Journal of Stored Products Research 78 (2018) 105–109

Contents lists available at ScienceDirect

Journal of Stored Products Research

journal homepage: www.elsevier.com/locate/jspr

Olfactory host location and host preference of *Holepyris sylvanidis* (Brèthes) (Hymenoptera: Bethylidae), a parasitoid of *Tribolium confusum* Jacquelin du Val and *T. castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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ARTICLE INFO

Article history:
Received 23 January 2018
Received in revised form
16 June 2018
Accepted 30 June 2018

Keywords:
Parasitoid
Stored product pests
Integrated pest management
Holepyris sylvanidis
Olfactometer

ABSTRACT

Parasitoids can suppress populations of their host and thus play a primary role in Integrated Pest Management. We studied foraging cues in *Holepyris sylvanidis* (Hymenoptera: Bethylidae), a larval parasitoid of *Tribolium* species, in a four-chamber olfactometer. *H. sylvanidis* is reported as a cosmopolitan parasitoid of Coleopteran including two major pests of stored products, the confused flour beetle *Tribolium confusum* and the red flour beetle *T. castaneum*. Our study reveals 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. Our findings may contribute to the development of biological control strategies of *T. castaneum* and *T. confusum* with parasitoids.

1. Introduction

The parasitic wasp *Holepyris sylvanidis* (Brèthes) (Hymenoptera: Bethylidae) is a larval parasitoid of *Tribolium confusum* Jacquelin du Val and *T. castaneum* (Herbst) (Coleoptera: Tenebrionidae), the economically most important stored product pests worldwide (Athanassiou et al., 2005; García et al., 2005). *H. sylvanidis* is a potential natural enemy for the biological control of important stored products pests, which makes any information on its biology important for application (Amante et al., 2017a; c). From an applicative point of view, *H. sylvanidis* female is capable to penetrate different thick layers of fine coarse grist when it searches for hidden larvae. Lorenz et al. (2010) reported that the host finding capacity is hindered by a decrease in particle size. To be more precise, the parasitoid can penetrate easily through 1 and 2 cm fine grist, at 4 cm the wasp is less successful and it can't penetrate deeper than 8 cm into coarse grist. Considering that the host, the confused flour beetle, often lives hidden under milled grain (Sokoloff, 1974), the capability of *H. sylvanidis* to penetrate the substrate became crucial to obtain good results when this wasp is considered for biological control. Eliopoulus et al. (2002) reported *H. sylvanidis* as the most "dominant" parasitoid collected in storage facilities in Greece, whereas in terms of frequency this parasitoid was the second. In terms of frequency Eliopoulus et al. (2002) showed that the major presence of this parasitoid was on flour although the wasp was also collected in grain and dried fruits. In the same study the author indicated *H. sylvanidis* as able to develop high populations both in grain and flour, reaching 59% and 61% of collected adults respectively.

Though, scope of using parasitoids is very limited in stored product insect management, understanding the innate response of parasitoids to odours derived from host infested material (chemical cues) assumes significance from an academic point of interest (Amante et al., 2017b). However, parasitoids can have applicative importance in biological control of pests in food industries. Grain producer tend to look at chemicals alternative to control pests (Flinn and Hagstrum, 2001) and nowadays only few pest control companies are considering the biological control as a remunerative

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https://doi.org/10.1016/j.jspr.2018.06.008

business. Furthermore, a lack of knowledge is still present especially regarding field releases and operators able to use this relatively new approach are very few. On the other hand problems for people, animals and environment could derive from residues left on the food and disperse in the environment. Because a lot of pests are resistant to major insecticides, the opportunity to find organic molecules or innovative systems to control pests has been taken into account by researchers since decades (Collins et al., 1993; Herron, 1990; Muggleton, 1987; Muggleton et al., 1991). Parasitoids represent one of the most adopted biological control systems of stored products and they belong almost entirely to the order Hymenoptera.

Hence, the response of *H. sylvanidis* females to different stimuli was evaluated in a four chambers olfactometer. In detail, we studied the reaction of *H. sylvanidis* to the whole host complex, to the plant components of the host complex, to the attacked host stages (young instar larvae, and old instar larvae) as well as to pupae and adults of *Tribolium.* In order to study a potential preference, all experiments were performed with each of the two *Tribolium* species, also in direct comparison. In addition, we examined the fecundity of *H. sylvanidis* with fourth instar larvae of *T. castaneum* and *T. confusum* as hosts.

2. Materials and methods

2.1. Insects

T. castaneum and *T. confusum* adults were kept in a 9 cm diameter Petri-dish on 20 g of a rearing substrate containing durum wheat flour (*Triticum aestivum*), rye flour, yeast and malt, or 20 g of wheat flour and 5% of brewers' yeast, respectively (Athanassiou and Kavallieratos, 2014). Freshly emerged adults were removed from the substrate three times (*T. castaneum*) or once (*T. confusum*) per week by sieving with a 0.6 mm sieve and kept on the rearing substrate as reported above in order to lay new eggs. For both *Tribolium* species, the flour containing the eggs was kept at 28 ± 1 °C and 65 ± 5% relative humidity (RH). After about 23 days of incubation, fourth instar larvae of both beetle species were obtained.

In order to rear the parasitoids, two females and one male of *H. sylvanidis* were kept in a 9 cm diameter Petri-dish. Ten fresh fourth instar larvae were added to each Petri dish twice a week (Ahmed et al., 1997). The parasitized larvae of the beetle were collected three times a week and kept at 28 ± 1 °C and 65 ± 5% RH. In order to support the natural behaviour of Bethylidae, which lay eggs on the paralyzed host larva in a hideaway, undulated cardboard was added to the Petri dish. As reported by Ahmed et al. (1997), *H. sylvanidis* females feed on their host in order to enhance egg production and subsequently oviposition. To reduce host feeding and increase oviposition, an energy and protein rich diet was provided containing water (1 ml), honey (0.1 g), pollen (7 pieces), and royal jelly (0.05 g) on a 1×1 cm filter paper.

2.2. Host finding

Experiments were performed with 1–3 days old, inexperienced, starved and mated females, which were kept individually in an Eppendorf vial until the start of the observation. According to Vet et al. (1990) we defined inexperienced parasitoid females as those, which had no previous experience with hosts beyond that occurring during the development. For mating, a male (1-day old) was introduced into the vial containing a female and left there for 24 h. Females were randomly assigned to the experiments and after each test they were returned to the stock cultures. Each female was used in the experiments only once.

Each set of experiments was replicated 30 times. The experiments were conducted in a static four-chamber olfactometer (Fig. 1; Steidle and Schöller, 1997). It consists of an opaque Plexiglas® cylinder (height 4 cm, diameter 19 cm) divided by vertical plates into four chambers. On the top of the cylinder, a walking arena (height 1 cm, diameter 19 cm) consisting of plastic gauze (mesh 0.2 mm) with a rim of heat resistant Plexiglas® (0.9 cm high) was placed and covered with a glass plate. For each test a Petri dish (5.5×1.5 cm) containing the odour sample was placed beneath the walking arena in one chamber, and the opposite chamber either contained an empty Petri dish or a Petri dish with another odour sample. The other two chambers remained empty as transition zones. The experiments were conducted in a dark room and the olfactometer was illuminated from above. At the beginning of each experiment, a wasp was placed in the centre of the walking arena. For a period of 600 s, its behaviour (walking, resting) and the position of the wasp were registered using the computer software “The Observer 5.0” (Noldus, Wageningen, The Netherlands). The olfactometer was rotated after each observation.

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) ($n = 5$).

2.3. Host acceptance

Host acceptance on fourth instar larvae of *T. castaneum* and *T. confusum* was studied in no-choice bioassays. Single females of *H. sylvanidis* were kept in a 12 cm Petri dish containing a piece of undulated cardboard together with one male. Fifteen fourth-instar larvae of either *T. castaneum* or *T. confusum* were supplied to each female weekly. After one week, pairs were transferred to a new

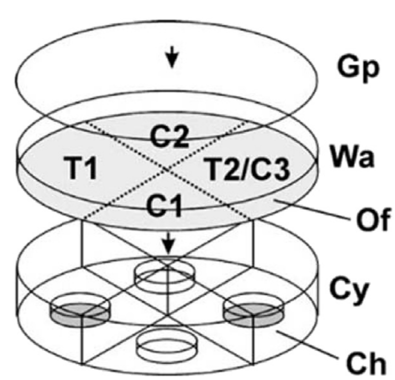

Fig. 1. Olfactometer for testing the response of wasps towards different odour sources. Height 5 cm, diameter 19 cm. Abbreviations: Gp-glass plate; Wa-walking arena, Of-odour fields; Cy-cylinder; Ch-chambers, T1 test field above odour sample, C1 and C2; transition zones above empty Petri dishes; T2/C3: field opposite of T1 with odour sample in Petri-dish, or with empty dish (Steidle and Schöller, 1997).