The potential of host-specific volatiles from *Tribolium confusum* larval faeces for luring the ectoparasitoid *Holepyris sylvanidis*

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Abstract

The ectoparasitoid *Holepyris sylvanidis* (Bethylidae) attacks larvae of different stored product pest beetles. Previous studies on the olfactory host search of *H. sylvanidis* revealed that female parasitoids are strongly attracted to volatiles released from *Tribolium confusum* larval faeces, in particular to (*E*)-2-nonenal and 1-pentadecene. We suggested that these host-specific key compounds may serve the parasitoid as long-range attractants for host location. In this context, we propose that the attractive volatile blend could be used to establish a new approach within the biological control of stored product pests by guiding the parasitoid to its host and thus, increasing the host finding success. We investigated the potential of the identified host-indicating volatile cues to attract *H. sylvanidis* from a distance by offering the two key compounds to female parasitoids. Their walking behaviour and the covered distance were analysed on a Kramer sphere. Moreover, in semi-field trials both attractive volatiles were loaded onto rubber septa which were placed next to 4th instars of *T. confusum* at 1.5 m distance from the parasitoids. We studied the host finding success of *H. sylvanidis* by (i) measuring the mean time to locate and parasitise *T. confusum* larvae and (ii) counting the number of parasitised and unparasitised host larvae as well as the number of newly hatched parasitoids compared to the control without additional olfactory cues. First results showed that *H. sylvanids* females can locate the provided host larvae from a distance. Parasitistion of host larvae started four days after the release of parasitoids. No effect of the additionally offered host-specific key volatiles ((*E*)-2-nonenal and 1-pentadecene) on the parasitoid´s host finding success was observed at the given conditions and used amounts of compounds. Further studies are required to determine the right odour blend and concentrations for attraction of parasitoids over a distance and finally to show that the addition of host-derived kairomones may support the host finding success of *H. sylvanidis*.

Keywords: biological control, stored-product pests, semi-field trial, long-range attractants, Bethylidae.

Introduction

Over the last years, social concerns about the usage of synthetic pesticides for protection of food commodities or stored products against insect infestation have been increased considerably, mainly due to possible side-effects for humans (e.g. food contaminations, health risks for users) and environment (e.g. persistence of chemical residues) as well as well as the risk of developing resistance within pest populations (Field, 1992; Arias-Estevez et al., 2008). That in turn has led to an increased demand for alternative non-chemical pest management strategies (Phillips and Throne, 2010). Within the field of integrated stored product protection, the use of natural enemies (e.g. parasitoids) of stored-product pests as biological control method represents a promising and environmentally-friendly approach, but more research on the biology and behaviour of parasitoids is needed (Flinn and Schöller, 2012; Trematerra, 2012).

For instance, Adler et al. (2012) showed that the release of the larval ectoparasitoid *Holepyris sylvanidis* at a two-week interval was sufficient to control the local population of the confused-flour beetle *Tribolium confusum* in a grain mill. As a result, additional heat treatment, that had to be adopted in the past, was not necessary during the further experiment. Therefore, and basend on previous studies and assumptions, *H. sylvanidis* is a potential candidate for the biological control of beetle larvae infesting stored products, in particular *T. confusum* and other *Tribolium* species (Evans, 1977; Fürstenau et al., 2016; Amante et al., 2017; Fürstenau and Hilker, 2017).

However, one important weak point in previous applications of parasitiods in general was the lack of specific traps and attractants which could help to monitor natural occurring beneficials as well as those additionally released. The development of suitable lures and traps for monitoring present populations of natural enemies requires profound and further research on the olfactory host finding process of the respective parasitoids with the aim to identify behavioural active, host-associated compounds for their application (Philipp and Thrones, 2010; Trematerra, 2012).

Previous studies on the odour-mediated host foraging behaviour of *H. sylvanidis* revealed that parasitoid females use volatiles released from larval faeces of *T. confusum* to locate its hosts. Two compounds of the faecal odour, (*E*)-2-nonenal and 1-pentadecene, were highly attractive to the parasitoid, particularly, in the presence of odour from host's feeding substrate, (i.a. wheat grist) (Fürstenau et al., 2016). Since the corresponding bioassays of this study were performed in a static 4-field olfactometer it still needs to be confirmed whether these two host-specific compounds ((*E*)-2-nonenal and 1-pentadecene) may act as long-range attractants for host location from a distance and whether these volatiles are possible candidates to lure and monitor *H. sylvanidis* individuals in the field. Therefore, the present study aimed to investigate (long-range) attraction effects of a two-component mix, consting of the specifically-host associated key compounds, on the host finding success and the efficiency of the parasitoid to locate *T. confusum* larvae in a semi-field trial from a distance.

Materials and Methods

Insects

Test insects (female *H. sylvanidis* and 4th instar host larvae of *T. confusum*) were taken from a permanent rearing at the JKI (Julius Kühn-Institute, Federal Research Centre for Cultivated Plants, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Berlin, Germany) as described previously (Fürstenau et al., 2016; Fürstenau and Hilker, 2017). According to Brindley (1930) and Sokoloff (1974), we defined 25-to-30-days-old *T. confusum* larvae (3-4 mm long) as 4th instars under our rearing conditions (25±1°C and 65±5 % relative humidity).

Preparation of the host-specific two-component mix (2CM)

The two-component mix, hereafter abbreviated to 2CM, was prepared by adding 2 mg (*E*)-2-nonenal (97%, purchased by Sigma Aldrich) and 1 mg 1-pentadecene (95%, purchased by TCI Europe) to 10 µl *n*-hexane (98%, purchased by Merck). The resulting solution was stored at -20°C until its use in subsequent bioassays.

Semi-field trial to evaluate the host finding success of H. sylvanidis from a distance

To investigate possible effects of two specifically host-associated compounds identified from *T. confusum* larval faeces on the foraging behaviour of *H. sylvanidis* females a semi-field trial was conducted. We evaluated the potential of 2CM to attract *H. sylvanidis* females over a 1.5 m-long distance and to improve the host finding success by guiding the parasitoid to its host. Experiments were performed in specifically manufactured boxes (0.75 x 2.0 x 1.0 m), consisting of a metal frame. Head and foot end as well as three side panels were made from gauze or cotton fabrics; four doors were embedded in the front side of the box. Each box was installed on a wooden panel. At the head end of the box we put a Petri-dish (Ø 5.5 cm) with fifty 4th instar host larvae of *T. confusum* on a plastic tray (23.5 x 30 cm) filled with wheat grist. Above the Petri-dish we put one odour dispenser which had been loaded with either 10 µl 2CM (treatment) or 10 µl of *n*-hexane (control) and evaporated for 24 h. As *H. sylvanidis* females generally transferres host larvae to hiding places (Ahmed et al., 1997) loose pipette tips were randomely put in each corner of one tray. Two boxes for test and control trials each were placed in two separate rooms to avoid biased results due to interferences between test and control treatments. During the semi-field trial, room temperature and relative humidity depended on the outside conditions and were recorded by a datalogger; the average room temperature and relative humidity were 22 ± 1°C and 33 ± 7%, respectively.

At the beginning of the two-weeks-lasting experiment, twenty 1-to-8-days-old, mated parasitoid females were released at the opposite side of the box, 1.5 m away from the Petri-dish with host larvae. On day 1, 4 and 6 after the release of parasitoids we checked whether we could find paralysed and/or parasitised *T. confusum* larvae in the pipette tips and outside the tray. Pipette tips having paralysed and/or parasitised host larvae were replaced by new ones; all parasitised larvae were transferred to the climate chamber. After seven days the tray filled with wheat grist, the Petri-dish with *T. confusum* larvae and the pipette tips were renewed and the number of remaining *T. confusum* larvae found inside the Petri-dish and the wheat grist were counted. Twenty new parasitoid females were released in each box. As described above for 1st week, the number of *T. confusum* larvae outside the tray and the proportion of parasitised and unparasitised host larvae were measured. The semi-field trial was stopped after 13 days; each trial was repeated twice.

Since *H. sylvanidis* females drag the paraslysed host larvae to hiding places for parasitation we defined as a successful host finding event when *T. confusum* larvae were found in the pipette tips or outside the tray. In addition to the mean number of successful host finding events, we also calculated the parasitation rate and the ratio of hatched parasitoid (females and males) compared to the control. Data were statistically analysed by a Welch Two Sample *t*-test for the rat of successful host finding events and a Wilcoxon rank sum test with continuity correction for the parasitation rate. All analyses were done using the statistical programm "R" version 3.4.1 (R Core Team, 2017) with packages "car" (Fox and Weisberg, 2011) and "pastecs" (Grosjean and Ibanes, 2014).

Results and Discussion

In the present semi-field experiments a mix of two highly attractive, host-associated odours, (*E*)-2-nonenal and 1-pentadecene, identified from the volatile blend collected from *T. confusum* larval faeces was offered to *H. sylvanidis* test females in combination with host larvae to test the influence of these additional odours on the parasitoid's host finding behaviour. We measured the rate of successful host finding events by counting the number of *T. confusum* larvae found in hiding places (pipette tips) and outside the tray 1, 4 and 6 days after the release of parasitoids. In both treatments the number of *T. confusum* larvae which had been displaced by *H. sylvanidis* increased with the duration of experiment (number of experimental days; Tab. 1). On day 1 after start of the experiment, all host larvae were still in their respective Petri-dishes in test and control treatment. Six days after releasing the parasitoids, 4.88 (±1.63) and 1.25 (±0.75) displaced host larvae were counted in the control and the 2CM-treatment, respectively. Overall, *H. sylvanidis* could locate and displace ca. 25% of fifty host larvae offered to parasitoids in the control-treatment; the rate of successful host finding events was twofold higher than in the 2CM-treatment (ca. 13%) but did not differ significant (Tab. 1). In both treatments the number of parasitised host larvae was lower compared to the number of displaced host larvae. In the control treatment ca. 8% of the fifty offered host larvae were parasitised whereas the parasitation rate was fourfold lower in the 2CM-treatment (ca. 2%). When calculating the parasitation rate we excluded all larvae which were not found at the end of the experiment. Regarding the high number of not recovered host larvae (4.25±2.04 larvae in the control and 4.38±1.97 larvae in test treatment, Tab.1) one could assume that the actual parasitation rate might be higher in both treatments.

The host finding ability under storage-like conditions has been examined previously for a few parasitoid species (Steidle and Schöller, 2002; Adler et al. 2012; Niedermayer et al. 2016). For example, Niedermayer et al. (2016) showed that the two larval parasitoids *Lariophagus distinguendus* and *Anisopteromalus calandrae* could locate approximately 20% of wheat kernels infested by the granary weevil *Sitophilus granarius* when they were released at 1 m distance. In comparison, the rate of successful host finding events of *H. sylvanidis* was higher in the control treatment (ca. 25%). Regarding environmental conditions, the field trial described before in comparison to our semi-field experiments differed considerably. The test parasitoids of *L. distenguendus* and *A. calandrae* were released into an open environment (two buildings of 150 m^2 and 45 m^2) where the host location process might be influenced by several local predominat conditions such as air flow, light incidenc, variable temperature and humidity (Niedermayer et al., 2016). In contrast, we conducted our experiments in a constant dark and closed environment as we used 2 m long boxes placed in two separated rooms. The host location and ability of *H. sylvanidis* under storage-like conditions is not known yet and needs to be tested in further studies. However, Adler et al. (2012) already demonstrated that a mass release of laboratory reared *H. sylvanidis* is sufficient to temporally suppress the growth of a natural occurring *T. confusum* population in a grain mill. This result indicates that the rate of successful host finding events might be the same or even higher than what we measured in the here presented semi-field trial.

Tab. 1: Effect of additionally deployed host-specific volatiles on the host finding success of Holepyris sylvanidis in semi-field experiments (N=2).

	N° of displaced *T. confusum* larvae (mean ± SE [a])			**N° of not found *T. confusum* larvae [b] (mean ± SE [a])**	**Rate (%) of host finding events (mean ± SE [a])**		**Parasitation rate (%) (mean ± SE [a])**	
	Days after parasitoid release					***p*-value [c]**		***p*-value [c]**
Treatment	**1**	**4**	**6**					
Control (hexane)	-	3.38 ±1.53	4.88 ±1.63	4.25 ±2.04	25.00 ±5.96	*0.126*	8.50 ±2.35	*0.054*
2CM [d]	-	0.88 ±0.40	1.25 ±0.75	4.38 ±1.97	13.00 ±4.26		4.00 ±1.00	

[a] SE = Standard error of the mean.
[b] Number of *T. confusum* larvae which were not found in Petri-dishes, wheat grist, pipette tips or outside the tray during and at the end of the experiment
[c] To compare the different treatments a Welch Two Sample *t*-test for the host finding success events and a Wilcoxon rank sum test with continuity correction for parasitation rate were applied.
[d] 2-component mix of specifically host-associated compounds ((*E*)-2-nonenal: 2 mg, 1-pentadecene: 1 mg)

Initially our suggestion was that the addition of host specific volatiles (2CM) loaded onto dispensers may facilitate the host search of *H. sylvanidis* by attracting and guiding the parastioid to its host and thus, increasing the host finding success. Surprisingly, the rate of successful host finding events (13%) and the parasitation rate (2%) were both lower in the test treatment, offering 2CM, compared to the control (rate of successful host finding events = 25%; parasitation rate = 8%; Tab. 1). A possible explanation for the different performance when using 2CM might be that concentrations of both compounds ((*E*)-2-nonenal = 2 mg and 1-pentadecene = 1 mg) used here were (much) too high and therefore possibly reppelled the parasitoids instead of attracting them. Regarding this, in preliminary studies on a Kramer sphere, however, we observed noticeable differences in the walking behaviour of *H. sylvanidis* individuals when 2CM was tested compared to the control without offering volatiles. Usually, two characteristics of the walking behaviour of insects on the Kramer sphere is a higher walking speed and therefore, a longer track length when the test individuals are strongly attracted to an odour source as Thiery and Visser (1986) have shown for the Colorado potato beetle *Leptinotarsa decemlineata* and its preferred host plant, *Solanum tuberosum*. In contrast, *H. sylvanidis* females walked slower and covered a smaller distance in presence of 2CM compared to the control treatment. Additionally, *H. sylvanidis* frequently turned back while walking on the Kramer sphere or rested more time in the test treatment (personal observations). The reverse movements of *H. sylvanidis* in presence of 2CM probably indicate that parasitoid female re-examined the area for a potential host. A similar behaviour was observed when females of the larval parasitoid *Tiphia vernalis* could not find immediately a host at the end of a trail of their preferred host, the Japanese beetle *Popillia japonica*. Parasitoid females stayed nearby the trail's end and searched the area to locate potential hosts in the soil (Rogers and Potter, 2002). Therefore, we can not exclude that the application of two highly attractive, host-indicating compounds (2CM) may influence the host finding behaviour of *H. sylvanidis* by attracting or repelling the parasitoid. Further studies on dispenser emission of 2CM are required to identify the correct blend (concentration, ratio etc.) and finally to show that these host-specific compounds can support the host finding success of *H. sylvanidis*.

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