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Host suitability of different instars of *Bemisia tabaci* biotype B for the parasitoid *Eretmocerus hayati*

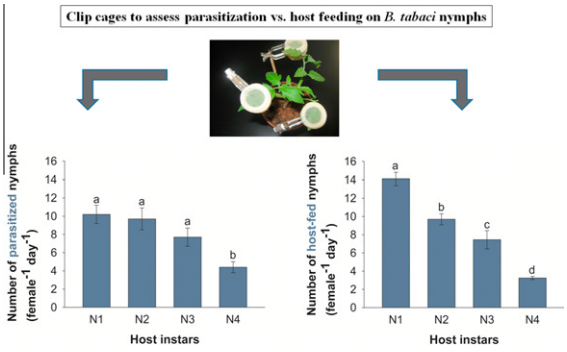
Nian-Wan Yang, Fang-Hao Wan *

State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, PR China

HIGHLIGHTS

- Suitability of *Eretmocerus hayati* on different *Bemisia tabaci* host instars were evaluated.
- *Er. hayati* parasitized and host fed most on first instar, least on early fourth instar.
- Development time was 13.5–17.4 days on different host instars.
- Preimaginal survivorship averaged at 75.2%.
- The species showed vigorous and destructive host feeding.

GRAPHICAL ABSTRACT



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ABSTRACT

The incidence of parasitism, host feeding, development time, survivorship, and sex ratio of the parasitoid *Eretmocerus hayati* Zolnerowich and Rose were evaluated when different nymphal instars of *Bemisia tabaci* Gennadius biotype B were offered as hosts. Experiments were conducted on tomato at 26 ± 1 °C, 65 ± 5% RH, and 14:10 (L:D) photoperiod. *Er. hayati* successfully oviposited and host-fed on all nymphal instars of *B. tabaci* on tomato with the exception of late fourth instars. However, the number of parasitized early fourth instars (4.4 nymphs female^{-1} day^{-1}) was significantly lower than that of first, second, and third instars, and the greatest incidence of parasitism was observed on first instars (10.2 nymphs female^{-1} day^{-1}), followed by similar number on the second (9.7 nymphs female^{-1} day^{-1}), and third instars (7.7 nymphs female^{-1}day^{-1}). The number of first, second, third, and early fourth nymphal instars fed on by females was 14.1, 9.7, 7.5, and 3.3 female^{-1} day^{-1}, respectively. Development time of *Er. hayati* was longest when first instars (17.4 days) were parasitized, intermediate for the second (16.3 days), and third (14.2 days) instars, and shortest for the early fourth (13.5 days) instars. The difference of development time between female and male was not significant. Survivorship (75.2%) did not differ statistically for parasitoids developing in whiteflies that were parasitized as different instars but offspring sex ratio (female/male) varied from 0.94 to 1.43. These values are well above those reported for other *B. tabaci* parasitoids, indicating that *Er. hayati* is a good candidate for biological control of *B. tabaci* in China.

* Corresponding author. Address: Institute of Plant Protection (South Campus), Chinese Academy of Agricultural Sciences, #12, Zhong-Guan-Cun Nan-Da-Jie, Haidian, Beijing 100081, PR China. Fax: +86 10 82105927.
E-mail address: wanfh@caas.net.cn (F.-H. Wan).

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1. Introduction

Bemisia tabaci Gennadius "biotype B" (=*Bemisia argentifolii* Bellows and Perring) (Homoptera: Aleyrodidae), is one of the most important pests of vegetables, field crops, and ornamentals throughout the world (Perring, 1996; Oliveira et al., 2001; Zhang et al., 2007; Wan et al., 2009). Since its invasion into China in the mid-1990s, the economical losses caused by this pest continuously increased (Wan et al., 2009). The extensive use of chemical insecticides in food crops has resulted in the development of resistance against several insecticides (Byrne et al., 2010; Feng et al., 2010; Schuster et al., 2010), increasing threats to the environment and human health.

Developing long-term integrated *B. tabaci* population management, with a strong natural enemy component, is a promising alternative. In a review by Gerling et al. (2001) concerning the worldwide natural enemy resources of *B. tabaci*, 79 species of predators and 55 species of parasitoids were recorded. A rich complex of at least 13 described species of parasitoids of *B. tabaci* in the genus *Eretmocerus* from the New World (Zolnerowich and Rose, 1998). Of the *Eretmocerus* spp., at least two species (*Er. eremicus* Rose and Zolnerowich and *Er. mundus* Mercet) are commercially available (Liu, 2007). Assessment of the potential of parasitoids already in China to control *B. tabaci* indicated that 18 species of parasitoid were present, but they did not exert sufficient control (Ren et al., 2001).

As the performance of indigenous natural enemies was judged inadequate, the strategy of providing new natural enemies to supplement indigenous species has turned to be a promising way to strengthen *B. tabaci* biological control. In the 1990s, foreign explorations were initiated within the framework of USDA biological control program (Kirk and Lacey, 1996) and over 56 populations of parasitoids were imported to USA (Kirk and Lacey, 1996; Kirk et al., 1993, 2000; Legaspi et al., 1996). A total of seven species of *Encarsia* and five species of *Eretmocerus* were released, of which all species of *Eretmocerus* and one species of *Encarsia* established in agricultural ecosystems (Goolsby et al., 2005a).

Eretmocerus hayati Zolnerowich and Rose is native to Pakistan. Like other species in the genus of *Eretmocerus* (Rose and Zolnerowich, 1997; Zolnerowich and Rose, 1998), *Er. hayati* is a solitary parasitoid ovipositing externally, under the nymphal host. Upon eclosion, the first instar larva penetrates the host, feeds, and pupates internally. This species is a biparental primary parasitoid, with both males and females developing in whitefly nymphs. It was released in Lower Rio Grande Valley of Texas, USA in 1995–1996 and later in the greater Phoenix metropolitan area and near Yuma, Arizona as part of biological control efforts for management of *B. tabaci* (Goolsby and Ciomperlik, 2008; Gould et al., 2008). Results from Texas indicated that the introduction of *Er. hayati* would contribute to meaningful reductions in *B. tabaci* abundance by substantially increasing the overall level of parasitism (Goolsby et al., 2005b; Goolsby and Ciomperlik, 2008). Based on the CLIMEX indices and observations on establishment in USA, *Er. hayati* offered the best prospects for introduction in Australia and China (Goolsby et al., 2005a), and, in October 2004, the first releases were made in Australia. Since then, levels of apparent parasitism have averaged 29.3% of fourth instars with only 24% of collections having no parasitism present, and *Er. hayati* contributes 85% of the overall apparent parasitism (De Barro and Coombs, 2009). *Er. hayati* was introduced into quarantine at the Institute of Crop Protection, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China from Texas in 2008 by the scientists of the State Key Laboratory for Biology of Plant Diseases and Insect Pests.

Host instar suitability is of practical concern in optimizing rearing resources and the success of field application. The biology of *Er. hayati* with respect to nymphal stages of *B. tabaci* has not been documented. The present study was undertaken to evaluate host instar effects on the incidence of parasitism, host-feeding, developmental time, survivorship, and offspring sex ratio of *Er. hayati* parasitizing *B. tabaci* biotype B.

2. Material and methods

2.1. Stock cultures of insects and host plants

Laboratory colonies of *Er. hayati* were established from a parasitized *B. tabaci* population maintained on melon plants in a greenhouse in the Vegetable IPM Laboratory, Texas Agricultural Experiment Station at Weslaco, TX, USA. A stock culture of *B. tabaci* biotype B was established using 300 individuals from a colony which had been maintained on tomato plants for the last 5 years without any exposure to pesticides, and obtained from the Institute of Vegetables and Flowers, CAAS.

Whiteflies and parasitoids were maintained on tomato plants, *Solanum lycopersicum* L. var. *lycopersicum* (Solanaceae), variety Zhong-Za No. 9, in an air conditioned glasshouse, under the condition of 26 ± 2 °C, and natural light regime (39°57′ N, 116°19′ E), at the Institute of Plant Protection, CAAS, Beijing, China. Plants reached approximately 15 cm height with 5–7 fully expanded leaves were used in the experiments.

2.2. Experimental parasitoids

Experimental parasitoids were obtained by exposing *B. tabaci*-infested tomato plant to 20 female wasps. Infested plants were maintained in an air-conditioned laboratory, where all experiments were conducted, with temperature set at 26 ± 1 °C, 65 ± 5% RH and light regime 14:10 (hours L:D). After 13–18 days, parasitoids pupae were collected and put in a Petri dish with a drop of honey (5%). Petri dishes were checked daily for emergence and wasps that had emerged were used 1 day later. All experimental females were observed mating and not experienced oviposition.

2.3. Host suitability study

Twenty unsexed whitefly adults were introduced into a clip cage secured on the top two or three leaves of 15 cm high tomato plants for a 12-h oviposition period to assure stage uniformity. When nymphs had developed to the desired stage (N1, N2, N3, N4, and LN4, which are first, second, third, early fourth, and late fourth instars, respectively.), only 40–50 nymphs were kept on each leaf, with additional and stage-undesirable nymphs being removed using an insect pin. A pair of 1-day-old *Er. hayati* was introduced into the clip cage confining the nymphs for each of the five whitefly stages for a 24-h oviposition period, after which the parasitoids were removed. Each treatment (host instar) had at least 20 replicates.

The number of whitefly hosts that were killed by parasitization and host-feeding were first checked 7–8 days after parasitoid removal. If the hosts were parasitized, the mycetome displacement was visible through the cuticle at the time of examination. If the hosts were killed by host-feeding, the bodies became flat and desiccated. The parasitized nymphs were marked. Subsequently, all "unparasitized" nymphs were turned over with an insect pin under a stereomicroscope to check for eggs that may have remained under the nymphs without successfully hatching. The parasitized nymphs (with visible mycetome displacement) were continuously monitored for development. When the parasitoids pupated, the leaves with the parasitized nymphs were detached from the plants. The end of the petiole of each leaf was individually inserted in a

water-saturated cotton ball wrapped with parafilm. The leaves were then individually placed in a Petri dish (9-cm in diameter and 1.5 cm in depth). Overall developmental time of the immature stages was calculated from the date of oviposition to adult emergence. Numbers of emerged adults were counted and sexed, and dead parasitoid pupae and whitefly pupae were also counted (Liu, 2007; Urbaneja and Stansly, 2004).

2.4. Data analysis

One-way ANOVA (SPSS 13.0 software package) was used to analyze the differences in the number of whitefly hosts parasitized and fed, parasitoid developmental time and survival rate among different host instars. Percentages (p) were arcsine transformed before analysis. Differences among means related to different host instars were compared with Tukey's Honestly Significant Difference (HSD) test at $\alpha = 0.05$. While differences between means related to female and male parasitoids were compared with independent samples T-test at $\alpha = 0.05$.

3. Results

3.1. Oviposition and host-feeding

Er. hayati successfully oviposited under all nymphal instars of *B. tabaci* with the exception of the late fourth instars (red-eyed nymphs or pupae, LN4) (Fig. 1A). However, numbers of parasitized early fourth instars (mean = 4.4 nymphs female^{-1} day^{-1}, SE = 0.6) was significantly lower than parasitized first, second and third instar (df = 3, 82; $F = 6.963$; $P < 0.001$). The greatest incidence of parasitism was observed on first instars (mean = 10.2 nymphs female^{-1} day^{-1}, SE = 1.0), followed by similar number of parasitized nymphs when oviposited under the second (mean = 9.7 nymphs female^{-1} day^{-1}, SE = 1.2) and third instars (mean = 7.7 nymphs female^{-1} day^{-1}, SE = 1.0).

Females host-fed on all nymphal instars of *B. tabaci* with the exception of late fourth instars but in significantly (df = 3, 76; $F = 43.287$; $P < 0.001$) different numbers (Fig. 1B). The females consumed only 3.3 (SE = 0.2) early fourth instar female^{-1} day^{-1} and 14.1 (SE = 0.7) first instar female^{-1} day^{-1}, followed by similar number of the second (mean = 9.7 nymphs female^{-1} day^{-1}, SE = 0.6) and third instars (mean = 7.5 nymphs female^{-1} day^{-1}, SE = 1.0).

3.2. Development

Er. hayati developed successfully from egg to adult on all instars of their whitefly hosts (except the late fourth instars which were not acceptable for oviposition, see Section 3.1) (Table 1). The development time of *Er. hayati* from egg to pupa on different host instars differed significantly (df = 3, 657; $F = 508.262$; $P < 0.001$): the older the parasitized hosts were, the shorter time was needed for development. The development times of pupal stage did not differ significantly (df = 3, 491; $F = 2.235$; $P = 0.083$) (Table 1). The difference of overall developmental time between female and male was not significant (independent-sample T -test, $p < 0.05$; $t = 0.016$–1.324, $P = 0.188$–0.987).

3.3. Survival rate and sex ratio

Survival rates varied depending on the host instars but the difference was not significant: mean egg hatching rate varied between 93.4% and 99.2%; larval survivorship varied between 88.9% and 96.7%, and of pupa varied between 85.1% and 90.1%, that for a total varied between 72.9% and 79.3% (Table 2). The mean sex ratio (female/male ratio) differed statistically on different host instars (df = 3, 76; $F = 7.421$; $P < 0.001$): it was 0.94 (SE = 0.08) on third instar and 1.43 (SE = 0.09) on first instar, followed by 1.37 (SE = 0.07) on the second and 1.20 (SE = 0.05) on the fourth instars.

4. Discussion

Er. hayati could complete its life cycle on *B. tabaci* kept on several different crops under a wide range of temperatures (20, 22, 26, 30, and 34 °C) in the laboratory (Kuang et al., 2011). This study is the first one to establish the host instar-specific biological parameters for *Er. hayati* parasitizing *B. tabaci* biotype B.

Er. hayati successfully oviposited under all nymphal instars of *B. tabaci* on tomato with the exception of late fourth instar, and parasitized more first, second, and third instar nymphs than fourth instar. The congeneric *Er. mundus* also parasitize all instars of *B. tabaci* biotype B and biotype Q except the mature fourth instar or pharate adult, although parasitized most third instar nymphs (Jones and Greenberg, 1998; Urbaneja and Stansly, 2004). Data on *Eretmocerus melanoscutus* Zolnerowich and Rose are controversial: Liu (2007) reported that females parasitized least under first instars and most under third instars, followed by similar numbers of parasitized nymphs when ovipositing under the second and early fourth instars, while Zang and Liu (2008) reported that the species parasitized more first and second instar nymphs than third and fourth instars.

The mean age of *Er. hayati* females at first exposure to a host was 24 h. *Er. hayati* exhibit no preoviposition period since females parasitize *B. tabaci* upon emergence (own observations). In this characteristic, *Er. hayati* is similar to *Er. mundus* which exhibits no preoviposition period, either (Gerling and Fried, 2000; Ardeh, 2004; Urbaneja et al., 2007). However, not all *Eretmocerus* spp. are similar: a majority of *Er. melanoscutus* females (93%) did not lay eggs until

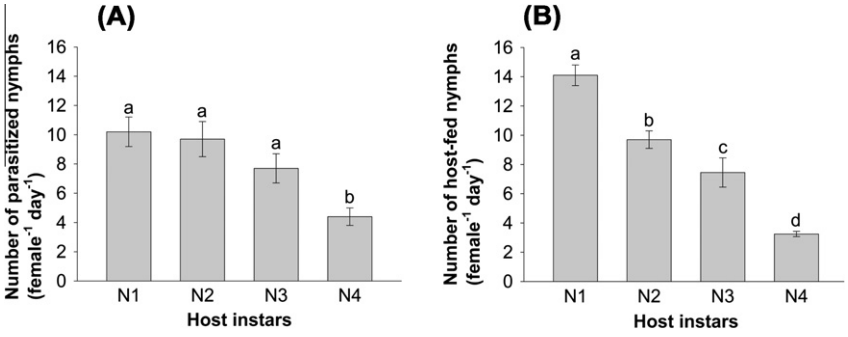

Fig. 1. Parasitization (A) and host-feeding (B) of *Er. hayati* under different instars of *B. tabaci* nymphs on tomato under laboratory conditions at 26 ± 1 °C, 65 ± 5% RH, and l4:10 (L:D) h. Host instars, N1, N2, N3, and N4 are first, second, third, and early fourth instars, respectively. *Er. hayati* did not oviposit and host-fed under late fourth instar.

Table 1
Developmental time (mean ± SE) of *Eretmocerus hayati* on different nymphal instars of *Bemisia tabaci* biotype B (n = number of replicates).

Host instars present[A] (days after oviposition)	Development time in days (n)[B]		
	Egg to pupa	Pupa to adult	Egg to adult
N1 (9)	12.3 ± 0.1 (179)a	5.1 ± 0.1 (124)a	17.4 ± 0.1 (124)a
N2 (11)	11.3 ± 0.1 (209)b	5.0 ± 0.1 (136)a	16.3 ± 0.2 (136)b
N3 (14)	9.1 ± 0.1 (170)c	5.1 ± 0.1 (144)a	14.2 ± 0.1 (144)c
N4 (16)	8.4 ± 0.1 (103)d	5.1 ± 0.1 (91)a	13.5 ± 0.1 (91)d

[A] N1, N2, N3, and N4 are first, second, third, and early fourth instars, respectively.
[B] Means followed by the same letter within the same column were not statistically different differences (HSD test; $P < 0.05$).

Table 2
Survivorship (mean ± SE) of *Eretmocerus hayati* on different nymphal instars of *Bemisia tabaci* biotype B (n = 20).

Parasitoid stage	Survivorship on different host instars (%)[a]			
	N1	N2	N3	N4
Egg	93.4 ± 2.1	93.7 ± 2.8	98.6 ± 0.9	99.2 ± 0.8
Larva	90.4 ± 4.3	96.7 ± 6.9	90.4 ± 4.0	88.9 ± 6.9
Pupa	86.2 ± 3.2	85.1 ± 2.4	87.2 ± 5.1	90.1 ± 6.0
Total	72.9 ± 4.8	74.1 ± 4.2	79.3 ± 6.1	74.1 ± 5.1

[a] None of them were statistically different by different host instars (HSD test, $P < 0.05$. df = 3, 90; $F = 0.252–2.816$; $P = 0.052–0.859$).

the second day after emergence, with a pre-oviposition period of 1–2 days (Liu, 2007).

Host-feeding by females of hymenopteran parasitoids is an asset in biological pest suppression (Jervis and Kidd, 1986). *Er. hayati* not only parasitize their hosts but also feed on them, increasing its impact by destructive host-feeding. Host-feeding of *Er. hayati* has not previously been reported in the literature. We observed that *Er. hayati* successfully host-fed on all nymphal instars of *B. tabaci* on tomato with the exception of late fourth instar, and fed more young instars than old ones. The results coincide with those found by Zang and Liu (2008) on *Er. melanoscutus*. An explanation could be that the parasitoids need to obtain a similar amount of nutrition (hemolymph) during their lifespan, and they would naturally consume more young (small) nymphs than old (large) ones if single instar was offered in no choice experiment (Zang and Liu, 2008).

Of the six species of *Eretmocerus* whose host-feeding has been documented, the daily mean consumption is 1.5 second-instar *B. tabaci* biotype Q by *Er. mundus* in 24 h (Urbaneja et al., 2007), 0.8–3.8 third-instar *Trialeurodes vaporariorum* Westwood by *Er. eremicus* (Zilahi-Balogh et al., 2006), 4.5 mixed *B. tabaci* biotype B instars by *Eretmocerus rui* Zolnerowich and Rose (McAuslane and Nguyen, 1996), and 0.5–1.1 second-instar *B. tabaci* biotype B by *Eretmocerus staufferi* Rose and Zolnerowich and *Eretmocerus tejanus* Rose and Zolnerowich in 72 h (Heinz and Parrella, 1998). *Er. hayati* consumed a lot more hosts in our experiment. Under natural conditions, parasitoids would be exposed to mixed-instar hosts. The effects of host density and choice in such circumstance are not yet known, but such information would help to improve the efficiency of *Er. hayati* as a biocontrol agent.

Development time of *Er. hayati* was longest when first instars were parasitized as compared to all other instars tested. The results were similar to those reported in the literature of three related species of *Er. mundus* (Foltyn and Gerling, 1985; Jones and Greenberg, 1998; Urbaneja and Stansly, 2004), *Er. rui* (McAuslane and Nguyen, 1996) and *Er. melanoscutus* (Liu, 2007). Gelman et al. (2005) stated that although *Er. mundus* eggs hatched under the second, third, and early fourth instar host nymphs, the larva remained under its whitefly host and did not penetrate until the whitefly had molted to its early fourth instar. The delay of development corresponded approximately to the time required for the whitefly host to develop to early forth instar, when the whitefly initiates the nymphal adult molt and adult development (Gerling, 1966; Hu et al., 2002).

There was no effect of host stage on preimaginal survivorship, as in *Er. mundus* (Urbaneja and Stansly, 2004) or *Er. melanoscutus* (Liu, 2007). However, survivorship varied with different species, evaluation methodologies, host biotypes, and host plants. Survivorship in *Er. mundus* is 84.2% on sweet pepper leaf disc (Urbaneja and Stansly, 2004), 69.5% on tomato and 76.6% on sweet pepper in clip cages when parasitizing *B. tabaci* biotype Q (Urbaneja et al., 2007). Survivorship in *Eretmocerus sp. nr. furuhashii* Rose and Zolnerowich ranges from 57.4% to 73.2% when parasitizing *B. tabaci* biotype B on different host plants (Qiu et al., 2005), and that of *Er. melanoscutus* is 93.4% on cabbage (Liu, 2007).

In conclusion, we demonstrated the suitability of *Er. hayati* to adapt to different environmental conditions, in this case host stage, and host plant and temperature in previous study (Kuang et al., 2011),that contribute to the value as a biological control agent of *B. tabaci*. Furthermore, our results showed that *Er. hayati* exhibits higher incidence of parasitism, host feeding, development time, and survivorship than that of *Encarsia bimaculata* Heraty and Polaszek (Qiu et al., 2006) and *Er.* sp. nr. *furuhashii* (Qiu et al., 2005), the two dominate species parasitizing *B. tabaci* in South China (Li et al., 2011); and *Encarsia formosa* Gahan (Zhang et al., 2003), which is abundant in north China in protected fields (Li et al., 2011). Research in the USA and Australia had confirmed the consistent performance of *Er. hayati* on *B. tabaci* in the laboratory and the field (Goolsby et al., 2005a; Gould et al., 2008; De Barro and Coombs, 2009). We consider that *Er. hayati* is a good candidate for biological control of *B. tabaci* in China. Still, the capacity to disperse between patches and search out prey, which are also critical attributes for success, are yet to be evaluated. Finally, the capacity to integrate a range of compatible control options in the agricultural ecosystem will be a key determinant of an effective whitefly control strategy.

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