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Host selection and parasitism behavior of *Lysiphlebus testaceipes*: Role of plant, aphid species and instar

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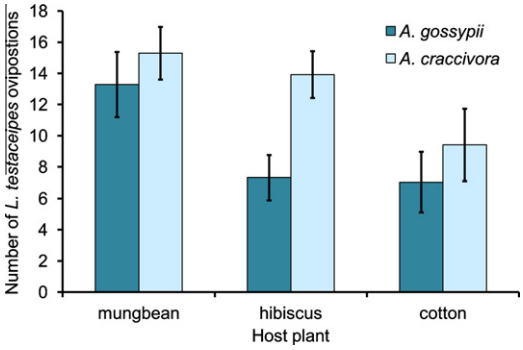
HIGHLIGHTS

- *Lysiphlebus testaceipes* parasitised more cowpea aphid than cotton aphid.
- Fourth instar and adult aphids were attacked more frequently than second instars.
- Cotton aphid cornicle secretion can cripple wasps, preventing further parasitism.

GRAPHICAL ABSTRACT



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ABSTRACT

The aphid parasitoid *Lysiphlebus testaceipes* is a potentially valuable biological control agent of *Aphis gossypii* a major worldwide pest of cotton. One means of increasing the abundance of a biological control agent is to provide an alternative host habitat adjacent to cropping, from which they can provide pest control services in the crop. Host selection and parasitism rate of an alternative host aphid, *Aphis craccivora* by *L. testaceipes* were studied in a series of experiments that tested its host suitability relative to *A. gossypii* on cotton, hibiscus and mungbean. Host acceptance, as measured by number of ovipositions was much greater in *A. craccivora* compared to *A. gossypii*, while more host aphids were accepted on mungbean than cotton. When given a choice *L. testaceipes* attacks more 4th instar and adult stages (63% and 70%, respectively) of both hosts than 2nd instar nymphs (47%). In a switching (host choice) experiment, *L. testaceipes* preferentially attacked *A. craccivora* on mungbean over *A. gossypii* on cotton. Observations of parasitoid contact with *A. gossypii* cornicle secretion suggest it provides a useful deterrent against parasitoid attack. From these experiments it appears *L. testaceipes* has a preference for *A. craccivora* and mungbean compared to *A. gossypii* and cotton, in this respect using *A. craccivora* and mungbean as alternative habitat may not work as the parasitoid is unlikely to switch away from its preferred host.

1. Introduction

A potential way to increase biological control of field crop pests is to add adjacent alternative habitats to the agricultural landscape with the aim of increasing in field parasitism levels by increasing the localized abundance of parasitoids (Landis et al. 2000). Prior to implementing such a system it is important to understand the host selection behavior of parasitoids both in the crop and alternative habitats.

Parasitoids select hosts based on their suitability (size, nutrition) for immature development, as determined by contact with semiochemical cues, antennal contact and ovipositor probing (Larocca et al. 2007). Host acceptance may be mediated by host

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defense mechanisms including avoidance, physical defense (e.g. kicking) and chemical defense (e.g. cornicle secretion in aphids).

Acceptance of a particular aphid species may be influenced by a range of features of the plant on which the aphid is feeding. Plant structures such as trichomes may interfere with host searching (Vinson 1976). Plant architecture can lead to parasitoids restricting their searching and avoiding concealed parts of the plant where aphids may aggregate (Stadler and Völkl 1991). Semiochemicals released by the plant may affect host selection behavior; in some cases hosts may only be parasitised when present on particular plants (Vinson 1976). Plants can indirectly affect parasitoids through the influence they have on aphid size (Wool and Hales 1997). Further, host selection behavior may be modified by status of the parasitoid itself, such as its size, physiological status (egg load) and prior experience, e.g. the host it emerged from (Iwasa et al. 1984; Chau and Mackauer 2001; van Emden et al. 2008; Desneux et al. 2009; Henry et al. 2009).

When multiple hosts are available, differences in availability, acceptance and suitability of hosts may result in variable patterns of parasitism and preference may not be constant across time (Chow and Mackauer 1991). In cases where the parasitoid oviposits in disproportionately more of the most abundant species it is said to display positive switching behavior (Murdoch 1969). In contrast, negative switching occurs when a parasitoid accepts disproportionately more of a rare species (i.e. it has a strong preference for that species) (Chesson 1984). Changes in levels of parasitism in response to the relative abundance of pest species compared to other hosts species in the agricultural landscape may determine if biological control will be successful.

Lysiphlebus testaceipes (Cresson) (Hymenoptera: Braconidae) is an aphid parasitoid with a reputed wide host range and is considered both a host and habitat generalist (Schuster and Starks 1974; Carver and Franzmann 2001; Starý et al. 2004). Field surveys (Schlinger and Hall 1960; Starý et al. 1988, 2004) and laboratory trials (Carver 1984) have reported high incidence of *L. testaceipes* parasitism of *Aphis gossypii* Glover, *Aphis craccivora* (Koch), *Rhopalosiphum maidis* (Fitch) and *R. padi* (L.) which are all common pests of field crops in Australia. Two of these aphids, *A. gossypii* and *A. craccivora*, are polyphagous (Blackman and Eastop 2000) while *R. padi* and *R. maidis* have more restricted host ranges. Both *A. gossypii* and *A. craccivora* are found on a number of agricultural crops and weeds and neither species is restricted to plant hosts from just one family, although *A. craccivora* tends to favor Leguminosae (Blackman and Eastop 2000). The generalist nature of *L. testaceipes* is a potential benefit as a biological control agent if it is able to establish large populations on alternative hosts and then provide pest control in nearby field crops, by switching its attack to the pest species.

The aim of this study was to investigate the host acceptance behavior of *L. testaceipes*. First, the role of host aphid species (*A. gossypii* and *A. craccivora*) and plant species (cotton, hibiscus and mungbean) on *L. testaceipes* host acceptance was investigated. Second, the effect of host instar on host acceptance was examined. Finally, we test if *L. testaceipes* displays positive switching behavior, by assessing if it will switch to preferentially parasitise the most abundant host in its habitat.

2. Methods

2.1. Experiment 1: effect of aphid host species and plant-type on oviposition

Host acceptance of *L. testaceipes* was tested by exposing wasps to two aphid species, either *A. gossypii* or *A. craccivora* on three plant types; cotton, hibiscus and mungbean. Host acceptance was accessed on the number of oviposition events recorded for each aphid by plant type combination.

2.1.1. Insects and plants

Separate glasshouse colonies (25 °C ±10, 50% ±25 R.H.) of *A. gossypii* and *A. craccivora* were established on each of three plants: cotton *Gossypium hirsutum* (L.), mungbean *Vigna radiata* (L.) Wilczek and hibiscus *Hibiscus rosa-sinensis* (L.). Colonies had been established on each respective host for 2 generations before use in experiments. *L. testaceipes* used in this experiment were reared from *A. gossypii* feeding on hibiscus. Parasitoids were originally collected from the Darling Downs region of Queensland, Australia, from *A. gossypii* on cotton. The parasitoids were subsequently reared on hibiscus and for the experiment they were obtained by collecting mummies from the hibiscus. To reduce prior exposure to host cues, wasps were dissected out of their mummies and kept individually in gelatin capsules (size 0) before use (van Emden et al. 1996). Virgin females were tested between 16 and 24 h after emergence. As parasitoid size may affect host choice behavior (Henry et al. 2009) a stereomicroscope (50× magnification) was used to select only wasps of a median body size (2 ± 0.05 mm) i.e. there were no outliers.

2.1.2. Experimental procedure

Host plant material was tested by using leaf discs of equal size (40 mm diameter) to standardize the search area for the parasitoid. Leaves were collected from plants grown in 6 L pots in the glasshouse. Plants were grown in a 50:50 ratio of soil and potting mix (Osmocote® Premium Potting mix) and fertilized monthly with liquid fertilizer (Miracle-Gro® All-purpose). Leaf discs were prepared from leaves collected from aphid free host plants, grown in a similar manner to the colony plants, but kept in a separate glasshouse. Discs were cut from the leaves with a hole-punch. Agar (2%) was poured into Petri dishes (Falcon®: 50 mm diameter, 9 mm depth) and a leaf disc was then placed ventral side up onto the setting agar with cut edges slightly embedded. Aphids were collected from each host plant, by removing infested leaves from colony plants in the glasshouse, and then moving these aphids onto the leaf discs with a fine brush. Thirty aphids of 2nd, 3rd or 4th instar were selected from the leaf samples and placed onto each leaf disc. The Petri dishes were then held at 25 °C for 16–20 h prior to use, to allow the aphids to settle and resume feeding.

Level of parasitoid host acceptance of each host aphid and food plant complex (2 aphid species × 3 host plants) was studied by observing the behavior of ten naïve (no prior host contact) wasps on each host complex. Testing was conducted in a Petri dish (80 mm diameter, 12 mm depth, non-vented) with the leaf disc placed centrally. For each test an individual female was released into the Petri dish arena for 30 min. Attack latency (time elapsed from release to first sting) and total stings (insertion of ovipositor into the host) in the remaining time were recorded. Observation of behavior was aided by a stereomicroscope (20×–50× magnifications). After the wasp had finished stinging and moved away from an aphid, the aphid was removed from the arena with a fine brush and stored in 70% ethanol.

To confirm observed oviposition, a sample (n = 10) of the stung aphids from each replicate were dissected and checked for presence of parasitoid eggs. In replicates where fewer than 10 aphids were stung, each stung aphid was dissected. Aphids were dissected with forceps, in a well slide filled with saline solution (1.5 g NaCl/200 mL) and 1% detergent (Jones et al. 2003). The slide was then examined using a stage microscope (63× magnification) for presence of a wasp egg(s).

2.1.3. Experimental design and statistical analysis

This experiment was run over 10 days with one replicate of each treatment completed per day. Order of treatments completed each day was randomized. For statistical purposes each day was considered a block. For each replicate, the proportion of aphids in which eggs were detected was used to adjust sting results to