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Food and Host Searching Decisions Made by *Ibalia leucospoides* (Hymenoptera: Ibaliidae), a Parasitoid of *Sirex noctilio* (Hymenoptera:Siricidae)

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Introduction

During their adult life, parasitoids—insects that lay eggs in or on other arthropods eventually killing them- spend a significant proportion of their lifetime searching for hosts, this behavior will determine their future success, simply because fitness is closely related to host encounter (Godfray 1994). However, adult parasitoids are free living animals that in most cases need to acquire food for metabolic functions such as maintenance, survival and locomotion, as well as for reproduction. Feeding on sugar-rich products or else on host fluids (i.e. host-feeding) may imply benefits in terms of future fitness, by for instance increasing egg laying opportunities through increased egg production or longevity (Chapman 1998; Speight et al. 1999). In a biological control context, providing sugar sources in the field could in some conditions, increase the efficacy of parasitoids used as natural enemies of pests (Heimpel and Jervis 2005).

In the field, parasitoid foods for non-host feeding species, is found as floral and extra- floral nectar or else honeydew excretions (Heimpel et al. 1997). If food and hosts are found in different patches, a choice must be taken by foraging females which can have significant effects on parasitoid fitness and overall parasitization rates. Generally, when the choice favors feeding, oviposition opportunities may be missed, but chances for future egg laying may be increased, through an increase in longevity and fecundity (Lewis and Takasu, 1990). When foragers opt for laying eggs rather than to feed, there is an immediate gain in fitness, but one which may

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occur at the cost of future gains (Heimpel and Collier 1996; Jervis and Kidd 1996; Bernstein and Jervis 2008).

Like many other insects, parasitoids rely heavily on chemical cues throughout the foraging process (Lewis and Takasu 1990, Wyatt 2003). The response elicited by odors, depends strongly on several extrinsic factors such as temperature, moisture, light intensity, molecule emission rate, wind speed and direction, distance between the individual and the source of odors, and other odors present in the air (Vinson 1976; Turlings et al. 1993). Hence, for the entire host searching and location process to occur successfully, a hierarchy of behavioral stimuli and associated responses with the host or foods and its habitat takes place (Turlings et al. 1993; Siqueira and Farias 2003).

Decisions made between searching for hosts or food, are also influenced by several state dependent aspects. Through detailed models, Bernstein and Jervis (2008) analyzed parasitoid choice for food or hosts and presented a theoretical framework for decision making in synovigenic parasitoids. Their main findings indicate that females confronted with high food availability should postpone feeding until reserves drop below some threshold and that they should never forage for food, when food availability is low (see also Tenhumberg et al. 2006). A key consideration from their models is that the distance between patches (i.e. travel distance) and also egg load can have important effects on parasitoid decisions.

Recent work has explored the role of food acquisition by the *I. leucospoides* on flight potential and host patch exploitation rules (Corley et al. 2010; Fischbein et al. 2011). This is because the species is a parasitoid of the woodwasp *Sirex noctilio* Fabricius, a major pest of pine worldwide (Carnegie et al. 2006) and in pines forestations food may be limiting parasitization rates. Here, we explored through a set of olfactometer assays, the behavioral response of female parasitoids to food or host cues and the influence recent feeding has on these decisions. Our aim is to further our understanding on parasitoid decision making, by exploring the behaviour of a species that emerges with a high ovigeny index (Fischbein 2011; ovigeny index, is defined as the proportion of the potential lifetime complement of eggs that is mature upon female emergence, Jervis and Ferns 2004). Additionally, we asked whether such response varied according to the feeding status of tested individuals. This adds information on the role food acquisition during rearing and housing may have on the initial decision making process displayed by females upon release.

Materials and Methods

Biology of *Ibalia leucospoides*

The parasitoid is a large cynipid wasp (16–17 mm long) that attacks eggs and larvae of the pestiferous woodwasp, *S. noctilio*. Since the introduction of *I. leucospoides* as a bio-control agent of woodwasps into Australia in the sixties, the parasitoid has become established throughout the invasion range of its host, mostly through subsequent accidental introductions (Madden 1988). Parasitism rates achieved by this species in some regions do not exceed 40% and the role of this species in controlling woodwasp populations is probably limited by the difficulties in finding their hosts (Fernández-Arhex and Corley 2005; Fischbein 2011). Females of *I. leucospoides* oviposit inside the eggs and 1st instar larvae of woodwasps, which are burrowed deep inside the xylem tissue of pines. It is well known that *I. leucospoides* uses chemical cues, derived from a fungal symbiont of *S. noctilio*, to locate host eggs and larvae within a tree (Madden 1968; Spradbery 1974; Martínez et al. 2006). Adults emerge, usually a year later, with a high number of mature eggs (~500, Fischbein 2011). *I. leucospoides* adults may feed avidly on diluted honey in captivity, although it remains unknown whether they feed in the field.

Parasitoid Rearing and Fungus Culturing

Parasitoids used in the experiments were obtained from logs collected in plantations located in the proximities of the city of Bariloche (Patagonia, Argentina) during the summers of 2005 to 2010. Pine trees (*Pinus contorta* var. latifolia) that showed signs of attack by woodwasps, were felled, cut into 1 m-long billets and housed at ambient conditions, in locker-type cages. Attacked trees are easily detected in the field, especially in heavily infested sites. All insects (hosts and parasitoids) were collected from the cages every day during their flight season. Spores of the symbiotic fungus *Amylostereum areolatum* were extracted from female woodwasp mycangia (Thomsen and Harding 2010), and were left to grow naturally in a culture medium based in agar agar, malt, yeast and pine extract (Martínez et al. 2006). Cultures were maintained during 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 excised from the culture dishes into 170 mm discs. The fungus culture was used as a chemical signal of possible oviposition sites to *I. leucospoides* (Martínez et al. 2006).

Experimental Procedures

We used a total of 248 female parasitoids. All insects used in experiments had emerged during the 48 h prior to experiments. One-hundred and twenty individuals were fed during 8 days on a honey solution (30% *v/v*, 3 mL; the “feeding treatment”). Whereas 128 individuals, were housed for 8 days with access to water only (the “unfeeding treatment”). The choice by *I. leucospoides* females was tested using 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 wick of cotton wool. The stimuli containers consisted in Perspex boxes (10 × 10 × 10 cm) attached to the end of the arms of the olfactometer. To avoid visual asymmetries, the device was placed inside a large white Perspex box (50 × 50 × 25 cm) covered by a red, see-through detachable Perspex cover.

The experiments consisted in introducing a single female into the base of the olfactometer and given short period to adapt (1 min), released and allowed for a maximum of 15 min to choose between the labyrinth arms. A choice was considered to be made, when the animal passed arbitrarily a line set 5 cm into each arm. The females which occasionally turned back were eliminated from further analyses. After each assay, the olfactometer was washed, dried and ventilated to avoid pseudo replication (Ramirez et al. 2000). Furthermore, the device was rotated inside the Perspex box and the position of the odor sources was exchanged between arms to avoid potential asymmetries in the set-up. All experiments were carried out at room temperature (21±0.1°C) and mean humidity (54±5%) in natural lighting conditions.

We carried out 3 different experiments to study the response levels of either treatment to: (a) fungus (culture fungus disc of 170 mm^2) against a control (no stimuli); (b) food (3 mL of honey solution, 30% *v*/*v*) against a control; and (c) a choice between food or fungus stimulus. The stimulus "fungus" is used as a proxy of host presence. This is because it is known that volatile infochemicals elicited by fungal growth are used by the parasitoid locate and even quantify host larvae (Martinez et al. 2006). To avoid volatile concentration decrease through time, the stimuli were replaced with new ones every assay. Responsiveness was measured as the percentage of females that chose a given arm.

Data Analysis

To determine the influence of the *feeding status* on *decisions* we used a generalized linear model assuming a binomial distribution of residuals. We also included the variable *experimental year* in the analysis, due to the fact that experiments were performed in different years. The data were fitted to the following model "*chosen source=feeding treatment (either fed or unfed) * experimental year*". All data analyses were carried out using the R statistical environment (R Development Core Team 2008).

Results

The response rate of parasitoids to the assays was generally high. Eighty percent of the individuals (fed $n=103$, starved $n=95$) made a choice towards an odor source. We found that *I. leucospoides* females responded similarly in different experimental years allowing us to remove this variable from our model. Instead, we noted that the nutritional state of the experimental animals with both stimuli offered, influenced their choice.

The results of the first experiment (*Fungus vs Control),* showed significant differences between fed and starved parasitoids (GLM; $\chi^2=4.15$, df=1, $p=0.042$, $n=71$, Fig. 1). In turn, while fed individuals showed no preference between fungus

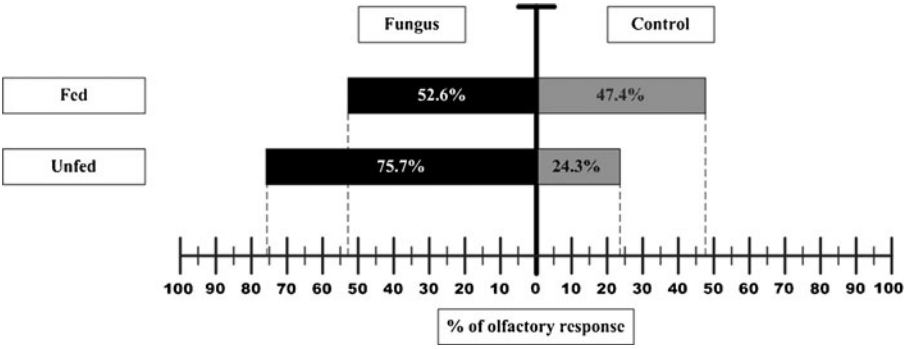

Fig. 1 Percent response of *I. leucospoides* females (fed and unfed) to a choice of fungus stimuli *vs*. control (no stimuli). Unfed parasitoid ($n=33$) significantly chose the fungus, whereas fed parasitoids ($n=38$) did not have any preference (GLM; $\chi^2=4.15$, df=1, $p=0.042$)

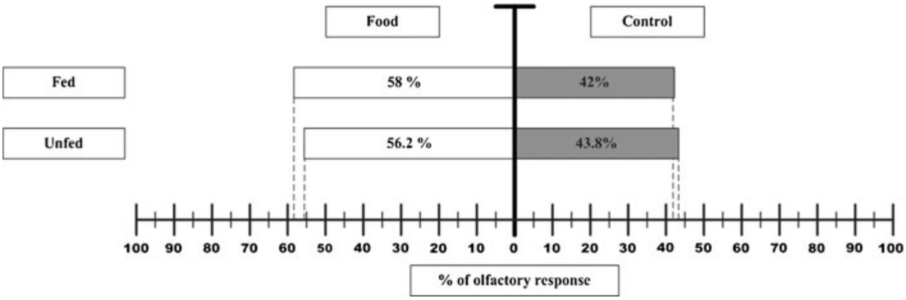

Fig. 2 Percent response of *I. leucospoides* females (fed and unfed) to a choice of food stimuli *vs.* control. None of the parasitoids tested showed preferences to food (GLM; χ^2=0.021, p=0.88; unfed n=32, fed n=31)

and control stimuli (n=38), unfed parasitoids significantly preferred the fungus over the honey (n=33).

The second experiment (*Food vs Control)* did not show any significant differences between treatments (GLM; χ^2=0.021, df=1, p=0.88, n=63). Individuals belonging to either group, did not display any clear preferences (fed n=31, unfed n=32, Fig. 2).

Finally, the result of the third experiment (*Food vs Fungus)* did not show any significant differences between unfed and fed individuals (GLM; χ^2=0. 55, df=1, p=0.45, n=64). Still, individuals of both treatments significantly preferred the fungus odor source (fed n=34, unfed n=30, Fig. 3).

Discussion

We studied under laboratory conditions, the olfactory response of *I. leucospoides* to cues associated with food- and host-foraging. The choice should reflect decision making of female parasitoids in the field and could potentially contribute to their rearing and release protocols. Our main findings suggest that the responses of these parasitoids are state-dependent. Food deprived females favored the fungus containing stimuli suggesting an urgency in egg discharge. In contrast, fed individuals did not show any clear preference throughout the assays; satiated females only displayed

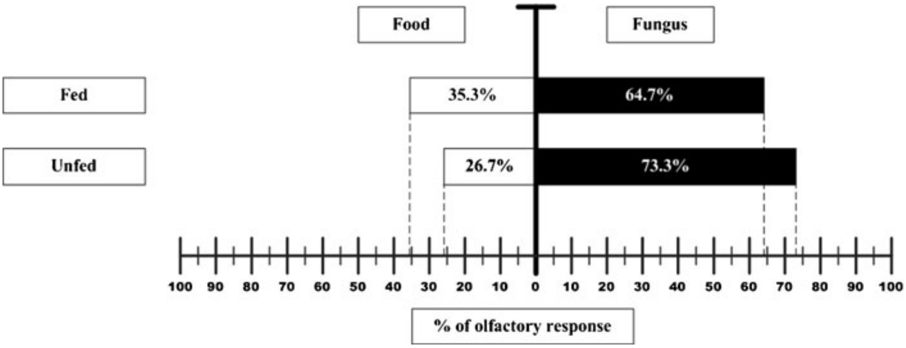

Fig. 3 Percent response to a choice between fungus (host) *vs.* food. None of the parasitoids evaluated showed any significant differences between the stimulus tested (GLM; χ^2=0.55, p=0.45; fed n=34, unfed n=30)

preference towards host cues when choosing between host- and food -associated cues. Recall here that in this species, adult females emerge from the logs, with and almost complete egg load.

When making decisions, parasitoids acquire and then integrate a variety of cues, depending on the environmental characteristics and/or their internal state and preferences (Godfray 1994). For example, it has been shown that *Venturia canescens* females can display adaptive plasticity in decision making. This wasp detects chemical cues associated with food, as well as those associated with hosts and has the capacity to integrate and respond to different external (e.g. resource odors) and internal (e.g. physiological state) information during the food-searching process (Desouhant et al. 2005). Our results indicate that choosing food or hosts by *I. leucospoides* females is also affected by their current feeding status.

Recent work has shown that feeding by *I. leucospoides* does not increase flight performance in laboratory conditions, as measured on tethered females of equivalent ages to those of the present study (Fischbein et al. 2011). The authors argued that flight capacity in this parasitoid is strongly dependent on morphological traits, in turn affected largely by host selection abilities and suggest that adults emerge with sufficient nutrient storage –obtained during larval development- to fuel somatic and locomotive needs. The fact that adult females emerge with and almost complete egg load may also contribute to explain the limited influence of food acquisition on dispersal in this parasitoid (Fischbein et al. 2011).

Similarly, a study in which female *I. leucospoides,* some with a feeding experience (*ad libitum* access to a 30% *v*/*v* honey-water solution for 48 h) were released and allowed to visit a set of host-rich logs in a semi-natural setting, showed that patch selection and residence time were strongly affected by host abundance in logs and the distance from the release point and not by feeding experience. Again, it was suggested that the lack of influence of feeding on patch residence time observed, was in line with the high ovigeny index and a need for a rapid egg discharge (Corley et al. 2010).

From an applied perspective, our study adds evidence on the fact that the performance of *I. leucospoides* in the field as a bio-control agent of damaging woodwasp populations is unlikely to be improved by the addition of artificial nectar sources or even feeding prior to release. Note that in the pine plantations were this parasitoid is freed, food is scarce due to the increased shading and extensive pine needle mulching found and may be considered a limiting factor to parasitoid success. While our findings do not explore in depth the mechanism behind the decisions taken by *I. leucospoides* females, or the physiology of nutrition management, it is clear that starved females responded best to host derived cues. Our results suggest that food provisioning in captivity, not only may not improve dispersal capacity or increase residence time on host patches, but may even discourage host searching in the field.

While *I. leucospoides* females avidly feed in laboratory conditions, food acquisition has little influence or else a negative one on female decision making. These findings emphasize past results that suggest that factors limiting enhanced parasitization success in pine plantations, are more likely related to host finding and selection, as well as to the species egg maturation strategy (Fernandez-Arhex and Corley 2005; Corley et al. 2010; Fischbein et al. 2011). Further studies, in which adult parasitoids are caught in the field, are needed to test whether any feeding actually occurs. Also, a more in depth comprehension of the environmental conditions under which this parasitoid has evolved (e.g. host and food abundance and distribution in their native habitat) will allow a better understanding of the behavior of this parasitoid species.

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