

Chemical information from the fungus *Amylostereum areolatum* and host-foraging behaviour in the parasitoid *Ibalia leucospoides*

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Abstract. Parasitoids locate hosts using reliable and predictable cues such as smells derived from host plants or from the hosts themselves. For host species that live with mutualistic organisms, such as several wood boring insects, cues derived from the symbionts are likely to be exploited by specific parasitoids. Through a set of bioassays, the behaviour of the parasitoid *Ibalia leucospoides* Hochenwarth (Hymenoptera: Ibalidae) is studied in response to the fungus *Amylostereum areolatum* Boidin (Basidiomycotina: Corticiaceae), a symbiont of its host, the wood wasp *Sirex noctilio* Fabricius (Hymenoptera: Siricidae). The results show that parasitoids are attracted to the fungus when growing naturally within pine logs, and also when growing on an artificial medium. Fungal volatiles also elicit increased parasitoid activity and may provide information on relative densities of hosts available for parasitization. It is speculated that the chemical information derived from the host fungal symbiont comprises reliable and detectable host-locating cues used by parasitoids to search for concealed hosts.

Key words. *Amylostereum areolatum*, host location, *Ibalia leucospoides*, mutualism, parasitoid, *Sirex noctilio*, symbiosis.

Introduction

Host location in insect parasitoids is central to their reproductive success. This is because parasitoids typically oviposit in or on individuals of their host species and feed on them during their larval stages (Godfray, 1994). The mechanisms leading towards successful host finding are thus the subject of strong selective pressures, as are those in the host species, which tend to reduce the chances of being found (Quicke, 1997).

Parasitoids rely on a variety of cues to locate their hosts, but chemical information is paramount (Vinson, 1981). Some parasitoid species may use host derived olfactory cues, including cues such as excreta and pheromones, and/or infochemicals derived from host plants (Jacqueline *et al.*, 1994; Storeck *et al.*, 2000; Douloupaka & van Emden, 2003; Buitenhuis *et al.*, 2004; Lo Pinto *et al.*, 2004). They may also

make use of volatiles from organisms associated with the presence of the host (Madden, 1968; Spradberry, 1974; Pettersson, 2001). Generally, parasitoids have evolved to use chemical cues that are both reliable (i.e. offer some certainty of the host presence and possibly host-patch quality) and detectable (i.e. may be detected over long distances by the sensory capabilities that the parasitoids possess). This may be regarded as a reliability–detectability problem (Vet & Dicke, 1992) because it is known that both characteristics are correlated negatively, precisely because of selection acting on the host species (Powell *et al.*, 1998).

The interaction between *Ibalia leucospoides* Hochenwarth (Hymenoptera: Ibalidae) and *Sirex noctilio* Fabricius (Hymenoptera: Siricidae) shows how this parasitoid uses what appear to be both reliable and detectable chemical cues. The wood wasp *S. noctilio*, lays eggs inside tree trunks and its larvae feed on wood decomposing under attack by a symbiotic fungus, *Amylostereum areolatum* Boidin (Basidiomycotina: Corticiaceae). Female wood wasps carry fungal spores in specific sacs (mycangia) and lay them together with the eggs, deep inside the wood. The parasitoid, introduces its ovipositor

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through the host oviposition hole and injects one egg into the host egg or first-instar larva. Madden (1968) and later Spradberry (1974) suggested that the parasitoids found *S. noctilio*'s eggs and larvae by chemical cues derived from fungal growth. Acetaldehyde, a chemical component produced by 3-week-old fungi, in coincidence with the presence of hosts available for oviposition, elicits typical host-searching antennal movements (Madden, 1974, 1981). It has been suggested also that *I. leucospoides* finds potentially host-rich pine trees responding to α and β -terpenes produced by trees attacked by wood wasps (Madden, 1988).

In the present study, the fungus–parasitoid interaction is examined in further detail, by focusing on the parasitoid behaviour elicited by *A. areolatum* derived allelochemicals, at a small spatial scale. Interest lies in whether fungal growth enhances parasitoid activity when combined with pinewood, induces orientation and provides information on host-patch quality. The working hypothesis is that the host mutualist should be used intensively by the parasitoid for host detection, location and patch-quality classification because it provides a reliable and detectable information source of the presence of its concealed host species.

Materials and methods

Parasitoid rearing

The parasitoids used in all the experiments were obtained in the proximities of the city of Bariloche, in North-West Patagonia, Argentina. Pine trees (*Pinus contorta* var. *latifolia*) attacked by *S. noctilio*, and subsequently by the parasitoid, were cut-down and kept under ambient conditions in locker-type cages with natural lighting. All insects were collected from the cages every day during their flight season. The parasitoids were allowed to mate and feed on a sugar/water solution (30% w/w) for 24 h before the experiments.

Fungus growth

To obtain the fungus cultures that would later serve in the experiments, *A. areolatum* spores were extracted from *Sirex noctilio* mycangia (Thomsen, 1996). These spores were grown in a culture medium based in agar–agar, malt, yeast and pine extract (Martínez & Fernández-Arhex, unpublished data). The fungus was left to grow naturally for 2–3 weeks inside a dark cabinet at room temperature (17–25 °C) before use in the experiments. The mycelium used in the bioassays was cut from the Petri dishes and comparisons were carried out simultaneously to prevent errors of pseudoreplication.

Experimental procedures

In an initial experiment, parasitoid behaviour was assessed in patches containing fungal volatiles only inside a rectangular

Perspex box arena (length 14 cm; width 7.5 cm; height 7 cm) covered with a lid. Inside, two 170-mm² diameter discs, one with the fungal culture and the other with growth medium only, were placed one in the centre on either extreme of the longer side of the arena. A single female parasitoid was placed individually inside a small Perspex vial that was carefully inverted in the centre of the arena and left to adapt for 5 min before removing the vial. Each experiment lasted for 5 min and the response variable measured was the first disc touched by the parasitoid. In total, 30 replicates (an individual parasitoid each time) were made. Experiments were carried out at room temperature (17–25 °C) in natural lighting and the arena was rotated to avoid visual asymmetries. Data were analysed by means of the chi-square test.

In a second experiment, naïve female parasitoids were exposed to freshly cut pine logs (*Pinus contorta* var. *latifolia*), 0.3 m in length, containing freshly cultivated fungal hyphae and clean logs (i.e. without fungus). The fungus was introduced into noninfected logs via holes (2 mm in diameter and 3 cm deep) made into the wood with a drill, similar to *S. noctilio* oviposition. The mycelium was taken from the artificial culture, inoculated into the holes and then allowed to grow for 2 weeks in cages set outside to ensure no contact with *S. noctilio*. The control logs had holes drilled but no mycelium inoculated. Both logs (treatment and control) were placed within a 1-m³ large wire mesh cage, totally covered in brown paper to make the cage dark, with the exception of a slit on the top that allowed the light of a fluorescent tube (20 W, length 57 cm) to illuminate the arena and a slit on one of the sides for the observer to watch. Individual female parasitoids were released from Perspex vials at the vertex of an imaginary triangle (30 cm on each side) of which the logs were standing on the other two vertices. Each replicate lasted 30 min and the log reached either by walk or by flight was recorded. Thirteen replicates were made in total. After each trial, logs were changed and placed in varying arrangements within the triangle. Data were analysed using the binomial test.

In a third experiment the modulation of parasitoid activity in the presence of *A. areolatum* volatiles was tested. The experimental arena was the same Perspex box as in the previous experiment, but this time a single 170-mm² diameter disc of culture medium was placed in the centre of the arena with or without growth of 2-week-old *A. areolatum* mycelium. Then, the bottom of the arena was covered with a wire mesh (0.2 mm) that allowed the volatiles to move freely and the insect to walk over the disc without touching the source. To quantify parasitoid activity, lines were drawn on the floor of the arena forming a grid of nine equally-sized rectangles. A single female parasitoid was placed inside a small Perspex vial that was carefully inverted in the centre of the arena and left to adapt for 5 min before starting the experiment (i.e. vial removal). A total of 40 replicates were carried out, and the response variable measured was the number of grid-lines crossed by the parasitoid during 3 min. Experiments were carried out at ambient temperature (17–25 °C) and natural midday lighting. Resulting data were compared by means of the *t*-test.

In a fourth experiment, the preference of *I. leucospoides* females was tested towards two different concentrations of the chemical volatiles of *A. areolatum*. We used a glass Y-tube olfactometer (base 13 cm, arms 7 cm, diameter 2 cm), which was connected to an air pump creating an air flow of 1 cm s^{-1} . Before the air entered the system, it was filtered through activated charcoal and then re-humidified by passing through damp cotton wool. The chemical stimuli containers consisted of Perspex boxes ($10 \times 10 \times 10 \text{ cm}$) attached to the end of the arms of the olfactometer. To avoid visual asymmetries, the device was placed inside a white Perspex box ($50 \times 50 \times 25 \text{ cm}$), covered by a red, see-through detachable Perspex cover.

A single parasitoid female was introduced into the base of the olfactometer and given 1 min to adapt and a maximum of 15 min to choose between the arms. A choice was designated when the female passed an imaginary line set 5 cm into each of the arms. After each replicate, the olfactometer was washed and ventilated to avoid pseudo-replication (Ramírez *et al.*, 2000). Furthermore, the olfactometer was rotated inside the Perspex box and the position of the odour sources was exchanged between arms to avoid potential asymmetries in the set up.

The bioassay consisted of three parts: (i) the response level of naïve females to a relatively low amount of *A. areolatum* volatiles; (ii) then to a relatively higher concentration of fungus; and (iii) the preference of females between these two concentrations. To obtain the different concentrations of volatiles in the arms of the Y-tube, the odour sources were small (7 mm^2) and large (170 mm^2) discs of the culture of *A. areolatum*, both aged 2 weeks. Thus, it was possible to obtain two different concentrations of chemical stimuli. To avoid the decrease of volatile concentration through time, the discs were replaced with new ones every hour. Responsiveness was measured as the percentage of females that chose the arm containing the fungal volatiles. Data were analysed using the binomial test.

Results

In the bioassay carried out in the small Perspex arena where attraction to fungal culture was tested at a short distance, 80% of the females preferred the source that had the fungus culture ($\chi^2 = 10.8$, d.f. = 29, $P < 0.05$) although, during the control series performed in the device with agar medium on both sides, insects chose randomly ($\chi^2 = 1.6$, d.f. = 14, $P > 0.05$).

Naïve females released in a cage with pine logs inoculated with fungus and the control significantly preferred to land on or to walk towards the one with the fungus growth (77%, $n = 13$, $P < 0.05$). Parasitoid activity, measured by the mean number of grid-lines crossed was 35 ± 7 lines in the presence of the fungi and 16 ± 5 lines without it. The presence of the fungal volatiles significantly increased the amount of lines crossed by the parasitoids ($t = -4.089$; d.f. = 38; $P < 0.05$).

During the test designed to establish the response towards a relatively low concentration of fungus carried by an air plume, significantly more females preferred the arm contain-

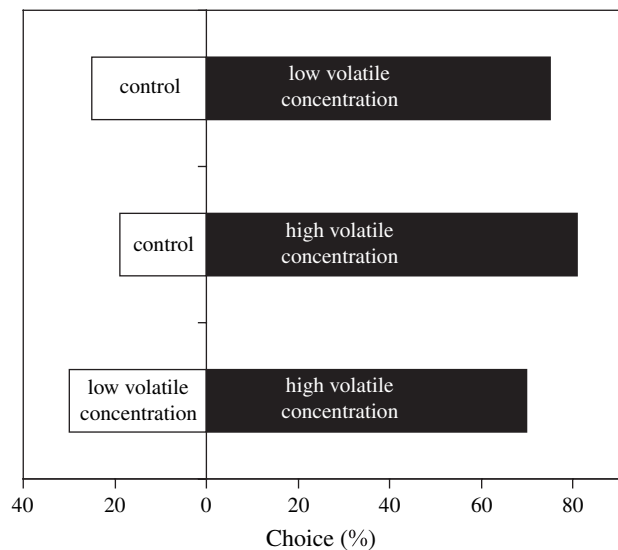


Fig. 1. Percent choice of parasitoids between arms of Y-tube olfactometer under three conditions: (i) small fungal culture discs ($n = 20$); (ii) large fungal culture discs ($n = 31$); and (iii) small vs. large fungal discs ($n = 30$).

ing fungus volatiles than the control (77%, $n = 20$, $P < 0.05$). Furthermore, when they were presented with a higher concentration of fungal volatiles, most of them chose the arm with fungus (75%, $n = 31$, $P < 0.05$). However, when parasitoids were tested towards either concentration simultaneously, they significantly chose the source containing the highest concentration (70%, $n = 30$, $P < 0.05$) (Fig. 1).

Discussion

The parasitoid *I. leucospoides* responds positively to the host mutualistic fungus *A. areolatum* when growing in a pine log and when growing on an artificial medium. Fungal volatiles also elicit increased activity in the parasitoid. *Amylostereum areolatum* may also provide information on relative densities of hosts available for parasitization.

The results from the present study suggest that the insect parasitoid *I. leucospoides* responds to volatile chemical compounds from *A. areolatum* during host foraging. First, naïve females are attracted towards fungal volatiles at a short distance. This behaviour is observed despite the absence of host or tree-derived chemical signals in the arena or olfactometer assays. In previous work, the role of the fungus in host location was assessed by watching parasitoids trying to oviposit in holes drilled by females in which no eggs were laid, but fungal spores were inoculated (Madden, 1968; Spradberry, 1974). Using this experimental procedure, there may still be on-site host marks that are used by the parasitoid in host location but, from the present study, it is clear that the parasitoid responds positively to the fungus free of host chemicals. As expected, a fungus rich environment elicits activity of

parasitoids and oviposition behaviour (V. Fernández-Arhex, personal observation).

Ibalia leucospoides prefers walking or flying towards logs that contain fungus, free from all other host infochemicals, rather than orientating towards clean logs. This finding suggests that parasitoids could detect fungus-derived volatiles in pine tree forests, and use them in host location. Because wood wasps disturb tree growth during their attack through a phytotoxic substance, it has been assumed that tree location by parasitoids would follow disturbed tree derived volatiles (Madden, 1981). It is plausible that this kind of volatiles are used by the parasitoids at a large distance, and fungus growing inside the wood of pine tree logs orientates parasitoids at shorter distances.

A noteworthy finding concerns the response of *I. leucospoides* to different volatile concentrations as generated by differently sized discs of fungus. Madden (1968) and Spradberry (1974) have shown that only 2–3 weeks of fungal growth attracts female parasitoids, which corresponds to the window for host parasitization. The present study shows that parasitoids can also detect differences in volatile concentrations that would relate to host abundance in the field. Although the fungus grows rapidly inside the wood, the higher concentration of 2–3-week-old fungi relates directly to a higher number of host oviposition drills on a tree, and hence to a higher number of potentially available hosts.

During their adult life, most parasitoids must interact in a multitrophic context and consequently base their foraging decisions on information coming from several trophic levels (Vet & Dicke, 1992; Powell & Poppy, 2001). Among the vast array of infochemicals that may be used by foraging parasitoids to reach their hosts, there are some that are more reliable, and others that are easier to detect. On the one hand, the most reliable infochemicals are those produced by the host but, as a consequence, released in very small quantities, and therefore are difficult to detect (Dicke, 1999). On the other hand, plant-derived stimuli are assumed to be easier to detect because of the much larger biomass of the plant compared with that of the host, but not as reliable because there is not always an appropriate host on attractive plants (Vet & Dicke, 1992). Although the symbiosis enhances reliability, the natural and independent growth of the mutualistic species promotes host detection. By contrast with host or plant-derived infochemicals, the use of volatiles derived from host mutualists provides both a reliable and detectable source of information. The wide use of fungus-derived chemical information for host location at several spatial scales and for patch quality evaluation by *I. leucospoides*, as suggested in the present study, provides such an example.

It is interesting to note that, among the Hymenoptera, the parasitoid habit may have arisen from ancestral mycetophagus species such as the *Siricidae* (Godfray, 1994; Quicke, 1997). Species unable to carry their own wood decomposing fungal mutualists used those of other species to feed on, and the shift from mycetophagy to feeding on the protein-rich larvae of the other species has probably led to parasitoidism. For ancestral parasitoids, fungus-derived allelochemicals may have played a natural role in locating the fungus. In parasitoids attacking present day *Siricidae*, such as

I. leucospoides, the chemical information derived from the fungal symbiont of its host may still comprise the most important host-locating cue. Further work on fungus-host–hymenopteran parasitoid interactions should enlighten our understanding of host location behaviour and evolution in these parasitic insects.

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