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**Interaction of *Serangium japonicum* (Coleoptera: Coccinellidae),
An Obligate Predator of Whitefly with Immature Stages of *Eretmocerus* sp.
(Hymenoptera: Aphelinidae) within Whitefly Host (Homoptera: Aleyrodidae)**

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Abstract: *Serangium japonicum* potential predator of *Bemisia tabaci* is capable of discriminating between nymphs with advanced stages of parasitoid and unparasitized nymphs. The consumption of whitefly nymphs by the adult as well 4th instar larvae beetle offered 5 days postoviposition of *Eretmocerus* sp. and unparasitized nymphs were almost the same indicating that beetle are unable to distinguish between parasitized and unparasitized nymphs of whitefly in early stages of parasitization. The consumption of parasitized nymphs of whitefly declined with the increase in age of parasitoid inside the whitefly nymphs. Whitefly nymphs 9 days of parasitoid postoviposition were significantly less consumed than unparasitized control nymphs of same age. The avoidance was more significant at the pupal stage of parasitoid (13 days postoviposition of *Eretmocerus*) and was more determinant in adults than the 4th instar of *S. japonicum*. It can be concluded that incorporation of *S. japonicum* in conjunction with whitefly parasitoids in greenhouse system can suppress the rapidly increasing population of whitefly, thus enabling the whitefly parasitoids *Eretmocerus* and *Encarsia* species to keep the whitefly to acceptable thresholds without disrupting the other biological control.

Key words: *Bemisia tabaci*, discrimination, parasitized, coccinellidae

INTRODUCTION

Bemisia tabaci and its B-biotype have been reported as a serious cosmopolitan pest in various parts of the world^[1-3]. It has recently become most notorious economically important pest of agronomic, vegetable and greenhouse grown crops including ornamental plants on worldwide basis^[4-7]. Currently, in China it has been reported to invade the large areas in greenhouse and fields since last 3-4 years^[8] and has been recorded from about 74 plant species in Beijing^[8] 176 plant species in Guangzhou^[9].

Coccinellid *Serangium japonicum* Chapin is an important predator of *Bemisia* in China^[10]. The predator is a voracious feeder of all stages of *B. tabaci*. It has great potential as an effective biological control agent in any biological or IPM program against *B. tabaci*. An other natural enemy, *Eretmocerus* sp. an aphelinid parasitoid has been also found to play a significant role in limiting the whitefly population. It is currently known to outperform over *Encarsia* with a rate of parasitization 39.29% under natural conditions^[9].

The presence of parasitoids, predators and entomopathogenic fungi can play a large role in dynamics of whitefly population. Previous experience with whitefly

and with other pests demonstrates that biological control may substantially contribute to sustainable management of damage caused by *B. tabaci* in both greenhouse and field cropping environment^[11].

The joint use of biocontrol control agents to avoid the hazards associated with non judicious use of pesticides, depend upon what type of effect pathogenic agents, parasitoids, predators have on each other and exert jointly and individually on pest population. In naturally occurring field population of whiteflies parasitoids and predators act in conjunction with other mortality factors to regulate pest population. Currently, many efforts are being made to evaluate potential effect of natural enemies upon each other. The impact of coccinellids predation with reference to parasitized whitefly instars have been studied in detail to facilitate the use of predators in conjunction with parasitoids. However little information is available about the interaction of *Serangium* spp. with *Encarsia* and *Eretmocerus* spp.

MATERIALS AND METHODS

The poinsettia strain of *B. tabaci* and colonies of parasitoid *Eretmocerus* sp. and predatory beetle *S. japonicum* for throughout use in experiment were

initially taken from culture already established in Insect Ecology Laboratory of South China Agriculture University. Subsequently, whiteflies were reared and maintained continuously on poinsettia placed within the cages (60x60x60 cm). *Eretmocerus* sp. and predatory beetle *S. japonicum* were successfully reared on poinsettia infested with whitefly nymphs under laboratory conditions (25±2°C, 60±10% RH and photoperiod 14:10) in separate cages for at least one generation prior to their use for comparative study in laboratory.

Three developmental stages of *Eretmocerus* sp. were offered to 4th instar and adult of *S. japonicum* in the following sequence I) 1st instar larvae of *Eretmocerus* ii) 3rd instar larvae of *Eretmocerus* iii) pupae of *Eretmocerus*. Initial cohorts of whitefly were established by attaching circular plastic cage (a 0.5 cm diameter hole at one side, top covered with mesh for ventilation) on bottom surface of 180 leaves attached to the plants. In each cage, 20 matting pairs of whitefly were introduced and were left to oviposit for 24 h at 25±2°C. Plants leaves bearing eggs and resulting immatures were kept in the laboratory in cages at 25±2°C, 60±10% RH, 14:10 (L: D). After 12 days of whitefly oviposition, 2 pair of *Eretmocerus* sp. were released in clip cages attached to lower surface of leaves for 24 h. Before carrying out the treatment the larvae and beetles were kept starved for 6h. Thereafter, leaves with parasitized and unparasitized nymphs were offered in petridishes (9x9 cm) referred as experimental arena at following stages:

- 1) Whitefly nymphs 5 days of parasitoids postoviposition (possibly the 1st instar larvae of *Eretmocerus*).
- 2) Whitefly nymphs 9 days of parasitoid postoviposition (possibly 3rd instar larvae of *Eretmocerus*).
- 3) Whitefly nymphs 13 days of parasitoids postoviposition (pupae of *Eretmocerus*).

In the treatment only parasitized nymphs were offered to the beetle whereas in control only unparasitized nymphs of same age of whitefly were provided for feeding. Number of whitefly parasitized and unparasitized nymphs offered were standardized to 30 nymphs in each parasitized and unparasitized control by using 000 size needle under the binocular microscope. Each treatment includes the 15 4th instar larvae and 15 adult beetles. Humidity was maintained in experimental arena by placing the moisten filter paper in each petridish to avoid the curling and dislodging the whitefly nymphs. After 24 h beetles and larvae were removed from the experimental

arena and each petridish was examined under the microscope. The number of the whiteflies still alive and predated by the beetle and larvae were recorded. Damage to whitefly nymphs and pupae of the *Eretmocerus* were recognized by cuticle damage or feeding under the microscope. This cuticle damage was a kind of hole made by chewing to get the access to the internal fluid of the whitefly nymphs.

Data analysis: Data was recorded as the number of prey attacked and was analyzed with paired difference t-test, significance was assessed at P=0.05

RESULTS

Laboratory studies showed that both the larvae and adult of *S. japonicum* are the voracious feeder, capable of consuming large number of immature whiteflies in short period of time. Adult beetles consumed averagely 22-23 parasitized whitefly instars out of 30 whereas consumption rate was 24-25 in the control. All the beetles tested feed on the whitefly nymphs regardless they are parasitized or not during 24 h of study. The consumption rate of parasitized and unparasitized whitefly instar was not significantly different (P=0.0630) indicating that beetle are unable to distinguish between parasitized and unparasitized pupae in early stages of parasitization.

The consumption of parasitized nymphs of whitefly declined with the increase in age of parasitoid inside the whitefly nymphs. Whitefly nymphs 9 days of parasitoid postoviposition were significantly less consumed than unparasitized control nymphs of same age (P=0.0007) with an average of 11.40 in parasitized treatment than 18.8 nymphs in unparasitized control (Table 1). The avoidance was more determinant at the pupal stage of parasitoid and was more significant than the 4th instar of *S. japonicum*. Only 2 of the 15 beetles tested attempt to attack parasitized pupae more than the other (P<0.0001). The avoidance of parasitized pupae by adult beetle also increased as the encounter with parasitized host increased. Only 2.06 parasitoid pupae were damaged by the beetles in the parasitoid treatment indicating the discriminatory ability of the beetle.

Table1: Mean number of whitefly nymphs consumed by adult *Serangium japonicum* in parasitized and unparasitized treatment

Parasitized Stages offered to adult <i>S. japonicum</i>	n (replicate)	Prey consumed	
		nymphs	control Adult
5 days postoviposition	15	30	24.73±2.91a 22.98±2.24a
9 days postoviposition	15	30	18.8±1.86a 11.40±1.47b
13 days postoviposition	15	30	17.0±1.25a 2.06±1.34b

Means compared by paired comparison t-tests; significance was assessed at P=0.05

Table 2: Mean number of whitefly nymphs consumed by fourth instar of *Serangium japonicum* in parasitized and unparasitized treatment

Parasitized stages offered to adult	n (replicate)	Prey consumed		
		nymphs	control	4th instar
<i>S. japonicum</i>				
5 days postoviposition	15	30	20.37±1.13a	21.60±2.07a
9 days postoviposition	15	30	25.08±2.22a	17.10±1.69b
13 days postoviposition	15	30	20.09±1.04a	3.21±0.72b

Means compared by paired comparison *t*-tests; significance was assessed at P=0.05

4th larval instar of *S. japonicum*: The behaviour of the 4th larval instar of the *S. japonicum* toward the parasitized and unparasitized nymphs was the similar to that of the adult beetle. 4th larval instar consumed the more parasitized nymphs of the whitefly provided after 5 days of postoviposition in contrast to unparasitized nymphs of same age, however there was not a significant difference (0.0497). 21.60 parasitized nymphs of whitefly were consumed out of 30 as compared in control the consumption was 20.37 (Table 2). This revealed that similar to adult beetles, 4th instar was also unable to discriminate between parasitized and unparasitized. The discrimination was more significant when whitefly nymphs were offered 9 days of post oviposition of *Eretmocerus* (P=0.0003).

Parasitized nymphs 13 days postoviposition of *Eretmocerus* (pupae of parasitoid) were also significantly avoided as compared to control (P=0.0001). Only 3 out of 15 larvae did not feed or attack during 24 h of experiment. In the beginning when starved larvae of the *S. japonicum* were placed in the arena containing parasitized pupae of whitefly, the larvae tried to attack the parasitized pupae of the whitefly in the similar manner as in the control with unparasitized pupae of whitefly. But the number of attack attempts decreases immediately after encounters with parasitized pupae of the whitefly in the treatment arena. Both the adult and 4th instar of *S. japonicum* starved after frequent encounters with parasitized pupae of *Eretmocerus*.

DISCUSSION

The avoidance of prey larvae with old instars of parasitoid has been widely reported in many studies. The age of the parasitoid within prey nymph has the pronounced effect on the avoidance behaviour of the coccinellid predators. This discriminatory behaviour of the coccinellids is related to the relative host specificity of these predators compared to predators feeding on wide range of prey diet. This phenomena was described by the Quazada and DeBach^[12] for the interaction between *Rodalia cardinalis* (Muslant) and cottony cushion scale

Icerya purchasi Maskell prey parasitized by the *Cryptochaetum iceryae*. The beetle showed complete avoidance behaviour and starved when left only with scales containing pupae of parasitic fly. Holemer *et al.*^[13] related the discriminating feeding of *D. pusillus* for whitefly nymphs with advanced stages of *E. transvena* and *Eretmocerus* sp. nr. *californicus* to extra oral digestion in coccinellids mostly found in small coccinellids. During feeding process, after penetration of mouthparts in body of prey, the body fluid is gradually sucked up with the frequent regurgitation that helps in stirring up the sucked material and then to digest the contents sucked. Penetration of parasitized nymph by the predator may become difficult than those of unparasitized hosts because of physiological changes in host cuticle as hardening of cuticle caused by the parasitoid inside the host. Additionally, the body contents of the prey nymphs are used up as the parasitoid matures leaving behind only the air spaces that interferes with successful uptake of fluid. Avoidance near pupation can also be contributed to lack of sufficient or almost nil food availability for the predator at the time of parasitoid pupation within host body. It has been noticed that whitefly instars containing *E. transvena* were avoided by adult as well as 4th instar of *D. pusillus* for predation than unparasitized whiteflies during laboratory studies^[13]. Lopez *et al.*^[14] has also reported the similar interaction between *Nephaspis bicolor* and aphelinid parasitoid.

The predatory coccinellids *Hippodamia convergens* preferred the unparasitized aphids *Aphis gossypii* over aphids mummies parasitized by *Lysiphlebus testaceipes* when give the opportunity to feed on equal number of both prey type. The preference however, was not particularly strong^[15]. Similarly in laboratory studies by Ramanni and Bhumannavar^[16], adult of *Axinoscymmus puttardriahi* and *Cybocephalus* sp. were able to discriminate between the parasitized and unparasitized whitefly nymphs and completely avoided feeding on parasitized nymphs, both in the presence and absence of unparasitized nymphs. Generally, however predators consume both unparasitized and newly parasitized hosts without preference, but discriminate increasingly against parasitized prey as the parasitoid develops^[17,18]. As obligate potential predator of whitefly, *Serangium* is capable of discriminating between parasitized nymphs with advanced stages of parasitoid and unparasitized nymphs (based on laboratory study), indicating that only the immature stages including eggs of parasitoid will be at the risk of predation in naturally occurring arthropod community. However, it may not have the negative impact on biological control. Studies in Hawaii indicated that

Nephaspis spp. were very effective at high prey densities while the aphelinid parasitoids were effective when the populations were small^[19,20]. Also, Heinz and Nelson^[21] found that addition of *D. pusillus* to the greenhouse subjected to *E. formosa* and *E. pergandiella* releases provided the greatest suppression of whitefly, even though *D. pusillus* did not discriminate between unparasitized whitefly nymphs and those harboring eggs or young larvae. *Serangium* more likely to feed among high densities of whitefly while aphelinid parasitoid are effective when population is small, thus there is a good potential for integration of both in management program for whitefly. In naturally occurring population of whitefly with voracious feeding potential, *Serangium* can suppress the rapidly increasing population of whitefly and enabling the whitefly parasitoids *Eretmocerus* and *Encarsia* species to keep the whitefly to acceptable thresholds without disrupting the biological control.

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