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Host preference by *Allothrombium pulvinum* (Acari: Trombidiidae) larvae on aphids: *Macrosiphum rosae*, *Aphis gossypii* and *Hyalopterus amygdali* (Homoptera: Aphididae)

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Abstract. In this study aphid-plant association and its effect on host preference of parasitic *Allothrombium pulvinum* larvae was examined with multiple-choice tests. Host species selection, host size selection and superparasitism with mite larvae were studied with two-choice tests. Three aphid species were used: *Macrosiphum rosae*, *Aphis gossypii* and *Hyalopterus amygdali*. In multiple-choice tests, larvae of *A. pulvinum* showed no significant preference for any aphid-plant association when given *M. rosae* on rose, *A. gossypii* on cucumber and *H. amygdali* on apricot simultaneously. Two-choice tests showed that larval mites preferred *H. amygdali* to *A. gossypii*, but had no preference when offered a choice between *A. gossypii* and *M. rosae* or between *H. amygdali* and *M. rosae*. In host size selection and superparasitism tests, significantly more mites selected the larger host (*M. rosae*). Furthermore, parasitised *H. amygdali* were preferred to unparasitised ones.

Introduction

The velvet mite, *Allothrombium pulvinum* Ewing (Acari: Trombidiidae) is a natural enemy of a variety of pest species (Saboori and Zhang 1996; Zhang 1991a). It is a univoltine species with a complex life cycle: non-feeding quiescent stages (prelarva, protonymph and tritonymph) alternate with active feeding stages (larva, deutonymph and adult). Larvae emerge in spring and are ectoparasites of aphids. *A. pulvinum* and two other well-known species, *A. fuliginosum* (Hermann) and *A. ovatum* Zhang & Xin have been reported from several species of aphids (Welbourn et al. 1983; Zhang 1996; Zhang and Faraji 1994; Dong et al. 1996, 1998; Dong 2001). *Allothrombium* deutonymphs and adults emerge in summer and autumn, respectively, and are free-living predators of aphids, spider mites and various other small arthropods. Adults may continue to moult before reproduction (Chen and Zhang 1991; Saboori and Zhang 1996).

Because of the potential importance of *A. pulvinum* as a biological control agent against pest aphids and mites, a series of studies has been undertaken on its sys-

tematics, morphology, seasonal distribution, life history, ecology, behaviour, parasite-host and predator-prey interactions (Zhang and Xin 1989, 1992; Zhang 1991b, 1992a, 1992b, 1992c, 1992d; Zhang and Chen 1993). Using two-choice tests, Zhang (1996) showed that larvae of *A. pulvinum* preferred *Myzus persicae* (Sülzer) to *Myzus ornatus* Laing, and *Macrosiphum rosae* (L.) to *Cryptomyzus alboapicalis* (Theobald). Zhang (1991b, 1996) also showed that larvae of *A. pulvinum* preferred larger (adult and fourth nymphal instars) to smaller hosts (first and second nymphal instars) of *Acyrthosiphon pisum* (Harris), *Liosomaphis berberidis* (Kaltenbach) and *Dreoanosiphum platanoidis* (Schrank). Zhang (1991b) hypothesized that preference for large hosts has an adaptive significance because this will reduce the risk of host changing during the larval stage. Within a host population, distribution of parasitic larvae was often uneven among host individuals. Zhang and Chen (1993) suggested that one of the mechanisms underlying this pattern was the preference for parasitised to unparasitised aphid hosts, resulting in superparasitism of the host. For example, Zhang (1991b) showed that larvae of *A. pulvinum* preferred parasitised to unparasitised *A. pisum*, regardless of host size.

In Iran *A. pulvinum* was first reported from the North (Zhang and Faraji 1994). Larvae studied by Zhang (1996) were from the UK, whereas Iranian larvae differ in some morphological aspects and host relations (Zhang and Faraji 1994). Saboori and Zhang (1996) studied the biology and laboratory rearing of Iranian larvae. They showed that it is the most abundant species of Trombidiidae in Mazandaran province (north Iran). In this study, aphid-plant association and its effects on host preference of *A. pulvinum* larvae was examined with multiple-choice tests, and host selection and superparasitism of several aphid species were examined with two-choice tests.

Materials and methods

Sources of mites and aphids

Larval mites of *A. pulvinum* were reared from eggs. Adult mites were collected on 21 January 2001 in Chalus, north Iran. Twelve females of *A. pulvinum* were brought back to the laboratory in a plastic Petri dish (diameter 25 cm and height 4 cm) with moist soil as substrate, at 4±1 °C, 80% RH and 12L:12D, during reproductive diapause. The Petri dish was sealed with a piece of napkin to prevent escape. After 58 d the temperature was raised to 14 °C and after one more day, it was raised to 23 °C. *M. rosae* from a natural population in the orchards of Isfahan were used to feed these mites. Rearing Petri dishes were checked daily, and aphids or water were added regularly to provide sufficient food and moisture. One female oviposited a mass of eggs on 3 April 2001, another female on 6 April 2001 and another on 9 April 2001. When eggs were laid, the mass was placed in a vial (diameter 2.5 cm and height 6 cm) plugged with cotton balls to prevent escape. Prelarvae were first seen on 20 April 2001. Larvae began to emerge on 1 May 2001. These unfed lar-

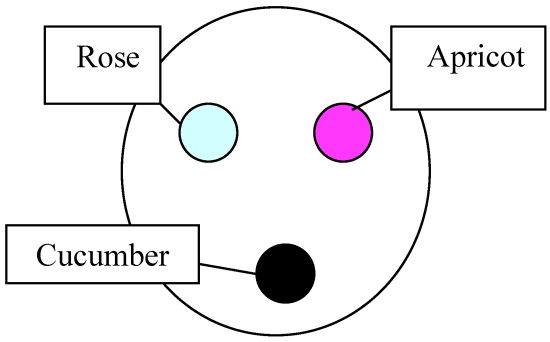

Figure 1. Arrangement of leaves in Petri dish

vae were used in various host selection tests. Some non-engorged larval mites that had detached from their first hosts (*M. rosae*) were also used in tests after they had starved for 12–24 h.

Three species of aphids were studied: *M. rosae*, *Aphis gossypii* Glover and *Hyalopterus amygdali* Geoffroy of the subfamily Aphidinae (Homoptera: Aphididae). *A. gossypii* individuals used in the experiments were from a laboratory culture maintained at 20 °C at College of Agriculture, Isfahan University of Technology. *M. rosae* and *H. amygdali* were collected from a natural population in the orchards of Isfahan. All experiments were conducted at 23±1 °C, 70% RH and 12L:12D.

Selection of aphids by A. pulvinum *larvae (multiple-choice tests)*

Host plants in this experiment were rose, cucumber and apricot. Based on Dong et al. (1998) selection tests were conducted in a large Petri dish (diameter 25 cm). Leaf discs (diameter 3 cm) of each plant were arranged as in Figure 1. Each leaf disc was fixed to the bottom of the dish using a ring of double sided adhesive tape. Ten apterus adult females of each aphid species (*A. gossypii* on cucumber, *M. rosae* on rose and *H. amygdali* on apricot) were offered simultaneously. Then, twenty newly hatched larval mites were released in the center of the Petri dish and the dish was capped with a perforated plastic lid. After one hour, the numbers of mites stuck on the rings of adhesive tapes and those on aphid species of each leaf were counted.

General experimental design and statistical analysis of two-choice tests

These tests were carried out in a glass vial (diameter 3 cm and height 0.8 cm). One host A and one host B were added to the vial first. Then a larval mite was allowed to choose between A and B. The vial was capped with a perforated lid to prevent escape. The vial was examined once or twice per hour until the mite parasite had selected one host, by continuing feeding until completion of this stage or death of the host. After replicating this choice test 25–30 times, the data were used to test

Table 1. Selection by larvae of *Allothrombium pulvinum* of simultaneously offered *A. gossypii* on cucumber, *H. amygdali* on apricot and *M. rosae* on rose. Numbers indicate mite larvae.

	Replicates						
[Aphid] on [Plant]	1	2	3	4	5	6	Pooled
A. gossypii on cucumber	6	1	4	2	2	7	22
H. amygdali on apricot	4	1	6	10	10	5	36
M. rosae on rose	7	10	4	2	2	5	30
X^2	0.55	8.95	0.15	6.55	6.55	0.55	2.891
P	0.476	0.016	0.493	0.08	0.08	0.476	0.290

the null hypothesis that the mite parasite does not choose between hosts A and B (H0: P=0.5) (binomial test; Zhang (1996)).

Host selection experiments

Mite individuals were given a choice between the following host species pairs: (A) *H. amygdali* vs. *A. gossypii*, (B) *M. rosae* vs. *H. amygdali*, (C) *M. rosae* vs. *A. gossypii*. There were 25 replicates per choice test. In a separate experiment, mite parasites were given a choice between a large apterus adult female and a small first or second nymphal instar of *M. rosae* (25 replicates).

Superparasitism experiment

Mites were given a choice between a parasitised and an unparasitised *H. amygdali*. Because aphid size may affect selection of hosts, the parasitised host and the unparasitised host were chosen to be of a similar size (either both were large or both were small). There were 30 replicates in this study.

Results and Discussion

Selection of aphid species by A. pulvinum *larvae (multiple-choice tests)*

Although the pooled numbers of mites trapped on adhesive tape surrounding aphids on their leaves decreased from *H. amygdali* on apricot > *M. rosae* on rose > *A. gossypii* on cucumber (Table 1), the difference was not significant (P>0.05), indicating that the mites have no preference for any aphid-host combination. Perhaps the various aphid-plant combinations are equally attractive to the mite parasites. Alternatively, they may have had neutral attractiveness. If *A. pulvinum* select their host by chance (Zhang 1996), an equal distribution of the mites over the various combinations is to be expected.

Host species selection in a two-choice test

When given a choice between *H. amygdali* and *A. gossypii*, 88% of the mite parasites selected *H. amygdali*, 12% selected *A. gossypii* (n = 17; P = 0.002). When given a choice between *H. amygdali* and *M. rosae*, 66% of the mites selected *M. rosae*, 34% selected *H. amygdali* (n = 21; P = 0.189). When given a choice between *M. rosae* and *A. gossypii*, 58% of the mites selected *M. rosae*, 42% selected *A. gossypii* (n = 20; P = 0.503). Perhaps the mite parasites prefer more inactive and less defensive hosts (i.e. *H. amygdali* vs. *A. gossypii*). Why a preference is found with two options offered, but not with three, remains to be elucidated.

Host size selection

When given a choice between a large apterus adult female *M. rosae* and a small first or second nymphal instar, 77% of the mites selected the large host, 23% selected the small host (n = 18; P = 0.031). Larval mites may prefer hosts of larger sizes because larger hosts are more likely encountered. Also, natural selection may favour individual mites that select larger hosts because mite parasites may kill small hosts before they complete their development (Zhang and Xin 1989).

Superparasitism

When given a choice between a parasitised and an unparasitised *H. amygdali* host, 74% of mites selected the parasitised host, 26% the unparasitised host (n = 23; P = 0.018). This preference for parasitised hosts was also found by Zhang (1991b).

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