* females. In our experiments designed to identify the attractive elements of the host complex, naive wasps did not react to uninfested whole grains and flour, but to most of the host stages of both *Tribolium* species. Whether the kairomonal cues identified could be used to manipulate the behaviour of these parasitoids in order to increase the effectiveness of biological control has to be investigated in future studies (Amante et al., 2017 a, b, c).

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