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Prey consumption by *Harmonia axyridis* of un-parasitized and *Encarsia formosa* parasitized *Bemisia tabaci* biotype B prey

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Abstract

Bemisia tabaci is a major pest, causing serious losses to many agricultural crops worldwide. In the present study, *E. formosa* did not parasitize *B. tabaci* egg and N_1, but the subsequent developmental stages were parasitized. *H. axyridis* larvae as well as adults consumed significantly higher number of un-parasitized than *E. formosa* parasitized *B. tabaci* N_2, N_3, N_4 and pharate adults prey. The consumed number of prey increased in the subsequent predator's larval instars, where the adult females consumed higher number of prey than the males. The predator consumed both un-parasitized and parasitized prey, where the number of un-parasitized prey was significantly higher than parasitized one. The present results provide more opportunities of using *H. axyridis* in pest management programs together with *E. formosa*. As the predator feed more on un-parasitized prey, there is greater potential of integration both natural enemies in a biological control program against *B. tabaci* for higher pest suppression.

Keywords: Prey stages, Intraguild predation, Parasitization, Predator-prey-parasitoid interaction

1. Introduction

The cotton whitefly, *Bemisia tabaci* (Gennadius) (Homoptera, Aleyrodidae) is one of the most important agricultural insect pests in the Middle East, Europe, North and Central America [1]. *B. tabaci* feed on more than 700 host plant species in 86 plant families [2], which include important field and greenhouse crops [3], and horticultural crops [4]. *B. tabaci* has a high reproductive capacity and destructive life habits that enable it to cause severe damage through plant feeding and transmit more than 90 types of virus diseases in commercial crops [5], among which tomato yellow leaf curl virus (TYLCV), tomato mottle virus (TMoV), and bean golden mosaic virus are some of the important ones (BGMV) [6].

Attempts made to control *B. tabaci* in the past have failed mainly because of its high reproductive rate and many generations per year [7], preferred habitat on the undersurface of leaves [8] as well as its rapidly developed high resistance to many widely sprayed insecticides [9]. The massive spraying of insecticides has killed/suppressed its natural enemies too, which has further aggravated the problem [10]. Host plant races are one variant of biotype [11] of *B. tabaci*, now referred to as biological types that exhibit variation in geographical distribution, host range, fecundity, dispersal behavior, insecticide resistance, natural enemy complexes and endosymbiont complement [12]. The present results will help devise new or improve the already existing biological control methods for the pest suppression.

The multi-colored Asian ladybeetle, *Harmonia axyridis* Pallas (Coleoptera, Coccinellidae) is an important non-specific predator of many insect pests [13]. It could make a good candidate for mass rearing and release in pest hot spot infestations in open fields and greenhouses, because it has a good searching ability and a high prey consumption rate [14]. Although, it originated in Japan, Korea, Formosa, China and other parts of Asia [15], due to the high efficiency of *H. axyridis*, the predator has been imported to many countries (e.g. France, USA, Greece, Egypt and Syria) [16].

Before initiating any bio-control program, it is important to investigate biology and prey consumption of a predator, its preference for a certain pest stage or pest species as well as interaction with other natural enemies [17]. *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) is an important parasitoid used worldwide for commercial control of whiteflies in greenhouse crops. *E. formosa* parasitizes several whitefly species including *Trialeurodes vaporariorum* [18] and *Bemisia tabaci* [19].

Intraguild predation (IGP) occurs naturally in managed and unmanaged ecosystems worldwide [20]. In nature many natural enemies occur together and interact with one another in same natural ecosystems. Such interactions and predation/parasitization may have beneficial (support one another), detrimental (reduce efficiency of one another) or neutral effects (do not effect one another) effect on overall pest management [21].

The present study aimed at the following objectives: 1) To find predatory effect of *H. axyridis* of un-parasitized and *E. formosa* parasitized *B. tabaci* different stages. 2) To compare predation of immature and adults of un-parasitized and *E. formosa* parasitized *B. tabaci* different stages.

2. Materials and Methods

The experiments were conducted at the Chinese Academy of Agricultural Sciences (CAAS) Beijing, China during 2006-07. A stock culture of *B. tabaci* was maintained on tomato plants, variety Zhong Za No. 9, with few individuals obtained from cotton plants, variety Shi Yuan No. 321, in a glasshouse at CAAS. The culture was maintained on tomato plants, variety Zhong Za No. 9, in rectangular aluminum cages (80x50x60 cm) meshed with muslin cloth from four sides for ventilation. The cages were held in a climatically controlled chamber at the Institute of Plant Protection (IPP), South Campus, CAAS Beijing, at a temperature of 25±2°C, relative humidity of 65±5% and a photoperiod of 16:8h (L:D) with an artificial light intensity of about 4000 lux. Tomato plants were regularly grown in small pots (10 cm diameter and 8 cm height) in a glasshouse. The two weeks old infested plants were used to infest one week new ones and to feed the predators.

For obtaining appropriate stages of *B. tabaci* for different experimentation, fresh tomato plants were exposed to adult's *B. tabaci* infestation in the cages. After a couple of days, the adults were removed and the plants were incubated under climatic conditions as per above. The plants were daily observed for obtaining the desired prey stages, i.e. eggs, N1-N4 and adults for the different experiments.

H. axyridis culture was initiated from few individuals obtained from the already established culture at IPP, CAAS. The rearing took place in the cages and under climatic conditions as per *B. tabaci*. *Aphis craccivora,* reared on bean plants, was used for rearing the predator. For continuous and adequate prey supply, aphid infested bean leaves were frequently replaced inside the cages. *H. axyridis* eggs, larvae and pupae as well as adult females and males were obtained from the cages for different experimentation. In all the experiments uniform sized fresh tomato leaves were used as arena of observation in the clip on cages (3.5 cm diameter at mouth, 2.0 cm diameter at base and 4.0 cm long).

2.1 Prey consumption by the larval instars

For these experiments, newly hatched *H. axyridis* first instar (L_1) larvae were picked up using a camel-hair brush, transferred singly to the clip on cages and daily offered with 20 eggs, 10 first nymphal instar (N_1), 10 second nymphal instar (N_2), 5 third nymphal instar (N_3), 5 fourth nymphal instar (N_4) or 5 pharate adults. For this, uniform sized tomato leaves infested with batches of similar aged eggs, nymphs and pupae of *B. tabaci* were randomly selected from the culture, and extra eggs/individuals were removed with a fine camel-hair brush. After determination of prey consumption requirement, the subsequent predator stages were offered with higher number of prey stages, where the second instar (L_2) was offered with 50 eggs, 30 N_1, 20 N_2, 15 N_3, 10 N_4 or 10 pharate adults; third instar (L_3) with 90 eggs, 50 N_1, 40 N_2, 20 N_3, 15 N_4 or 10 pharate adults; and fourth instar (L_4) with 100 eggs, 80 N_1, 70 N_2, 60 N_3, 50 N_4 or 40 pharate adults of *B. tabaci* as per L_1. The larvae were transferred daily into new cages with fresh prey and the consumed number of prey in the old cages was noted. All the test stages of *H. axyridis* were less than 12h old and starved for 12h before using in the different experiments. There were 20 replications for each prey stage.

2.2 Prey consumption by the adults

Freshly emerged *H. axyridis* adults (max. 24h old and starved for 12h) were selected for these experiments. The adults were daily transferred into fresh cages and daily offered fresh prey of 100 eggs, 80 N_1, 70 N_2, 60 N_3, 50 N_4 or 40 pharate adults on fresh tomato leaves as described above for larvae. The consumed number of nymphs or puparia was recorded from 1st-10th day of female's as well as male's longevity. There were twenty replications for each predator sex and prey stage.

2.3 Prey consumption of parasitized prey

E. formosa was obtained from an old stock maintained whitefly infested cotton plants in a glasshouse at IPP, CAAS and maintained on tomato plants infested with *B. tabaci* in the rearing cages and climatic conditions mentioned as mentioned above. In a series of experiments, similar aged batches of eggs, 1st, 2nd, 3rd and 4th nymphs and pharate adults of *B. tabaci*, reared on 2-3 weeks old tomato plants, were separately exposed to freshly emerged *E. formosa* adults for oviposition.

The experimental procedure and number of parasitized prey offered to the immature and adult predators was the same as mentioned above for un-parasitized prey. Prey consumption data was recorded during the entire development of all larval stages and from 1st-10th days of adult's longevity with feeding on all stages of prey. There were twenty replicates for each predatory immature as well as both adults per prey stage.

2.4 Statistical analysis

The data obtained from the different experiments were subjected to T-test and significance levels determined at $p \leq 5\%$, means were compared using Statistic 8.1 software program.

3. Results and Discussion

3.1 Prey consumption by the larval instars and adults

E. formosa did not parasitize *B. tabaci* in the embryonic and first larval instar stages, but the subsequent developmental stages were parasitized. Since the egg and N_1 were not parasitized, un-exposed and exposed terminology was used for these two prey stages rather than un-parasitized and parasitized.

The consumed number of un-exposed and exposed *B. tabaci* eggs to *E. formosa* by *H. axyridis* larvae and adults were not significantly different within same predator stage (Fig 1). Egg consumption increased during subsequent larval instars. The females consumed higher number of eggs than males.

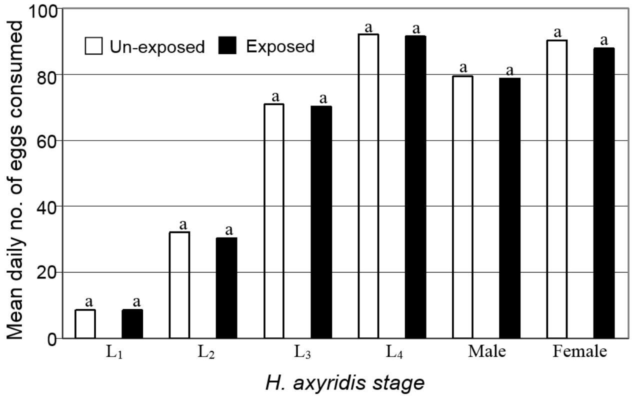

Fig 1: Prey consumption by *Hormonia axyridis* larvae and adults of un-exposed and *Encarsia formosa* exposed *Bemisia tabaci* eggs at 25+2ºC, 65+5% RH, 16:8h photoperiod. Bar heads with similar letters within same predator stage are non-significantly different at 5% level of significance (T-test).

The immature and adult predators, within same predator stage, consumed non-significantly different number of un-exposed and exposed *B. tabaci* N_1 to *E. formosa* (Fig 2), where consumption, both un-exposed and exposed, was higher during older larval instars and higher for females than males.

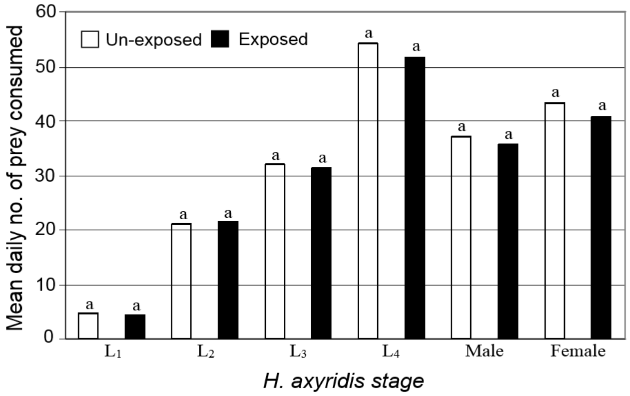

Fig 2: Prey consumption by *Hormonia axyridis* larvae and adults of un-exposed and *Encarsia formosa* exposed *Bemisia tabaci* N_1 at 25+2ºC, 65+5% RH, 16:8h photoperiod. Bar heads with similar letters within same predator stage are non-significantly different at 5% level of significance (T-test).

E. formosa parasitizing all four instars of the *T. vaporariorum* and was able to complete development [22]. But, in our experiments *E. formosa* didn't parasitize *B. tabaci* in the embryonic and first nymphal stage, which might be due to the small size of *B. tabaci* eggs and N_1 than that of *T. vaporariorum.*

The predator larvae and adults consumed almost number of *B. tabaci* eggs and N_1, both not exposed or exposed to *E. formosa* for parasitization. But, *H. axyridis* larvae as well as adults consumed significantly higher number of un-parasitized N_2, N_3, N_4 and pharate adults than the parasitized prey stages. The consumed number of prey increased during subsequent larval instars, where it was higher for females than the males.

3.2 Prey consumption of the parasitized prey

The larvae and adults of *H. axyridis* consumed significantly higher number of un-parasitized *B. tabaci* N_2 than parasitized one within same predator stage (Fig 3). The consumed mean number of un-parasitized prey was 4.1, 15.8, 23.0, 43.6, 29.5, 33.6 and parasitized prey was 0.9, 9.8, 14.9, 25.2, 20.9, 22.2 by L_1, L_2, L_3, L_4, adult males and females, respectively. Prey consumption was higher during subsequent larval instars, where the adult females consumed higher number of prey than males.

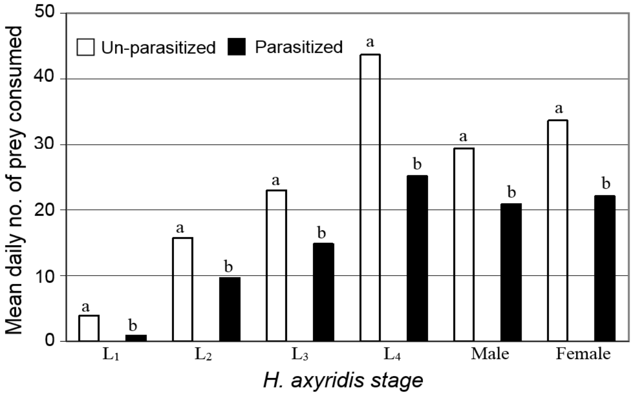

Fig 3: Prey consumption by *Hormonia axyridis* larvae and adults of un-parasitized and *Encarsia formosa* parasitized *Bemisia tabaci* N_2 at 25+2ºC, 65+5% RH, 16:8h photoperiod. Bar heads with different letters within same predator stage are significantly different at 5% level of significance (T-test).

H. axyridis adults and larvae fed readily on *A. asychis* parasitized *M. euphorbiae* mummies, although both predator stages also fed heavily on aphids [23]. The predator larvae ate nearly twice as many of the former when offered both aphids and mummies, and so significantly increased the ratio of mummies to aphids. Since *H. axyridis* feed on parasitoid mummies, so intraguild predation of the parasitoid by the predator could weaken overall biocontrol when both were present together. Intraguild predation of parasitoids by predators disrupt bio-control by parasitoids in a variety of systems [24], including greenhouses [25]. However, the predator's larval preference for aphids increase the ratio of mummies to aphids or no preference of the adults for mummies or aphids suggests bio-control might be improved by the predator's addition to the community.

The consumed mean number of un-parasitized N_3 prey by the *H. axyridis* L_1, L_2, L_3, L_4, adult males and females within same predator stage were significantly higher with 1.5, 10.1, 12.0, 22.2, 20.7, 24.7 than parasitized prey with 0.3, 3.2, 6.9, 12.2, 11.9, 15.2, respectively (Fig 4). Prey consumption, both un-parasitized and parasitized, increased during subsequent larval instars, and it was higher for adult females than males.

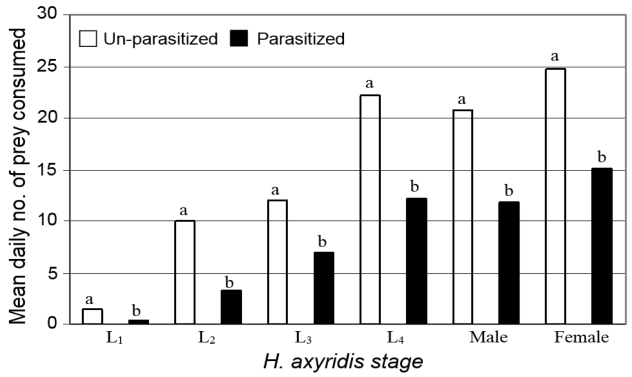

Fig 4: Prey consumption by *Hormonia axyridis* larvae and adults of un-parasitized and *Encarsia formosa* parasitized *Bemisia tabaci* N_3 at 25+2ºC, 65+5% RH, 16:8h photoperiod. Bar heads with different letters within same predator stage are significantly different at 5% level of significance (T-test).

H. axyridis L_1, L_2, L_3, L_4, adult males and females consumed, within same predator stage, significantly higher mean number of un-parasitized *B. tabaci* N_4 with 1.1, 2.7, 10.5, 16.5, 14.6, 17.3 than parasitized prey with 0.0, 1.1, 6.0, 7.4, 5.2, 7.5, respectively (Fig 5). The older larval instars consumed higher number of prey than the younger ones, and the adult females consumed higher number of prey than males.

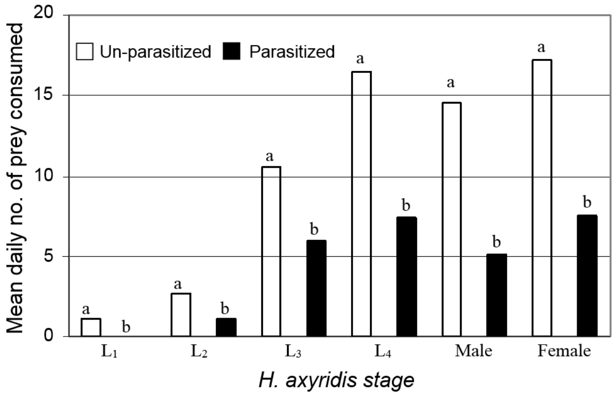

Fig 5: Prey consumption by *Hormonia axyridis* larvae and adults of un-parasitized and *Encarsia formosa* parasitized *Bemisia tabaci* N_4 at 25±2°C, 65±5% RH, 16:8h photoperiod. Bar heads with different letters within same predator stage are significantly different at 5% level of significance (T-test).

Within same predator stage, *H. axyridis* larvae as well as adults consumed significantly higher number of un-parasitized *B. tabaci* pharate adults than parasitized one, where it was a mean number of 0.0, 1.2, 6.0, 10.0, 7.1, 9.1 un-parasitized prey and 0.0, 0.0, 3.3, 3.9, 2.9, 3.7 parasitized prey for L_1, L_2, L_3, L_4, adult males and females, respectively (Fig 6). Prey consumption, both un-parasitized and parasitized, increased with larval instars and was higher for adult females than males.

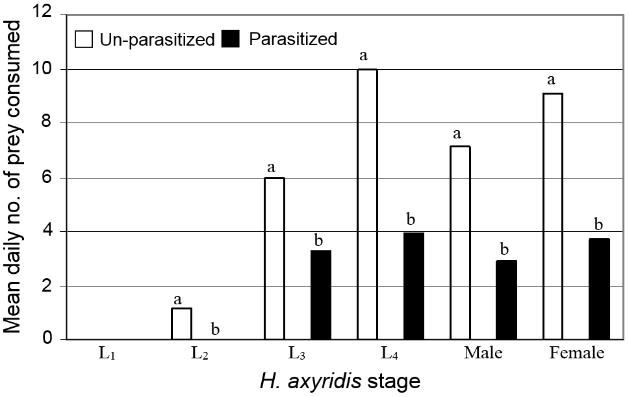

Fig 6: Prey consumption by *Hormonia axyridis* larvae and adults of un-parasitized and *Encarsia formosa* parasitized *Bemisia tabaci* pharate adults at 25±2°C, 65±5% RH, 16:8h photoperiod. Bar heads with different letters within same predator stage are significantly different at 5% level of significance (T-test).

Trophic interactions between natural enemies attacking *B. tabaci* have been reported earlier by several researchers. *Delphastus pusillus* (LeConte) consumed parasitized whitefly hosts containing younger stages of aphelinid parasitoids indiscriminately, but it avoided preying on hosts containing older parasitoid larvae and pupae [26]. Similarly, two species of the coccinellid Serangium avoiding preying on older aphelinid parasitoid larvae and pupae within *B. tabaci* hosts, which indicates that the magnitude of parasitism depends on the timing of IGP [27]. Mirid predator *Dicyphus hesperus* Knight an indiscriminant IG predator of *E. formosa* pupae in greenhouse whiteflies [28]. This behavior of the predator was not affected by the parasitoid age [29].

Some earlier researchers [24] found intraguild predation on immature parasitoids in many predator-parasitoid-prey systems. They found all of the predators in these systems preying on various stages of immature parasitoids within whitefly hosts even under non-parasitized prey alternative choice condition.

In similar studies, *Delphastus pusillus* (LeConte), a whitefly coccinellid predator, avoided parasitized *B. tabaci* fourth instar by the aphelinid parasitoid, *Encarsia tranvena* (Timberlake) (= *E. sophia*) (Girault and Dodd) and *Eretmocerus* sp. nr *californicus* Howard in favor of un-parasitized whitefly [30].

In no-choice and choice experiments, lower predation by *Delphastus catalinae* (Horn) on parasitized *B. tabaci* nymphs containing *Encarsia sophia* (Girault and Dodd) pupae than on larval stages or on un-parasitized whitefly nymphs was recorded [31]. The adults did not discriminate between prey types (parasitized or un-parasitized) in choice tests. In both choice and no-choice tests, the second instar *D. catalinae* larvae discriminated *B. tabaci* nymphs containing parasitoid larvae, and the third and fourth instar predator larvae attacked less the whitefly nymphs containing parasitoid pupae than larvae.

In free-choice and no-choice laboratory experiments, prey preferences of three generalist predators, *Geocoris punctipes* (Say), *Orius insidiosus* (Say), and *Hippodamia convergens* Gue´rin-Me´neville, when offered *B. tabaci* fourth instar nymphs and nymphs parasitized by *Eretmocerus sp. nr. Emiratus*, the three predators showed significant preferences for larval and pupal stage parasitoids over early fourth instar nymphs, but *G. punctipes* and *O. insidiosus* didn't discriminate between a choice of larval parasitoids and late fourth instar nymphs [32].

Although the studies of the present and earlier researchers have yielded mixed results, e.g. the coccinellid predators avoided or preferred parasitized prey over non-parasitized one or vice versa. Our results have increased the options of using *H. axyridis* in *B. tabaci* pest management programs together with the *E. formosa* parasitoid. Since the predator fed more on un-parasitized prey, there is high potential for integration of both natural enemies in a biological control program against *B. tabaci* for better pest suppression. And although IGP by *H. axyridis* on parasitized *B. tabaci* nymphs might weaken the overall pest management program. But, still its advantages are higher than its disadvantages.

As IGP interferes with the efficiency of natural enemies, so it might be detrimental to biological control in exerting their suppressive effects on pest populations [21, 23] However, contradictions exist in which the addition of an IG predator may pose no effect on pest suppression [33] or enhance overall biological control [27, 34].

4. Acknowledgment

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