

***Psytalia concolor* (Hymenoptera Braconidae): role of host movement and host substrate in ovipositor-probing behaviour**

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Abstract

Laboratory investigations into the effect of host movement and host substrate on ovipositor-probing behaviour in *Psytalia concolor* (Szépligeti) were conducted. *Ceratitis capitata* (Wiedemann) (Diptera Tephritidae) fully-grown larvae were used as hosts, while the host substrate was represented by an alfalfa meal-based medium. Five treatments were carried out, using an oviposition dish containing: (1) both mobile host larvae and medium, (2) only mobile host larvae, (3) only medium and (4) only immobilized host larvae, with (5) an empty dish serving as a blank control. Results showed that significantly more *P. concolor* initiated oviposition-probing behaviour in dishes containing both mobile host larvae and medium (100%); in contrast, significantly fewer wasps probed dishes containing host larvae alone (43.3%) or only medium (13.3%). The proportion of *P. concolor* probing dishes containing only mobile larvae was significantly higher than dishes containing medium alone. There was no significant difference among proportions of wasps probing dishes containing medium alone (13.3%) or immobilized larvae (6.7%) or empty control dishes (3.3%). Therefore, a combination of both mobile host larvae and host substrate induced the highest level of probing responses in *P. concolor*, suggesting that more than one cue is necessary to elicit the optimal response. Moreover, the finding that mobile larvae induced a higher level of ovipositor-probing behaviour than immobilized larvae suggests that host movement likely played a major role in eliciting ovipositor probing behaviour in *P. concolor*.

Key words: larval parasitoids, host habitat, host searching, Opiinae, *Psytalia concolor*, *Ceratitis capitata*.

Introduction

In parasitoids, host finding behaviour is usually subdivided into two main phases: location of the host's habitat (habitat location) and detection of the host within its habitat (host location) (van Alphen and Jervis, 1996). Host location comprises a combination of search modes, as the insect responds to a hierarchy of different stimuli while locating a host (for a review see Vinson, 1985 and van Alphen and Jervis, 1996). Apart from chemical and visual stimuli, vibrations produced from hosts which are feeding or moving have been reported to play a major role in host location in a number of parasitoid species (for a review see Meyhöfer and Casas, 1999). In addition, for parasitoids that attack a host concealed within plant tissues, ovipositor-probing often constitutes an essential element of host searching behaviour (van Dijken and van Alphen, 1998; Duan and Messing, 2000).

Psytalia concolor (Szépligeti) is a koinobiont endoparasitoid of many Tephritidae (Diptera) larvae (Marchal, 1910; Fischer, 1971). Found in Tunisia on *Bactrocera oleae* (Gmelin) (Marchal, 1910) this parasitoid has been used in other Mediterranean areas for biological control of this phytophagous by inundative and propagative releases [for a synthesis see Neuenschawander *et al.* (1986) and Raspi (1995)]. In Italy, this species is usually found living on *B. oleae* only in Sicily, southern Sardinia and, sporadically, in southern Tuscany (Raspi, 1995; Raspi *et al.*, 1996).

Previous studies on other braconid species that oviposit in the larval stage of their host (Lawrence, 1981; Henneman, 1997; Duan and Messing, 2000) showed that they use the host movement to locate host larvae

once the host micro-habitat is found, while chemical cues derived from hosts and/or their substrate seem to play a relatively small role in inducing ovipositor-probing behaviour. In *P. concolor* the ovipositor plays an important role in host location and host discrimination processes (Canale and Raspi, 2000), but so far a comparative study of factors inducing ovipositor-probing behaviour is lacking. Information on the cues eliciting such behaviour would improve knowledge of the ecological mechanisms regulating host selection by the parasitoid. Thus the aim of the present study was to investigate the role of host movement and host substrate on the initiation of ovipositor-probing behaviour in *P. concolor*.

Materials and Methods

Parasitoid and host rearing

The parasitoid *P. concolor* and its host *Ceratitis capitata* (Wiedemann) (Diptera Tephritidae) were reared as described by Raspi and Loni (1994). Upon emerging from host puparia, adult parasitoids were held inside cylindrical plexiglas cages (diameter 40 cm, length 50 cm) at a density of 100 specimens (males and females) per cage, 21 ± 1°C, 48 ± 10% relative humidity and 12:12 (L:D) photoperiod. All the experiments were conducted using 5-7-days-old mated naïve females (i.e. females that never had contact with the host). Fully-grown *C. capitata* larvae were used as hosts, while the host substrate used in all tests consisted of alfalfa meal-based medium (for further details see Raspi and Loni, 1994) that had already been in contact with the hosts for 10 days. The test larvae

and/or medium designed to be exposed to parasitoid females were placed in a Petri dish (diameter 6 cm; the depth was 0.4 cm in order to allow the females to oviposit). The top of the dish was covered with an organandy screen, which was tightly fixed by a plastic ring fit around the rim of the dish. Such artificial oviposition dishes are widely used for rearing opiine larval parasitoids (Wong and Ramadan, 1992) and mimic the host's natural microhabitat (infested fruit).

Testing procedures

Five treatments were carried out to evaluate the role of host movement and host substrate in eliciting ovipositor-probing behaviour in *P. concolor*: (1) an oviposition dish containing 20 mobile host larvae and 5 g of medium, (2) an oviposition dish containing only 20 mobile host larvae (without any substrate; larvae were washed in running tap water to remove residues of the rearing diet), (3) an oviposition dish containing only 5 g of medium (host larvae were removed before the tests), (4) an oviposition dish containing only 20 immobilized host larvae (washed in running tap water and killed by 2 hrs freezing; larvae were then set aside for 30 min after removal from the freezer in order to allow return to normal body temperature) and (5) an empty dish serving as a blank control. All treatments were conducted in the laboratory under climatic conditions previously described. For each treatment, the oviposition dish was placed on a platform in the center of a cylindrical plexiglas cage (diameter 30 cm, length 30 cm). A single female was released directly onto the screen cover of the oviposition dish using a cylindrical glass tube (diameter 2 cm). The ovipositor-probing responses evoked upon contacting the screen cover were observed. A probe was recorded when the female raised its abdomen and drove its ovipositor into the screen cover. A trial was considered finished when the wasp probed or left the oviposition dish, or after 10 min had elapsed. Wasps that left the oviposition dish within 10 sec after release without initiation of probing activity were discarded. Overall, this group of discarded wasps accounted for < 4% of tested wasps. For each treatment, after every 5 trials new larvae and/or diet were used. A total of 30 females was tested for each treatment.

Data analysis

Likelihood chi-square tests were used to evaluate treatment effects on ovipositor probing-responses of parasitoids among different treatments (Sokal and Rohlf, 1981).

Results and discussion

Significantly more *P. concolor* initiated oviposition-probing behaviour in dishes containing both mobile host larvae and medium (100%); in contrast, significantly fewer wasps probed dishes containing mobile host larvae alone (43.3%) or only medium (13.3%) (overall $\chi^2 = 102.87$, $df = 4$, $P < 0.0001$) (figure 1). The number of *P. concolor* females probing dishes containing only mobile larvae was significantly higher than dishes containing medium alone ($\chi^2 = 30.19$, $df = 1$, $P < 0.0001$) (figure 1).

There were no significant differences among proportions of wasps probing dishes containing medium alone (13.3%) or immobilized larvae (6.7%) or empty control dishes (3.3%) (figure 1).

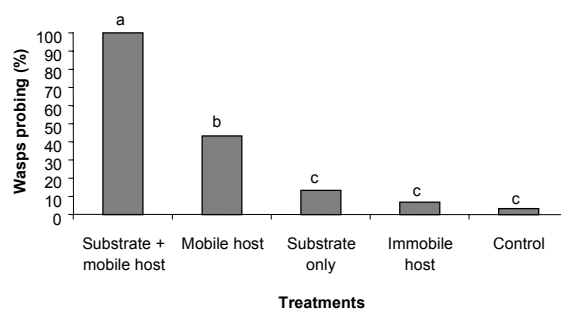


Figure 1. *P. concolor*. Ovipositor-probing responses to artificial dishes containing both *C. capitata* larvae and substrate, *C. capitata* larvae alone, substrate alone, immobilized *C. capitata* larvae alone or neither (control). Different letters indicate differences in response at 1% probability level according to the pairwise χ^2 test. Thirty wasps were tested for each treatment.

Results from this study showed that a combination of both mobile host larvae and host substrate induced the highest level of probing responses in *P. concolor*. The same result has been observed for other braconid species (Duan and Messing, 2000). For *P. concolor* cues from mobile larvae induced a significantly higher response than cues from host substrate alone. However, this finding is at variance with data published by Duan and Messing (2000), who reported that in *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera Braconidae) cues from the host substrate induced a significantly higher response than cues from live larvae alone, while in its congener *D. tryoni* (Cameron) host substrate elicited the same level of probing response as cues deriving from host larvae alone. In our experiment with *P. concolor*, we further observed that mobile larvae induced a higher level of ovipositor-probing behaviour than immobilized larvae or empty control dishes. Since all larvae had been carefully washed in water, it is likely that host movement plays an important role in eliciting ovipositor probing behaviour in *P. concolor*. But it should also be noted that the method by which the host is immobilized (freezing) may also affect the cues emanating from the latter (Prince, 1976). In addition, other cues associated with the metabolism of artificially immobilized hosts (such as body temperature and CO₂ radiation) are usually altered, possibly leading to production of different chemicals compared to substances emitted by a healthy host (see Meyhöfer and Casas, 1999, and references therein).

Host-induced vibrations (by larvae feeding or crawling inside the substrate) have long been suspected as playing a role in location by parasitoids of concealed hosts. Moreover, contact or short-range volatile chemicals derived from host substrate fermentation and/or excretion by the larvae are reported to be important in host location process (see review in Quicke, 1997). Although

the present study did not investigate the exact mode of host detection by *P. concolor*, in this parasitoid species the optimal response of the females appears to be achieved if both mobile host larvae and substrate are present, suggesting that host movement is only one type of cue which parasitoid may exploit to locate a host (Meyhöfer and Casas, 1999). Therefore, future investigations using new technologies such as non-contact laser vibrometers and vibration controllers (Meyhöfer *et al.*, 1994) may help to clarify the exact role of physical cues in the evolution of host location strategies of *P. concolor*. Moreover, the results of this work complement the morpho-ethological study by Canale and Raspi (2000) on *P. concolor*, where a morphological survey on the external feature of the tarsi revealed the presence of sensilla that could be directly involved in reception of vibrational signals (see also Meyhöfer *et al.*, 1997).

Finally, it should be noted that our results were obtained by using an artificial host medium. A comparison with the use of real host fruit was not evaluated in this study. It cannot be ruled out that in nature different host substrates which produce different quantities and qualities of chemical cues (due to host infestation) could affect the efficacy of the parasitoid in attacking the host larvae within a patch (Duan and Messing, 2000). Furthermore, the study reported here focuses primarily on investigating host detection within a patch; in nature it is likely that a complex of stimuli, deriving from both host habitat and host larvae, can influence the host finding behaviour.

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