Penetration ability of *Holepyris sylvanidis* into the feeding substrate of its host *Tribolium confusum*

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Abstract

The bethylid wasp *Holepyris sylvanidis* (Brèthes, 1913) is an antagonist of the confused flour beetle *Tribolium confusum* Jacquelin du Val 1868, a severe pest in the food processing industry and in grain products, primarily in flour mills and bakeries. Females of the larval ectoparasitoid *H. sylvanidis* have to detect hosts that feed in different depths inside stored products like flour or grist. This study addresses the questions (1) whether successful host finding by *H. sylvanidis* is dependent on the location of *Tribolium* larvae in the substrate and (2) whether the type of substrate affects host finding. In laboratory experiments, *T. confusum* larvae in a Petri dish accessible to the wasps were placed 1, 2, 4 or 8 cm deep in either fine or coarse ground wholemeal grist of wheat (main particle size: <0.2 mm or 1.4 – 3 mm) in fifteen replicates per substrate and depth. Parasitoids were released onto the surface of the substrate. *Tribolium confusum* larvae were not able to leave the Petri dish, however they could be pulled outside into the grist by *H. sylvanidis*. Within the behavioural sequence of parasitisation, pulling away of host larvae is the typical behaviour preceding oviposition. In order to determine host finding success by the parasitoid, the number of missing host larvae was assessed 2 wks after release of the wasps. In fine grist larvae were attacked down to 4 cm depth; however, larvae placed deeper (8 cm) were not found anymore. In contrast, host larvae in coarse grist were still detected at 8 cm depth. The results suggest that host finding by *H. sylvanidis* is hindered by decrease in particle size of the substrate. Nevertheless, *H. sylvanidis* may be considered a promising candidate for biological control of *T. confusum* larvae feeding in coarse grist and in thin layers of fine grist.

Keywords: *Holepyris sylvanidis, Tribolium confusum*, Biological control, Penetration ability, Host finding

1. Introduction

The acceptance of synthetic, not naturally occurring chemical materials used for pest control decreases. The interest in organic food increases. Therefore, biological pest control becomes more important. *Holepyris sylvanidis* (Brèthes, 1913) is a larval ectoparasitoid of *Tribolium confusum* Jacquelin du Val 1868, a severe pest in the food processing industry and in grain products. *H. sylvanidis* was described by different authors and can be reared in the laboratory for some time (Abdella et al., 1985; Ahmed and Islam, 1988; Ahmed et al., 1997). In regard to the application of the parasitoid against the confused flour beetle parasitizing of *T. confusum* larvae in deeper layers of the substrate would be desirable. This investigation was carried out to elucidate whether successful host finding by *H. sylvanidis* is dependent on the location of *Tribolium* larvae in the substrate and whether the type of substrate affects host finding.

2. Materials and methods

2.1. Insects

The insects used for the tests originated from the laboratory insect cultures of the institute, where *T. confusum* is cultivated on fine ground wholemeal grist of wheat at 25 ± 1°C, 65 ± 5% r.h., *H. sylvanidis* is reared on larvae of *T. confusum* in hollow wheat kernels (hollowed out by *Sitophilus* spp.) at 25 ± 1°C, 57 ± 5% r.h. The fourth instar larvae of *T. confusum* were used for the tests since *H. sylvanidis* prefers this larval stage for parasitisation (Ahmed et al., 1997). At the beginning of the experiments the parasitoids had a maximum age of ten days and were mated at least once before.

2.2. Experimental setup

The tests were carried out with both fine ground wholemeal grist of wheat (milling level 1) and coarse ground wholemeal grist of wheat (milling level 7) (Table 1). The grist contained all compounds of the grain and was freshly prepared prior to each experiment (grist mill Billy 200, Hawo's Kornmühlen GmbH, Bad Homburg, Germany).

Table 1 Milling levels and appropriate particle compositions of the two types of wholemeal grist of wheat (fine and coarse) used in the experiments

Milling level [1)]	Particle sizes (µm)	Portion of the total quantity (%)	Maximum particle size (µm)
1	≤ 200	64.0	1400
	201 - 710	31.7	
	711 - 1400	4.3	
	> 1400	0	
7	≤ 200	9.4	2800 - 3000
	201 - 710	9.5	
	711 - 1400	17.1	
	> 1400	64.0	

[1)] Milling levels are given by the grist mill Billy 200 (Hawo's Kornmühlen GmbH, Bad Homburg, Germany)

Before performing the tests the grain had been stored at -18°C for at least 10 d in order to kill potential arthropod pests. After thawing the moisture content of the wheat grains was adjusted to 14 ± 1%.

Experiments with both types of grist were carried out to reveal if *H. sylvanidis* is able to penetrate 1, 2, 4 and 8 cm deep into the substrate. Each depth (or layer thickness of the grist) was investigated in a separate 2-L glass jar. Each jar contained a Petri dish in the middle of the bottom with ten larvae of *T. confusum* and 1 g wholemeal grist of wheat for feeding purpose. The lower part of the Petri dish (Ø 3.5 cm) was covered with a slightly larger lid (Ø 5.5 cm). A V-shaped wooden stick (Ø 3 mm) placed in the lid of the Petri dish was used as spacer to preserve a gap between the two parts of the Petri dish. Through this gap the wasps had access to the host larvae. The host larvae themselves were unable to leave the Petri dish. Subsequently, the Petri dish in the glass jar was covered with a 1, 2, 4 or 8 cm thick layer of grist. Jars were shaken carefully to obtain a uniform density of the grist. Ten female *H. sylvanidis* and two males were released onto the surface of the grist. A drop of honey on the wall of the glass jar above the grist was added as food for the wasps. Each jar was closed with a piece of cotton cloth and rubber bands and kept in continuous darkness for 2 wks at 25 ± 1°C, 57 ± 5% r.h. in a climatic chamber. Each set of experiments (each type of grist combined with each depth) comprised fifteen replicates.

2.3. Collected data

After 2 wks the grist and the Petri dish were removed. The number of the remaining larvae of *T. confusum* in the Petri dish was recorded. According to Abdella et al. (1985), Ahmed and Islam (1988) and Ahmed et al. (1997) *H. sylvanidis* paralyses the host larva and always transports it away to a potential hiding place prior to depositing an egg on the larva. Therefore, if the number of host larvae in the Petri dish was less than ten this test was judged as a successful host finding. In so far, there was no distinction between one or more removed larvae leading to a positive judgement. To establish the judgement the neighbouring grist around the Petri dish was investigated for removed and parasitized host larvae.

2.4. Evaluation of the test

For each set of experiments the frequency of host finding by females of *H. sylvanidis* was determined. The frequency indicates in how many of the 15 replicates the parasitoids achieved to find host larvae. Fisher's exact test served for statistical evaluation ($P < 0.05$, SigmaStat 3.11.0) of the frequency of host finding in relation to depths and types of grist.

3. Results

Experiments with grist of both types revealed the tendency that the frequency of host finding decreased with increasing depth: the deeper the host larvae were hidden in the grist, the more seldom they were removed from the Petri dish by *H. sylvanidis* (Fig. 1). Significant differences in frequency were detected in fine grist between the tested depths 1 cm vs. 4 cm and in coarse grist between 1 cm vs. 8 cm as well as 2 cm vs. 8 cm. Comparing the two types of grist for all tested depths, 1, 2, 4 and 8 cm, the frequency of host finding was always higher in coarse grist. At a depth of 8 cm the frequency of host finding in fine grist was zero - no wasp achieved to remove one of the ten offered larvae, while in coarse grist the frequency of host finding was still six.

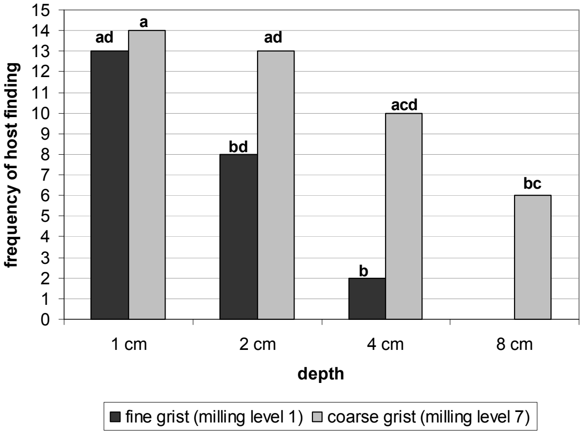

Figure 1 Frequency of host finding by *Holepyris sylvanidis* in all tested depths and types of wholemeal grist of wheat; tested locations of *Tribolium confusum* larvae: 1, 2, 4 and 8 cm deep in grist; tested types of substrate : fine grist (milling level 1) and coarse grist (milling level 7); number of replicates per depth and substrate: n = 15; bars (frequency of host finding) with different letters are statistically significant at $P < 0.05$ (Fisher's Exact Test); duration of the tests: 2 weeks; test conditions: 25 ± 1°C, 57 ± 5% r.h., continuous darkness.

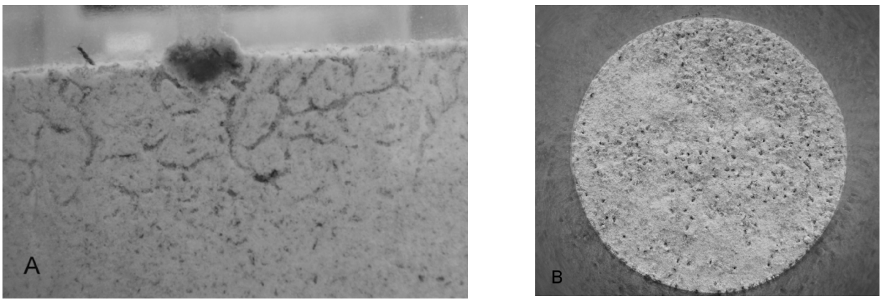

Figure 2 Small tunnels dug in the fine ground wholemeal grist of wheat (milling level 1) by *Holepyris sylvanidis*, (A) side view, (B) few from above.

Repeated observations of the single tests showed that females of *H. sylvanidis* were able to dig tunnels into the substrates (Fig. 2, A/B) and use them more than once. This was particularly obvious in fine grist.

4. Discussion

An important aspect for the optimisation of the application of biological control measures against *T. confusum* is the capability of a suitable antagonist to penetrate into the feeding substrate of its host. The experimental results showed that females of *H. sylvanidis* are able to penetrate differently thick layers of fine and coarse grist to find and parasitize hidden larvae of *T. confusum*. Host finding success depended on the thickness of the grist layer and the type of the grist. The wasps penetrated easily through 1 and 2 cm fine grist, at 4 cm they were less successful and they were not able to penetrate 8 cm. In coarse grist the wasps seemed easily to be able to pass through 4 cm, whereas host finding clearly decreased at 8 cm thickness.

A possible explanation for the influence of the particle size of the grist on the penetration ability might be the magnitude of the hollow space between the particles. Larger particles offer larger hollow spaces and the material can not be so densely packed as fine grist. Therefore, the effort for the wasps to dig tunnels into coarse grist is much smaller than that into fine grist. Presumably more than one wasp uses a tunnel and one wasp uses a tunnel more than one time, respectively. This may explain why the maximum depth of penetration and the magnitude of the frequency are more pronounced in coarse grist.

The mostly prevailing particle size of the coarse grist was 1.4 mm – 3.0 mm (Table 1). The females of *H. sylvanidis* had a body length of 2.96 ± 0.27 mm (Frielitz, Berlin, personal communication). Due to their small body size the wasps seem to be able to use small crevices and hollow spaces to move through the grist. Presumably the wasps can penetrate deeper than 8 cm into coarse grist. Further experiments would be necessary to determine the maximum depth of penetration.

It can be assumed that the deeper the host larvae were hidden in the grist the more difficult it was for the parasitoids to locate them. Obviously the wasps use volatile substances emitted by the excrements of larvae of *T. confusum* (unpublished data). These substances diffuse through hollow spaces in the grist and can be detected by the parasitoids on the surface or in the upper layers of the substrate. Possibly, the orientation towards chemical cues is decreased by sorption due to the larger internal surface of fine grist.

Also *Trichogramma embryophagum* (Hartig) was able to parasitize eggs of a host, *Ephestia* spp., down to 5 cm of wheat (Schöller et al., 1996), *T. evanescens* Westwood even down to 55 cm (Schöller et al., 1994). Schöller (2000) determined a maximum penetration depth of 30 cm in rye for *Habrobracon hebetor* (Say). Al-Kirshi et al. (1997) found larvae of *Trogoderma granarium* Everts parasitized by *Laelius pedatus* (Say) in 90 cm depth in wheat. Steidle and Schöller (2000) showed the capability of *Lariophagus distinguendus* (Förster) to parasitize *Sitophilus granarius* larvae hidden in wheat kernels in 4 m depth.

In this study *H. sylvanidis* penetrated thinner layers of substrate because the grist consisted of much smaller particles and was, therefore, more difficult to be penetrated than whole kernels of grain.

On the other hand the capability to penetrate into a certain depth of fine grist is crucial for female *H. sylvanidis* since its host, the confused flour beetle, often lives hidden under more or less thick layers of milled grain in mills or food factories (Sokoloff, 1974). Often the insect hides in small cracks and crevices, in aeration ducts, in or under machinery, in areas that are difficult to clean.

In this study, it was clearly shown that the larval parasitoid *H. sylvanidis* is able to penetrate various kinds of grist to find and parasitize its host, the confused flour beetle *T. confusum*. On this ground *H. sylvanidis* is a promising candidate for biological control of this pest insect especially in cases where the larvae are hidden under thin layers of the substrate. As demonstrated in this study the survival strategy of these wasps comprises its capability of actively host searching and parasitizing even in deeper layers of flour and grist.

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