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Host stage preference by *Aphidius colemani* and *Aphidius matricariae* (Hymenoptera: Aphidiidae) as parasitoids of *Aphis gossypii* (Hemiptera: Aphididae) on greenhouse cucumber

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Abstract: *Aphidius colemani* Viereck and *A. matricariae* (Haliday) (Hymenoptera: Aphidiidae) have a high potential as effective biological control agents for the cotton aphid, *Aphis gossypii* Glover in greenhouse crops. In this study, the host stage preferences of *A. colemani* and *A. matricariae* were determined in a growth chamber at 25°C, 65 % relative humidity and during a photoperiod of 16L: 8D hours. In choice and no-choice experiments, individual females of *A. colemani* and *A. matricariae* were offered first, second, third and forth instar nymphs of *A. gossypii*, as well as adults. The results showed that there were significant differences between the mean percentages of parasitized hosts at different stages ($P<0.05$). Under no-choice conditions, the highest value was registered for parasitism of third instar nymphs of *A. gossypii* by *A. colemani* and of third and fourth instar nymphs by *A. matricariae*. In choice experiments, females of *A. colemani* and *A. matricariae* consistently preferred third and fourth instar nymphs of *A. gossypii*. Mean percentages for parasitism by *A. colemani* and *A. matricariae* on the third nymphal instar of *A. gossypii* were 23.6 ± 3.69 and 18.4 ± 2.42, respectively. The progeny body size of *A. colemani* and *A. matricariae* at emergence increased with host stage at the time of parasitization. The greenhouse release of parasitoids would be best timed to coincide with the period when third and fourth nymphal instars of cotton aphid are most abundant.

Keywords: *Aphidius colemani*; *Aphidius matricariae*; host stage preference; cotton aphid; greenhouse cucumber

Introduction

The cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae) is a cosmopolitan, polyphagous species widely distributed in tropical, subtropical and temperate regions (Kersting *et al.*, 1999). In many regions, *A. gossypii* is one of the most important greenhouse crop pests (Havelka, 1978; Kersting *et al.*, 1999). Chemical control has generally been used as the major tool for controlling aphids on cucumbers (Parrella *et al.*, 1999). However, insecticide resistance and growing concerns about environmental hazards due to frequent use of insecticides have prompted the development of biological control of aphids. Biological control of aphids is increasingly applied on greenhouse crops (Parrella *et al.*, 1999; van Lenteren and Woets, 1988).

The two aphid parasitoids, *Aphidius colemani* Viereck and *Aphidius matricariae* (Haliday), have been considered good candidates for biological control of aphids such as *A. gossypii* (Bennison, 1992; van Steenis, 1993; Goh & Yoo, 1997), and their biology and use as biological control agents have been extensively reviewed (Goh & Yoo, 1997; Toussiddou *et al.*, 1999; van Lenteren & Woets, 1988). Aphid parasitoids may show considerable potential

as biological control agents for *A. gossypii* since *A. colemani* parasitism rates of up to 70% have been reported in June surveys of melon greenhouses in South Korea (Goh & Yoo, 1997).

Understanding the host stage preferences of parasitoids is a precondition for successful biological control programs, as it helps mass-rearing efforts and may also help to explain observed outcomes in the field. Both *A. colemani* and *A. matricariae* attack different stages of *A. gossypii*. It is not however known which stage is preferred for oviposition. This knowledge is essential for the mass-rearing of these parasitoids. We therefore sought to determine the stage range during which *A. gossypii* are vulnerable to attack from *A. colemani* and *A. matricariae* under no-choice and choice conditions.

Materials and methods

Rearing methods and experimental conditions

Insects were reared and experiments performed in a growth chamber at 25±1°C, 65±5% Relative Humidity and with a photoperiod of 16L: 8D hours. The cotton aphid and its parasitoids, *A. colemani* and *A. matricariae*, were originally collected from cucumber greenhouses in Tehran, Iran, in June 2004. Aphids were reared on cucumber (*Cucumis sativus* cv. Negin). Seedlings of *C. sativus* were grown to the 4-5 leaf stage in a mixture of sand (33%), clay (33%) and peat moss (33%) in 25cm pots. *Aphidius colemani* and *A. matricariae* were separately reared on *A. gossypii* in 30×60×35cm cages. Colonies of *A. colemani* and *A. matricariae* were replenished with field-collected individuals in spring and autumn. Aphids and parasitoids were reared on cucumber in the laboratory for several generations before they were used in the experiments.

No-choice test

Cucumber leaves containing 50 aphids at various stages (first, second, third and fourth instar nymphs and newly emerged adults) were placed in an experimental arena made of translucent glass containers (20×20×3cm). Recently-mated *A. colemani* and *A. matricariae* females were separately introduced into the test arena for 24 hours. After 24 hours exposure, parasitoids were removed. Two weeks after parasitoid exposure, the number of mummies was recorded. Parasitism percentage (number of mummies/total number of hosts) was calculated for each stage and parasitoid species. The procedure was repeated 5 times for each parasitoid species.

Choice test

To determine which aphid nymphal instars were accepted for oviposition by *A. colemani* and *A. matricariae*, 50 aphids - 10 of each instar - were randomly placed on cucumber leaves inside an experimental arena made of translucent glass containers (20×20×3cm). One recently-mated female wasp from each species was separately introduced into the test area for 24 hours, and then removed. After one day, second to fourth instar nymphs and adult aphids were moved to other leaves, surrounded by translucent plastic containers using a soft brush, and allowed to develop. The number of mummies was recorded. The procedure was repeated 5 times for each parasitoid species.

Statistical analysis

Data from experiments were analyzed using ANOVA and means were compared by Duncan's multiple range test (SAS Institute 2003). Standard error values are provided for all means.

Results

Under no-choice and choice tests, both *A. colemani* and *A. matricariae* females were able to parasitize all nymphal instars and the apterous adults of the cotton aphid (Figure 1, A & B).

However, the percentages of aphids parasitized by the two parasitoid species varied significantly between the different aphid stages.

Under no-choice conditions, *A. matricariae* showed a greater preference for third and fourth instars of the cotton aphid than for other stages (Figure 1A). Percentage of parasitism by *A. colemani* was highest on third instar *A. gossypii*, indicating that the third instar of cotton aphid was the most easily utilized by *A. colemani* for oviposition. In the choice test, both parasitoid species showed a preference for the third and fourth instars.

When *A. colemani* and *A. matricariae* were exposed to the third instar of cotton aphid in no-choice conditions, mean percentages of parasitism were 23.6±3.69 and 18.4±2.42, respectively (Figure 1A). There was a significant species effect on percentage of parasitism when parasitoids were exposed to the second instar of cotton aphid under choice conditions (F=7.538; df=9; P<0.05), whereas this relationship was not significant for the first (F=0.182; df=9; P>0.05), third (F=1.44; df=9; P>0.05), and fourth nymphal instars (F=1.11; df=9; P>0.05) or at the adult stage (F=1.00; df=9; P>0.05) (Figure 1B). The body size of *A. colemani* and *A. matricariae* progeny increased with host stage at the time of parasitization.

Discussion

Female wasps of *A. colemani* and *A. matricarie* oviposited in all 4 nymphal instars and also in recently-emerged adult cotton aphids. The high percentage of parasitism in the third and fourth nymphal instars in the choice experiment shows that female wasps preferred older nymphs for oviposition. Defensive behaviour in these instars therefore had no significant effect on parasitism. There were no significant differences between percentages of parasitism in the first and second nymphal instars and adults (Figure 1B). Similarly, the walnut aphid parasitoid, *Trioxys palliodus* (Haliday), preferred third and fourth instar nymphs of the walnut aphid, *Chromaphis juglandicola* (Kaltenbach), (Rakhshani *et al*., 2004). In contrast, *Binodoxys angelicae* (Haliday, 1833) preferred first instar nymphs of *Aphis pomi* de Geer (Cierniewska, 1976). *Trioxys cirsii* (Curtis) and *Monoctonus pseudoplatani* (Marshall) showed a preference for younger nymphs of the sycamore aphid, *Drepanosiphum platanoides* (Shrank) and rarely parasitized older nymphs (Hamilton, 1974).

Although older nymphs are generally the most hazardous, aphids parasitized in the 3rd and 4th instar nymphs can emerge as adults and reproduce before being killed (Stary, 1988). Lin and Ives (2003) showed that the preference of *A. colemani* for large hosts resulted in the greatest reduction in the population growth rate of *Aphis glycines* Matsumura, because by attacking adults, *A. colemani* kills the aphids that have the greatest immediate effect on the population growth rate. Thus, parasitism of the aphid instar plays an important role in biological control. Differences in parasitoid preference for the host stage depend on several factors. Mackauer (1983) notes that host stage preference is not constant: it is affected by experimental conditions and is a functional response of the parasitoid to population density. Host stage selection can also considerably affect the population growth of both the host and parasitoid species and can therefore have a definitive effect on whether potential hosts are successfully controlled by parasitoids (Hagvar and Hofsvang, 1991).

Further laboratory studies are required to determine the effect of different factors (e.g. various temperatures, relative humidity etc.) on the preference for and effect of various stages of the host on functional response and other aspects of aphid parasitoid behaviour.

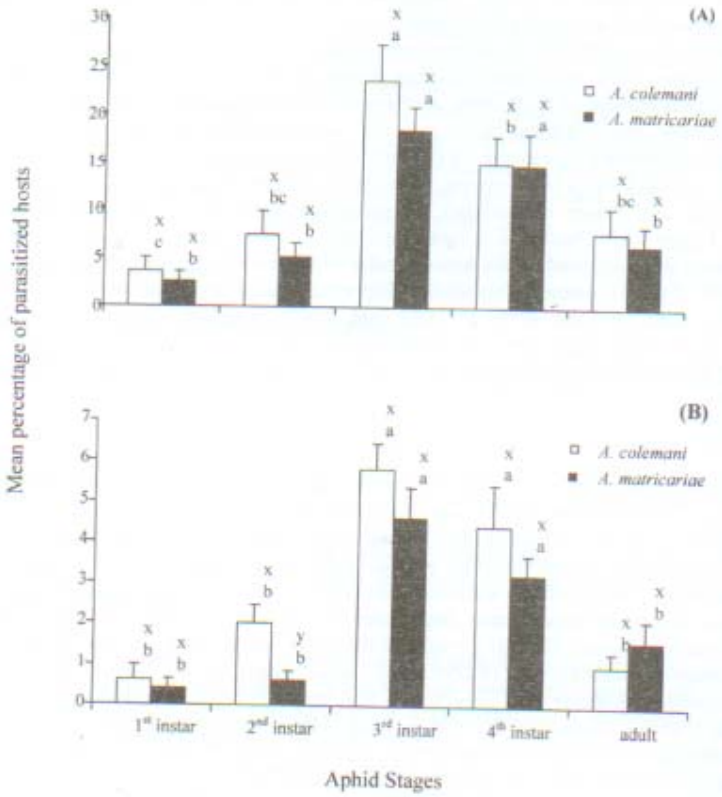

Figure 1. Percentage (mean ± SE) of different life stages of *Aphis gossypii* parasitized by *Aphidius colemani* and *Aphidius matricariae* in no-choice (A) and choice (B) tests. Letters **a**, **b**, and **c** indicate differences within species; letters **x** and **y** indicate differences between species ($P<0.05$).

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