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Host-associated kairomones used for habitat orientation in the parasitoid *Lariophagus distinguendus* (Hymenoptera: Pteromalidae)

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Abstract

Males and females of the parasitic wasp *Lariophagus distinguendus* respond to volatiles emitted by the larval faeces of one of their hosts, the granary weevil *Sitophilus granarius*. Previous studies have shown that attractive chemicals are emitted by astigmatid mites living in the host faeces and that these cues are attractive only to experienced parasitoids. In the present study we demonstrate that larval faeces of the host and headspace extracts of the faeces are attractive for both sexes of the parasitoid even when the mites were experimentally excluded from the beetle rearings. The response to volatiles from mite-free host faeces is innate. In order to elucidate the chemistry of this odor, headspace extracts were fractionated by adsorption chromatography. Tests using combinations of fractions of different polarities revealed that both the non-polar pentane and the polar methanol fractions were necessary to maintain the attractiveness. This indicates that the attractive odor is composed of a complex blend of components with different polarities. The composition of the polar fraction was analyzed by gas chromatography-mass spectrometry, whereas elucidation of the structure of non-polar components has not been possible so far. By orientating toward the same host-related volatiles used by females for host finding, *L. distinguendus* males may be arrested in patches of potentially high female density and thus increase their chance of mating.

Keywords: *Lariophagus distinguendus*; Mate finding; Host finding; *Sitophilus granarius*; Larval faeces; Kairomone

1. Introduction

Parasitic Hymenoptera use a variety of infochemicals for the location of food, mates, or oviposition sites (Godfray, 1994). For host finding and recognition, female parasitoids orientate toward volatile and non-volatile chemicals released by their host (e.g. cuticular hydrocarbons, pheromones) or its products (faeces, silk, exuviae), by the host's food plant (volatiles induced by feeding or oviposition), or by organisms associated with the host presence (bacteria, fungi) (Vet and Dicke, 1992; Quicke, 1997; Steidle and van Loon, 2002; Hilker and Meiners, 2006). The response of the parasitoids to these host-related kairomones can be determined by physiological and genetic parameters but may also be affected by environmental factors and particularly by experience (Vet et al., 1995). Numerous studies have demonstrated that parasitoids are able to associate profitable environmental cues, such as volatiles, with the presence of their hosts (Turlings et al., 1993; Vet et al., 1995).

Long-range orientation during mate finding in parasitic wasps is supposed to be mediated mainly by pheromones (Godfray, 1994; Quicke, 1997; Kainoh, 1999). Female-derived sex attractants of high volatility have been shown to attract males over long distances (e.g., Swedenborg et al., 1994; McNeil and Brodeur, 1995; Jewett and Carpenter, 1999). However, in several parasitoid species females appear to produce only relatively low-volatile pheromones that stimulate male courtship behavior but do not mediate long-range attraction (e.g., Finidori-Logli et al., 1996; Ruther et al., 2000; Steiner et al., 2006). In these species, males have to rely on infochemicals other than pheromones for long-range orientation toward females. One option for these males to reach the vicinity of females is to use the same host-associated volatiles that

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the females use for host finding. However, this subject has been widely neglected in the literature so far. One of the very few parasitoid species in which a male response to host-associated volatiles has been demonstrated is *Lariophagus distinguendus* (Förster) (Hymenoptera, Pteromalidae), a polyphagous larval and pupal ectoparasitoid of stored-product-infesting beetles (Steidle and Schöller, 1997). Males and females of this parasitoid use volatiles from the larval faeces of one of its hosts, the granary weevil *Sitophilus granarius* L., for host and mate finding (Steidle and Schöller, 1997; Ruther and Steidle, 2000). The response by the parasitoids is associatively learned (Ruther and Steidle, 2000, Steidle et al., 2003). Furthermore, all active compounds do not originate directly from the host faeces but some originate from the host-associated astigmatid mite *Tyrophagus putrescentiae* (Schrank) living in the faeces (Ruther and Steidle, 2000). These astigmatid mites can be especially abundant in so-called hot-spots, sites of intense primary infestation by beetle pests (Sinha, 1961).

However, hosts of *L. distinguendus* also occur in areas of lower infestation where moisture-sensitive mites are much less abundant. Field observations demonstrated that hosts are located and parasitized by *L. distinguendus* under these conditions as well (Steidle, personal observation). Thus, the question arises—which kairomones are used by *L. distinguendus* for host and mate finding when no mites are present?

The present paper aims to investigate kairomones for host- and mate-finding in *L. distinguendus* under conditions of low host infestation in the absence of host-associated mites. Therefore, we studied whether *L. distinguendus* males and females are attracted innately to larval faeces of the host *S. granarius* from cultures in which secondary infestation by astigmatid mites had been experimentally prevented. To characterize the chemical composition of potential kairomones, fractionated headspace extracts from larval host faeces were monitored for bioactivity and active fractions were analyzed by coupled gas chromatography-mass spectrometry (GC-MS).

2. Materials and methods

2.1. Insect rearing

To establish a mite-free *S. granarius* culture, batches of weevils were washed under warm water to remove any astigmatid mites present and dried with a hair dryer. Cultures of hosts and parasitoids were kept in the laboratory at a constant temperature of 25 °C, a relative humidity of 55 ± 5%, and a photoperiod of 16:8 h (L:D). Under these rearing conditions secondary infestation by moisture-sensitive mites is prevented. Nevertheless, weevil cultures were regularly examined for the presence of mites both optically with a stereomicroscope and chemically by GC-MS analyses of headspace extracts from larval faeces (see below). Only larval faeces that did not contain typical mite volatiles such as neral, geranial, neryl formate, and tridecane (Kuwahara, 1991; Ruther and Steidle, 2000) were used for behavioral experiments and chemical analyses.

To obtain host larvae of known age, 100 ml of adult *S. granarius* were kept on 1000 ml wheat grain (*Triticum aestivum* L., var. Batis) in plastic containers (19 cm width × 19 cm length × 6 cm height). Weevils were allowed to lay eggs in the grains for 7 days and were then removed. To rear *L. distinguendus*, approximately 100 freshly emerged parasitoids were placed in Petri dishes (9 cm ∅ × 1 cm height) with 60 ml weevil-infested grains containing 21- to 28-day-old host larvae and kept there until their death. This host age is known to be optimal for parasitization by *L. distinguendus* (van den Assem, 1971). Parasitoids emerged 18–21 days after host larvae had been parasitized.

2.2. Insects for bioassays

Only males and females without any mating or oviposition experience were used in the experiments. To obtain naïve individuals, parasitized grains containing parasitoids shortly before emergence were kept individually in 1.5 ml microcentrifuge tubes. After emergence, parasitoids were held in single-sex groups of at most 15 individuals in Petri dishes lined with moistened filter paper. One hour before experiments, parasitoids were individually placed in microcentrifuge tubes for acclimation at room temperature. Individuals tested were 1–2 days old.

2.3. Static four-chamber olfactometer

The behavioral response of inexperienced *L. distinguendus* to potential volatile attractants in the larval host faeces was investigated by using a static four-chamber olfactometer as described by Ruther and Steidle (2000). No airflow was generated. In one chamber, the odor sample was placed in a small Petri dish (5.5 cm diameter). The opposite chamber was used as the control chamber and the remaining two chambers adjacent to the test chamber were considered as buffer zones. The olfactometer was covered with a walking arena (19 cm ∅ × 1 cm height) made of plastic gauze (mesh 0.5 mm) and finally with a glass plate to prevent parasitoids from escaping. At the start of each bioassay, a single parasitic wasp was released into the arena and the time it spent within each of the four sectors, above the chambers (arrestment time) was recorded for 10 min by using Observer program 3.0 (Noldus, Wageningen, The Netherlands). Parasitoids that walked for less than 50% of the total observation time were not included in the statistical analysis. The olfactometer was rotated clockwise by 90° after each replicate to prevent biased results due to possible side preferences of the parasitoids. The walking arena and glass plate were regularly cleaned with ethanol and demineralized water. Odor sources were changed after five individuals had been tested.