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The role of contact chemoreception in the host location process of an egg parasitoid

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A B S T R A C T

Taste allows insects to detect palatable or toxic foods, identify a mate, and select appropriate oviposition sites. The gustatory system strongly contributes to the survival and reproductive success of many species, yet it is rarely studied in insect parasitoids. In order to locate and assess a host in which they will lay their eggs, female wasps actively search for chemical cues using their sensory organs present mainly on the antennae. In this paper, we studied the role of antennal taste sensilla chaetica in the perception of contact semiochemicals in *Trissolcus brochymenae* (Hymenoptera: Platygastridae), an egg parasitoid of the brassicaceae pest *Murgantia histrionica* (Heteroptera: Pentatomidae). Methanolic extracts obtained from male and female hosts elicited action potentials in taste neurons housed in antennal sensilla chaetica, indicating that these sensilla are involved in the perception of non volatile host kairomones. In behavioural assays, wasp females displayed an intense searching behaviour in open arenas treated with host extracts, thus confirming that these kairomones are soluble in polar solvents. We further investigated the extracts by Gas Chromatography-Mass Spectrometry (GC-MS) and found that they contain several compounds which are good candidates for these contact kairomones. This study contributes to better understanding contact chemoreception in egg parasitoids and identifying gustatory receptor neurons involved in the host location process.

1. Introduction

Hymenopteran parasitic wasps are known for their importance in successfully regulating the population density of a wide range of economic pests (Gauld and Bolton, 1988; Godfray, 1994; van Driesche et al., 2008). They locate suitable hosts through a hierarchical process which can be divided in the following steps: selection of preferred habitat, localization of host community, individual host localization, host recognition and acceptance (Vinson, 1998). Searching wasps can exploit a variety of stimuli while foraging for hosts, but chemical cues, called infochemicals or semiochemicals, appear to play a major role. Parasitoids mainly exploit volatile semiochemicals in the initial steps of the host location process whereas contact semiochemicals become progressively more important when parasitoids are in close proximity to the host. Most of the studies on chemical ecology of insect parasitoids have focused on perception of volatiles (olfaction) (Dougherty et al., 1992; Godfray, 1994; Guillot and Vinson, 1972; Gullan and Cranston, 1994; Harrisson et al., 1985; Höller et al., 1993; Kaissling, 1971, 1987; Prokopy, 1981; Quicke, 1997; Salerno et al., 2012; Vinson, 1985). On the contrary, relatively few studies have assessed the role of contact chemoreception (gustation or taste) in host location and/or recognition in various parasitoid species (Bénédet et al., 1999, 2002; Colazza et al., 2014; Salerno et al., 2009; van Lenteren et al., 2007; Vinson, 1991). In particular, gustatory perception of contact semiochemicals is still

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a neglected research area in egg parasitoid species. One reason for this could be the minute size of such insects which technically hampers the use of sophisticated electrophysiological techniques to investigate contact chemoreception (van Baaren et al., 2007).

Egg parasitoids target a specific host stage which develops rapidly and whose quality decreases over time (Vinson, 2010). Therefore, egg parasitoids have developed specialized strategies for a successful parasitic attack, based on the combined exploitation of chemical cues which are directly or indirectly related to host eggs (Colazza et al., 2010; Fatouros et al., 2008; Vinson, 1998). Indirect host related contact cues originating from host adults or juveniles in general elicit an arrestment response in egg parasitoids, which is characterized by an intense searching behaviour in the host contaminated areas (Conti and Colazza, 2012). In Trichogrammatidae and Platygastridae (=Scelionidae) families, the main indirect host related cues exploited by females are kairomones associated with the egg laying host, such as scales left on the substrate by lepidopteran females, or chemical footprints left behind by heteropteran hosts while walking on the substrate (Borges et al., 2003; Colazza et al., 1999, 2010; Conti et al., 2003, 2004; Salerno et al., 2006).

To exploit semiochemicals, parasitoid wasps use sensory organs present mainly on their antennae where chemosensory (olfactory and taste) sensilla have been described (Quicke, 1997). A taste sensillum is generally uniporous and usually contains several chemosensory cells and one mechanosensory cell. In egg parasitoids associated with heteropteran hosts, four types of gustatory sensilla have been described: sensilla chaetica, grooved pegs, papillary sensilla and sensilla conica (Bin et al., 1989). Among these types, uniporous gustatory sensilla chaetica are particularly suited to electrophysiological recordings (Iacovone et al., 2015). The sensilla chaetica are almost perpendicular to the ventral side of the antennal surface, and can be easily identified by their external morphology (Bin et al., 1989; Isidoro et al., 1996; Romani et al., 2010). Furthermore, it has been suggested that these sensilla, which are more rigid than the others, touch the substrate first, and therefore may detect information directly from the host (Bin, 1981). A combination of ultrastructural and behavioural investigations, carried out on several parasitoid families including Platygastridae (=Scelionidae), have also supported this hypothesis (Bin et al., 1989; Isidoro et al., 1996; Romani et al., 2010).

In this paper we evaluate the role of sensilla chaetica in the perception of contact kairomones that trigger arrestment behaviour in *Trissolcus brochymenae* Ashmead (Hymenoptera: Platygastridae). This specialist egg parasitoid is a potential biological control agent of the harlequin bug, *Murgantia histrionica* Hahn (Heteroptera: Pentatomidae) (Conti et al., 2003, 2004, 2010; Frati et al., 2013) which is a pest of cabbage species and other brassicaceous crops (McPherson and McPherson, 2000). The arrestment response behaviour of *T. brochymenae* wasps to areas contaminated with chemical footprints of *M. histrionica* is characterized by an initial prolonged motionless period with the antennae kept in contact with the surface. Hexane extracts from virgin and mated adults of *M. histrionica* also induce an arrestment response, enabling *T. brochymenae* wasps to discriminate between host genders as well as to assess the physiological condition of the host female (Salerno et al., 2009). Under the microscope it is possible to observe that the tip and the ventral side of the antennal surface are the first to touch the host contaminated substrate (personal observation). After the arrestment, the wasps start to walk slowly while drumming the surface with the antennae adopting a walking pattern characterized by a klinotactic response, namely a variation in orthokinetic and klinotaxic locomotion activity, so that the resulting tortuous path keeps the wasps in the contaminated areas (Colazza et al., 2007). In this work, we focused on sensilla chaetica of the antennae which are erected and likely to contact the substrate first. These sensilla, similarly to *Trissolcus basalis* (Bin et al., 1989), are located on antennomeres 6–11. Specifically, a group of three sensilla is present on antennomere 6; groups of four sensilla are present on antennomeres 7–10. Finally, eight sensilla are irregularly distributed on the antennomere 11 (Roberto Romani unpublished data).

The chemical identity of the host kairomones exploited by *T. brochymenae* is not known, but investigations based on behavioural activity of hexane extracts from dissected body parts of *M. histrionica* suggest that the host cuticle is probably the kairomonal source (Salerno et al., 2009). Here, using a combination of electrophysiology and behavioural assays, we investigated the sensitivity of *T. brochymenae* sensilla chaetica to *M. histrionica* host extracts. To better understand the chemical ecology of the host parasitoid interaction, particularly the nature of host kairomones that elicited a response in the egg parasitoid, body extracts of *M. histrionica* were purified and a chemical analysis of the most active fraction was carried out by Gas Chromatography-Mass Spectrometry (GC-MS).

2. Materials and methods

2.1. Insects

T. brochymenae originated from a laboratory strain collected near San Diego (CA) from *M. histrionica* eggs which were laid on *Isomeris arborea* Nutt. (Capparidaceae). The parasitoids were reared in a growth chamber (25 ± 1 °C, 60 ± 5% RH, L16:D8) on eggs of *M. histrionica*, maintained in 85 mL glass tubes and fed with small drops of Safavi sugar-water diet (Safavi, 1968). Harlequin bugs were originally collected from cabbage (*Brassica oleracea*) in the Beltsville (MD) area, and were reared as described by Conti et al. (2004). Both colonies were maintained in quarantine conditions in the Entomology laboratories of the University of Perugia (Italy).

2.2. Semiochemical extraction

To evaluate the behavioural and electrophysiological responses of *T. brochymenae* females to the host extract, mated females and males of *M. histrionica* were first frozen at −18 °C for about 30 min. From these insects, we collected under a binocular stereo microscope (Leica DMLB, Italy) the legs (clipped off at the coxa level), the wings, the pronotum and the scutellum. The inside of the pronotal cuticle was cleaned as thoroughly as possible to remove traces of adhering tissues. The extraction from dissected body parts rather than from the entire body had been chosen to avoid potential contamination by compounds derived from metathoracic glands (Mattiacci et al., 1993). For electrophysiological purposes, we needed to find the most suitable solvent for the elution of biologically active molecules comprising the host extract. The solvent had to be non neurotoxic, current conducting and polar. For both sexes, dissected body parts from *M. histrionica* adults were placed together in 15 mL glass vials and extracted with methanol at 30 °C for 24 h (Fig. 1A). For behavioural assays, body parts from 3 adults were extracted in 10 mL of solvent, whereas for electrophysiological experiments, we immersed the body parts of 45 individuals in 15 mL of methanol.

After removal of the body parts, the extract was evaporated under a gentle nitrogen stream and dissolved in water, 5% methanol in water (v/v), or 1% methanol in water (v/v) in order to reach the concentration of 3 DAE (dissected adult equivalent)/mL (Fig. 1B). Extracts so diluted were then behaviourally analysed for their biological activity. Preliminary electrophysiological tests using solutions in 5% methanol led us to hypothesize that at this concentration this solvent can negatively affect the response of gustatory receptor