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Host location and acceptance in *Psyttalia concolor*: role of host instar

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Abstract

Laboratory investigations on the role of host instar in host location and acceptance of naïve females of *Psyttalia concolor* (Szépligeti) (Hymenoptera Braconidae) were conducted. *Ceratitis capitata* (Wiedemann) (Diptera Tephritidae) larvae were used as hosts. A simple no-choice experimental test was performed, using oviposition dishes containing: (1) second instar host larvae or (2) late third instar (fully-grown) host larvae. The parasitoid:host ratio was 1:1. Results showed that significantly more *P. concolor* located hosts in dishes containing late third instar *C. capitata* larvae (100%) and fewer wasps located second instar larvae (16.7%). The relative failure of *P. concolor* to locate the latter larval stage in laboratory conditions it is likely to be related to the fewer vibrations produced by second instar hosts during feeding and/or movement. However, no host rejection behaviour was observed, with all located late third instar and second instar larvae being parasitized. Therefore, both instars will serve as suitable hosts for *P. concolor*.

Key words: larval parasitoids, host acceptance, host stage selection, Opiinae, *Psyttalia concolor*, *Ceratitis capitata*.

Introduction

Foraging parasitoids may encounter different host developmental stages which are potentially vulnerable to attack, but may differ in their profitability. For both idiobiont and koinobiont parasitoids, smaller hosts may require less time for handling and represent less of a risk of injury resulting from the defensive behaviour of the host (van Alphen and Jervis, 1996). Moreover, for females of both idiobionts and koinobionts may be advantageous to oviposit preferentially in/on older larvae, because the mortality occurring in the younger host larvae, resulting from predation and/or intraspecific competition, is usually higher (van Alphen and Jervis, 1996). In general, for koinobionts parasitoid small hosts may offer progeny a lower mortality risk from encapsulation (van Alphen and Drijver, 1982).

The Opiinae are parasitoids that oviposit in either the egg or larval stage of their host and emerge from the host puparium (Wharton, 1997). Among these, *Psyttalia concolor* (Szépligeti) is a koinobiont endoparasitoid of many Tephritidae (Diptera) larvae (Marchal, 1910; Fischer, 1971). This parasitoid has been used in Italy and other Mediterranean areas for biological control of *Bactrocera oleae* (Rossi) (Diptera Tephritidae) by inundative and propagative releases [for a synthesis see Neuenschawander *et al.* (1986) and Raspi (1995)]. In Italy, *P. concolor* can be found spontaneously, in late autumn, on *B. oleae* in Sicily, southern Sardinia and in various localities of coastal Tuscany (Raspi, 1995; Raspi *et al.*, 1996; Loni *et al.*, 2005).

As far as we know, only scanty bibliographical sources are available regarding the capacity of *P. concolor* to successfully locate and parasitize, both in the field and laboratory conditions, different instars of their hosts. Raspi and Canale (2000) reported that *P. concolor* can successfully parasitize second instar larvae of *Ceratitis capitata* (Wiedemann) (Diptera Tephritidae) in laboratory conditions, but so far a comparative study on the role of host instar in host location and acceptance is lacking. In a bio-control program that involves a parasitoid, a step entails establishing which instar of the target species will serve as the most suitable host, especially if the aim is the augmentative release. Therefore, in this paper we report data of a laboratory experiment where two different *C. capitata* larval instars were offered to *P. concolo*r, in a simple no-choice experimental test.

Materials and methods

Parasitoid and host rearing

Parasitoid *P. concolor* and host *C. capitata* were reared as described by Raspi and Loni (1994). After emergence from the host, adult parasitoids were stored in cylindrical plexiglas cages (diameter 40 cm, height 50 cm) at a density of 100 specimens (males and females, sex ratio 1:2) per cage [21 ± 1 °C, 48 ± 10% relative humidity and 16:8 (L:D) photoperiod]. A semisolid diet (honey mixed with pollen) and water were offered to the parasitoids.

Testing procedures

The treatments were performed in the laboratory under climatic conditions previously described. The experiment was conducted using naïve *P. concolor* females (i. e. females that never had contact with the host) 5-7-days-old (after the mating have been observed). A simple no-choice experimental design was used with either late third instar (fully-grown) larvae (7-9 mm in length) or second instar larvae of *C. capitata* (3 ± 0.3 mm in length). In controlled laboratory conditions the second instar larvae (amphipneustic larvae) were obtained 6 days after distributing the medfly eggs on the artificial rearing medium. The parasitoid to host ratio was 1:1 (the desired ratio was obtained by placing one female with one host larva). In the experiment, the test larvae

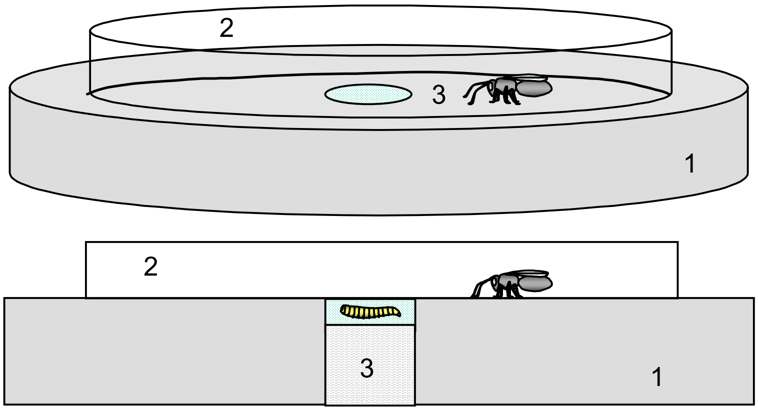

Figure 1. Schematic representation of the oviposition unit used in the experiment (more details are available in the text). Legend: (1) PVC dish; (2) transparent plexiglas lid; (3) dish hole containing both the host and substrate, covered by organdy screen.

with a small quantity of the substrate (about 0.5 g) in which they were grown, were placed in an oviposition dish (PVC tube, diameter 8 cm; depth 1 cm) inside a hole (diameter 0.8 cm; depth 0.5 cm) created in the centre of the dish (figure 1). The top of the hole was covered with an organdy screen, which was tightly fixed by inert glue around the rim of the hole. Similar artificial oviposition dishes are widely used for rearing opiine larval parasitoids (Wong and Ramadan, 1992) because they mimic the host's natural microhabitat (infested fruit). A single female was released directly onto the rim of the screen cover of the oviposition dish using a cylindrical glass tube (diameter 2 cm); subsequently the dish was covered by a cylindrical transparent plexiglas lid (diameter 6 cm; height 1 cm). A record of successful host location and ovipositions was kept. A host location was recorded as successful when the searching female, stationary on the patch where the host was placed, raised its abdomen and drove its ovipositor into the host (Canale and Raspi, 2000). An oviposition was considered successful when the female inserted its ovipositor into the host for at least 30-40 sec, because in this case the wasp usually lays an eggs. In contrast ovipositor insertions for 5-10 sec were considered as host rejection (Canale and Raspi, 2000). At the end of the experimental period, the host larvae were dissected in Rüngen's solution (Canale, 1998) and inspected for presence of parasitoid eggs. Only hosts containing at least one parasitoid egg were considered as parasitized. A trial was considered finished when the wasp successfully oviposited, or rejected the host, or after 30 min had elapsed without successful host location. A total of 30 females were tested for each treatment (each female was tested only once). The likelihood chi-square test and Student's *t* test were used to evaluate statistical differences among the different treatments (Sokal and Rholf, 1981).

Results and discussion

The results show that within 30 minutes significantly more *P. concolor* females located hosts in dishes containing late third instar *C. capitata* larvae (100%) compared to dishes containing second instar larvae (16.7%) (χ^2 = 40, df = 1, P < 0.0001) (table 1). We observed no host rejection behaviour, noting that all located late third instar and second instar larvae were parasitized (table 1). Furthermore, we can assert that the two different *C. capitata* host instars tested in the current study are equally suitable for oviposition by the parasitoid *P. concolor*. It is generally accepted that this parasitoid can be commonly reared on fully-grown *C. capitata* larvae (Raspi and Loni, 1994, and references therein) and also that it can successfully parasitize second instar larvae of both *C. capitata* (Raspi and Canale, 2000) and *B. oleae* (on infested olive fruits) (Canale, 1999). The best host location response of *P. concolor* itself and other braco-

Table 1. Host location and acceptance of second and third instar *C. capitata* larvae by *P. concolor*. In the column "hosts located", values with different letters indicate significant differences in response at 1% probability level according to the χ^2 test. In the column "latency period" values with different letters indicate significant differences in response at 5% probability level according to the Student's *t* test (values within brackets are standard deviations). Parasitoid: host ratio was 1:1 (one parasitoid female and one host larva). Thirty naïve wasps were tested for each treatment.

C. capitata instar	Hosts offered	Hosts located	Hosts rejected	Hosts accepted/located	Latency period (sec)
2nd instar larva	30	6 (16.7%) a	0	6/6 (100%)	72.3 (27.6) a
3rd instar larva	30	30 (100%) b	0	30/30 (100%)	34.1 (17.2) b

nid species is achieved if both mobile host larvae and substrate are present (Duan and Messing, 2000; Canale, 2003). However, in our comparative study it was evident that this parasitoid had more difficulties in locating the younger larval host stages of *C. capitata*, even though they were exposed in combination with substrate. Our direct behavioural observations revealed a typical and marked antennal drumming activity of *P. concolor* on the dish surface in all records, but the latency period (i. e. time between the female release onto the dish and the initiating of searching activity) was significantly longer in presence of second instar (72.3 sec) compared to fully-grown host larvae (34.1 sec) (t = 7.59, df = 29, P < 0.05) (table 1). Frequently (26.7% of tested specimens) we observed females drumming their antennae upon the patch where the second instar host was located without subsequently showing any ovipositor-probing behaviour. This behaviour may suggest that chemical and/or physical cues derived from the combination of second instar host/substrate were not sufficient to elicit an ovipositor-probing response (Canale, 2003).

Apart from chemical stimuli (see review in Quicke, 1997), vibrations produced by hosts which are feeding or moving have been reported to play a major role in host location in a number of parasitoid species of concealed hosts (see review in Meyhöfer and Casas, 1999). In *P. concolor*, Canale (2003) use of fully-grown *C. capitata* larvae showed that mobile hosts induced a higher level of ovipositor-probing behaviour than immobilized hosts, indicating that in this parasitoid species host movement plays an important role in host location. On this basis, it is likely that the above-mentioned failure of *P. concolor* to locate second instar hosts could be due to the relatively fewer vibrations produced by this larval stage during feeding and/or movement, weakly transmitted through a rigid material like the PVC. Additional experiments by using vibrometers are obviously necessary to support this assumption (Meyhöfer and Casas, 1999).

However, it should be noted that our results were obtained in experimental laboratory conditions and a validation by means of a comparison using fruits infested by different host instars was not evaluated in this study. The complex of *P. concolor* hosts known to date, and the fruits infested by such hosts, represent a homogeneous system of tritrophic interaction (Schmidt, 1991) attributable to Tephritidae that live on small spherical fruits, generally drupes (Monastero, 1931; Feron, 1952; Debouzie and Mazih, 1999), or on small-sized globular inflorescences like *Capparis spinosa* L. (Biliotti and Delanoue, 1959). As far as we know, evidences on the host developmental stages that are attacked by the parasitoid in the field are not available in the literature. In our opinion, it is likely that on infested fruits the older host larvae are not easily accessible by the parasitoid females, because the shortness of the ovipositor (about 4 mm) (Canale and Raspi, 2000) hampers parasitization of the larva buried deeper in the fruit. Moreover, in our experiment the dissection of accepted second and late third instar larvae revealed that they contained a single parasitoid egg, confirming that *P. concolor* places a single egg for each parasitization. It is acknowledged that in this parasitoid species laying a single egg on second instar *C. capitata* larvae ensure an offspring (Raspi and Canale, 2000), whereas on fully-grown hosts the number of 2 parasitoid eggs/parasitized host appears to represent the condition for ensuring an offspring (Canale, 1998; Loni, 2003). On this basis, in nature this parasitoid may have a greater general fitness in attacking younger host larvae.

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