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Article

## Prey preference, reproductive performance, and life table of *Amblyseius tsugawai* (Acari: Phytoseiidae) feeding on *Tetranychus urticae* and *Bemisia tabaci*

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### Abstract

*Amblyseius tsugawai* is a phytoseiid species distributed in Asia. In the present study, laboratory experiments were conducted to investigate its preference to prey stages when feeding on *Tetranychus urticae* and *Bemisia tabaci*, to evaluate its development and reproduction, and to estimate its life table parameters on each of the two prey species. Our results indicate that *A. tsugawai* shows preference to larvae of *T. urticae*, and to eggs of *B. tabaci*. When the two preferred stages were provided together, *A. tsugawai* randomly selected its prey. No significant difference in developmental times of *A. tsugawai* was observed when reared on *T. urticae* (6.75 days) and *B. tabaci* (7.05 days). In contrast, cumulative fecundity of *A. tsugawai* was only 14.6% when reared on *B. tabaci* (4.6 eggs/female) than on *T. urticae* (31.5 eggs/female). Impact of prey for both females and males on reproduction were also observed. Comparing to the treatment when both parents were reared on *T. urticae*, *A. tsugawai* fecundity was reduced by ca. 48.2% when the male was reared on *B. tabaci*, and was reduced by 86.5% and 88.8% when the female or both parents were reared on *B. tabaci*, respectively. *Amblyseius tsugawai* is the first indigenous phytoseiid species of China to show a positive intrinsic rate of population increase (0.013) when reared on *B. tabaci* as the only prey. The potential of *A. tsugawai* in greenhouse whitefly control is discussed.

**Key words:** whitefly, biological control, life table, prey preference

### Introduction

Predatory mites are important natural enemies of small arthropod pest mites and insects (Gerson *et al.* 2003; Zhang 2003; Hoy 2016). As the second largest group of biological control agents after the hymenopteran, the family Phytoseiidae includes about 30 species commercially produced worldwide (Van Lenteren 2012). However, this number is very small comparing to the total number of species presently described (>2300) (Chant & McMurtry 2007; Prasad 2012; Demite *et al.* 2014). There is a need for further investigations to discover more potential biological agents for practical use.

*Amblyseius tsugawai* Ehara (Acarina: Phytoseiidae) was first described from specimens collected on apples in Japan in 1959 (Ehara 1959). Subsequently, it has been reported from multiple surveys in orchards and vegetables in Japan (Kishimoto 2002; Toyoshima 2003; Toyoshima & Amano 2006; Gotoh *et al.* 2007; Gotoh & Mori 2011; Toyoshima *et al.* 2008; Sonoda *et al.* 2012; Funayama & Sonoda 2014; Wari *et al.* 2014; Funayama *et al.* 2015; Ishii *et al.* 2018), China (Wu 1987; Xia *et al.* 2002) and Korea (Ryu 2007).

This species is known to be a generalist predator, feeding on variety of foods, including spider mites (Funayama & Sonoda 2014; Funayama *et al.* 2015), thrips (Shibao *et al.* 2004), tea pollen, and

eggs of Mediterranean flower moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) (Kishimoto 2015). Moreover, in our preliminary studies, we observed that *A. tsugawai* can be reared on *Carpoglyphus lactis* (L.) (Acarina: Carpoglyphidae), and it showed high predation potential on *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) (Yang *et al.* 2018).

The whitefly *B. tabaci* is one of the most serious agricultural pests that injure various crops, especially greenhouse vegetables and ornamental plants worldwide (De Barro *et al.* 2011). In China, *B. tabaci* is also an important pest that causes up to 75% yield loss when it occurs at severe densities (Hu *et al.* 2011; Chu & Zhang 2018). Two phytoseiid species, *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) and *Amblydromalus limonicus* Garman & McGregor (Acari: Phytoseiidae) are effective biocontrol agents that have been widely used in Europe and North America for *B. tabaci* control (Nomikou *et al.* 2001, 2002; Calvo *et al.* 2011; Knapp *et al.* 2013). But no record of their local distribution in China has been reported. In addition, *A. swirskii* appear to be a strong competitor that may have negative impact on local phytoseiid species if introduced (Guo *et al.* 2016).

Alternatively, we have been screening for domestic predators that can efficiently control *B. tabaci*. Predatory mites, such as *Amblyseius orientalis* Ehara (Acari: Phytoseiidae) and *Amblyseius eharai* Amitai & Swirski (Acari: Phytoseiidae), were identified as potentially capable of feeding on *B. tabaci*, but they hardly produced any eggs when only using this prey (Ji *et al.* 2013ab; Zhang *et al.* 2015). The ability of *A. tsugawai* to feed on *B. tabaci* also encouraged us to evaluate its potential as a candidate for biological control of this pest.

In the present study, laboratory experiments were carried out to investigate preference of *A. tsugawai* to prey stages, prey preference of *A. tsugawai* to *T. urticae* and *B. tabaci*, life table parameters of *A. tsugawai* when reared on *T. urticae* or *B. tabaci*, and *A. tsugawai* reproduction when the parents were fed with different prey species. Its potential in greenhouse whitefly control and directions for further evaluations were also discussed.

## Material and methods

### *Mites and whitefly colonies*

*Amblyseius tsugawai* and *T. urticae* individuals were obtained from the colonies at the Laboratory of Predatory Mites, Institute of Plant Protection, Chinese Academy of Agricultural Sciences (CAAS). The *A. tsugawai* colony was established in 2014 using individuals collected from weeds near a wheat field, Kaifeng, Henan, and maintained on *C. lactis*. The *T. urticae* colony was reared on 2-week-old bean (*Phaseolus vulgaris* L.) seedlings. *Bemisia tabaci* individuals were obtained from Laboratory of Insects, Institute of Vegetables and Flowers, CAAS, and reared on tobacco (*Nicotiana tabacum* L.) seedlings. The mites and insects used in the present study were all maintained at 25±1°C, 70%±5% RH and 16L: 8D, while experiments were conducted under the same conditions.

Experiments were conducted in small units, and each was built with 3 layers of transparent acrylic boards (60×40×5mm). A piece of bean leaf was placed on the bottom layer and the intermediate layer that has a 30mm diameter hole in the center. Mites and pests were placed in this hole for experiments. Then the third layer was covered on the top. Finally, the experimental unit was sealed with the three acrylic board layers tightly clipped on both ends.

### *Prey preference of A. tsugawai*

Prey stage preference of *A. tsugawai* was determined using different stages of the two prey, *T. urticae* (eggs, larvae, and protonymphs) and *B. tabaci* (eggs, 1<sup>st</sup> instar larvae). The preference of *A. tsugawai* was determined on the most preferred stages of the two prey. Females were singly

transferred to experimental units, starved for 24 h, before being subjected to prey stage mixture consisting of 10 individuals of each stage. At least 15 females were tested for each prey stage combination, and each female was allowed to prey for 12h.

The number of each prey stage consumed was recorded, and the prey stage preference index ( $\alpha$ ) was estimated using the equation of Wilson & Gutierrez (1980):

$$\alpha = \left( \frac{N_{ai}}{N_i} \right) / \text{Max} \left( \frac{N_{ai}}{N_i} \right) \quad (1)$$

Where  $N_i$  is the number of the  $i^{\text{th}}$  prey kind available, and  $N_{ai}$  is the number of the  $i^{\text{th}}$  prey type consumed. For each test, the preference index of the most preferred prey stage/species equals 1. Mean predation and prey preference indices to each prey stage/species were compared using paired t-test in experiments for *B. tabaci* stages and most preferred stages of two species, and using one-way ANOVA followed by Turkey HSD multiple comparison test for the experiment for preferences among *T. urticae* stages. All analyses were conducted using SPSS 21.0.

#### *Development, reproduction, survival, and life table parameters of A. tsugawai*

The experiment was initiated with a cohort of newly laid *A. tsugawai* eggs, all produced within 24 h. Each egg was singly transferred to an experimental unit and subjected to one of the following two prey treatments: 15 mixed larvae and protonymphs of *T. urticae* per day (110 replicates); 20 *B. tabaci* eggs per day (155 replicates). No prey was provided for eggs. When hatched, each individual was moved into a new experimental unit with its prey supply daily. The amount of prey exceeded maximum daily consumption rate of the predator based on preliminary observations.

Experimental units were observed every 12 h to record *A. tsugawai* development. When reaching adulthood, predators were sexed, and each female was paired with a single male. Each pair (female-male) was reared until death of the female. Males were replaced if died. Experimental units were observed daily and eggs laid by each female were recorded. Developmental duration, longevity, reproductive parameters (preoviposition duration, oviposition duration, fecundity), and proportion of female offspring were compared using t-test.

Life table parameters of *A. tsugawai* were estimated based on age-stage two sex life table (Chi & Liu 1985; Chi 1988), using TWOSEX-MS Chart. Age-specific survival rate ( $l_x$ ) and age specific fecundity rate ( $m_x$ ) were calculated based on daily fecundity and survival. The intrinsic rate of population increase ( $r_m$ ), net reproductive rate ( $R_0$ ), finite rate of population increase ( $\lambda$ ), mean generation time ( $T$ ), and gross reproductive rate ( $GRR$ ) were estimated using Eq. 2–6:

$$\sum_{x=0}^{\infty} e^{-r_m(x+1)} l_x m_x = 1 \quad (2)$$

$$R_0 = \sum_{x=0}^{\infty} l_x m_x \quad (3)$$

$$\lambda = e^{r_m} \quad (4)$$

$$T = \ln R_0 / r_m \quad (5)$$

$$GRR = \sum_{x=0}^{\infty} m_x \quad (6)$$

#### *Impact of prey species on survival and reproduction of A. tsugawai*

Immature stages of *A. tsugawai* were reared on either *T. urticae* (mixed larvae and protonymphs) or *B. tabaci*. When reaching adulthood, predators were sexed and females and males reared on each prey were kept apart for subsequent experiments. The following four possible combinations involving females and males reared on each of the prey species were created: ♀-*T. urticae* × ♂-*T. urticae*, ♀-*T. urticae* × ♂-*B. tabaci*, ♀-*B. tabaci* × ♂-*T. urticae*, and ♀-*B. tabaci* × ♂-*B. tabaci*. Each combination was replicated for 15 times. Each pair was allowed to mate for 24 h in the absence of any food. Then the males were removed, and the gravid females were reared on the same prey as they were fed during immature stage. The experimental units were observed daily and

the number of eggs laid was recorded until female death. A single-factor ANOVA was used to compare the preoviposition and oviposition durations, fecundity, egg hatching rates, and offspring sex ratios. Means were compared using the Tukey HSD test at  $\alpha=0.05$ .

## Results

### *Prey preference of A. tsugawai*

For *T. urticae*, *A. tsugawai* females consumed significantly more larvae than eggs and protonymphs ( $p=0.043$ ), while for *B. tabaci* they consumed significantly more eggs than 1<sup>st</sup> instar larvae ( $p=0.025$ ). When the most two preferred stages of the two prey species were provided together, females consumed 6.2 larva of *T. urticae* and 5.3 eggs of *B. tabaci* eggs in 12h on the average, but no significant difference ( $p=0.203$ ) in either predation or preference index was obtained between the two kinds of prey (Table 1).

**TABLE 1.** Prey stage preferences of *Amblyseius tsugawai* adult females to stages of *Tetranychus urticae* and *Bemisia tabaci*.

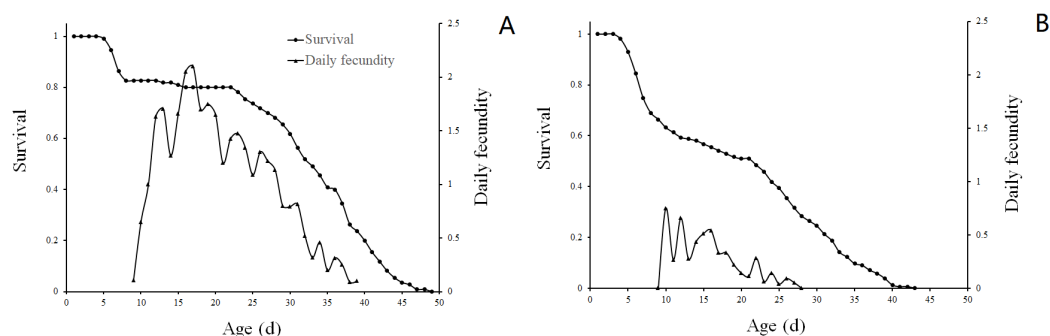
Experiment No.	Replicates	Prey Species	Prey Stage	Predation	Preference Index*
1	21	<i>T. urticae</i>	egg	3.2±0.4b	0.58±0.07b
			larva	5.0±0.5a	0.87±0.06a
			protonymph	4.0±0.5ab	0.67±0.06b
2	15	<i>B. tabaci</i>	egg	5.2±0.5a	0.91±0.05a
			1st instar larva	3.5±0.4b	0.66±0.08a
3	20	<i>T. urticae</i>	larva	6.2±0.4a	0.89±0.05a
		<i>B. tabaci</i>	egg	5.3±0.5a	0.75±0.06a

\* For each experiment, 10 individuals of each choice were provided, and preference index (means ± s.e.) within a column followed by different lowercase letters are significantly different at  $p<0.05$ .

### *Development, reproduction, survival, and life table parameters of A. tsugawai*

*Amblyseius tsugawai* was able to complete its life cycle on either prey species. Mean developmental time was 0.3 days longer on *T. urticae* than on *B. tabaci*, while immature survival was reduced from 82.7% to 63.9%. When reared on *T. urticae*, the predator immature mortality was mainly observed during the larval stage, whereas on *B. tabaci*, higher mortality was recorded during the deutonymph stage (Table 2, Fig. 1). In addition, most individuals that died during immature stages were also retarded in development. For examples, eggs that failed to hatch all shriveled on 5<sup>th</sup> or 6<sup>th</sup> days, so that their mortality was recorded on the 5<sup>th</sup> or 6<sup>th</sup> day (Fig. 1).

Adult longevity of *A. tsugawai* females and males were 22.6 days and 23.5 days, respectively, shorter when reared on *B. tabaci* than on *T. urticae*. In addition, females reared on *B. tabaci* recorded a 27.6% longer preoviposition duration and a 68.3% shorter oviposition duration, along with 85.4% lower fecundity (Table 3). Female age-specific survival rate ( $l_x$ ) and age-specific fecundity ( $m_x$ ) were presented in Fig. 1. The peak daily fecundity was observed on day 18 and day 9, ca. the 9<sup>th</sup> and 1<sup>st</sup> day of oviposition duration, on *T. urticae* (2.1 eggs/day) or *B. tabaci* (0.8 eggs/day), respectively. The life table parameters estimated from the two treatments were summarized in Table 4. Intrinsic rates of population increase were 0.145 and 0.013 when reared on *T. urticae* and *B. tabaci*, respectively.



**FIGURE 1.** Age-specific survival and fecundity curves of *Amblyseius tsugawai* reared on (A) mixed larvae and protonymphs of *Tetranychus urticae*, and (B) eggs of *Bemisia tabaci*.

**TABLE 2.** Juvenile survival and development (means  $\pm$  s.e.) of *Amblyseius tsugawai* reared on *Tetranychus urticae* (mixed larvae and protonymphs) and *Bemisia tabaci* (eggs) at 25°C.

<i>Amblyseius tsugawai</i> stage						
Prey species	Response variable	Egg	Larval	Protonymph	Deutonymph	Total Immature
<i>Tetranychus urticae</i>	survival	0.982	0.861	0.979	1.000	0.827
	(n)	(110)	(108)	(93)	(91)	(91)
	developmental duration (d)	1.87 $\pm$ 0.03	1.24 $\pm$ 0.08a	1.91 $\pm$ 0.05a	1.73 $\pm$ 0.06a	6.75 $\pm$ 0.08a
<i>Bemisia tabaci</i>	survival	0.974	0.867	0.863	0.876	0.639
	(n)	(155)	(151)	(131)	(113)	(99)
	developmental duration (d)	1.87 $\pm$ 0.02	1.03 $\pm$ 0.06b	1.88 $\pm$ 0.05a	2.29 $\pm$ 0.08b	7.05 $\pm$ 0.09b
P for developmental duration		-	0.02	0.838	<0.001	0.021

Developmental durations with means  $\pm$  s.e. followed by different lowercase letters are significantly different at  $p < 0.05$  between the two prey species, compared using student t-test.

**TABLE 3.** Reproductive parameters of *Amblyseius tsugawai* when reared on *Tetranychus urticae* (mixed larvae and protonymphs) and *Bemisia tabaci* (eggs) at 25°C.

Response variable	Prey species				
	<i>Tetranychus urticae</i>		<i>Bemisia tabaci</i>		P
	n	mean $\pm$ s.e.	n	mean $\pm$ s.e.	
Female longevity (days)	41	33.2 $\pm$ 1.2a	42	25.7 $\pm$ 1.1b	<0.001
Male longevity (days)	50	34.4 $\pm$ 1.0a	57	26.3 $\pm$ 1.2b	<0.001
Preoviposition duration (days)	41	2.9 $\pm$ 0.1a	32	3.7 $\pm$ 0.3b	0.007
Oviposition duration (days)	41	18.3 $\pm$ 0.8a	32	5.8 $\pm$ 0.8b	<0.001
Cumulative fecundity (eggs/female)	41	31.5 $\pm$ 1.6a	32	4.6 $\pm$ 0.6b	<0.001
Prop. female offspring	41	0.57 $\pm$ 0.08a	32	0.42 $\pm$ 0.03b	<0.001

Means  $\pm$  SE followed by different lowercase letters in the same row were significantly different at  $p < 0.05$  compared using student t-test.

**TABLE 4.** Life table parameters of *Amblyseius tsugawai* when reared on *Tetranychus urticae* (mixed larvae and protonymphs) and *Bemisia tabaci* (eggs) at 25°C.

Life table Parameter	Prey species	
	<i>Tetranychus urticae</i>	<i>Bemisia tabaci</i>
Intrinsic rate of increase ( $r_m$ )	0.145	0.013
Net reproductive rate ( $R_0$ )	11.75	1.23
Finite rate of increase ( $\lambda$ )	1.156	1.013
Mean generation time ( $T$ )	16.96	15.41
Gross reproductive rate ( $GRR$ )	15.6	2.25

#### *Impact of prey species on survival and reproduction of A. tsugawai*

Impact of prey species for the parents on reproduction parameters of *A. tsugawai* were summarized in Table 5. The females had the longest oviposition period and the highest fecundity when both females and males were reared on *T. urticae*. When only females were reared on the same prey, the oviposition period and fecundity decreased by ca. 31.5% and 48.3%, respectively, compared to above combination. When females or/and males were reared on *B. tabaci*, a more significant decrease in the reproductive parameters was observed with the oviposition period and fecundity being ca. 6 days and ca. 4 eggs, respectively. The two treatments also recorded lower egg viabilities. Across all four treatments, proportion of female offspring was positively correlated with fecundity (corr = 0.951, p = 0.049).

**TABLE 5.** Reproductive parameters of *Amblyseius tsugawai* as affected by prey for both parents at 25°C.

Prey for female		<i>T. urticae</i>	<i>T. urticae</i>	<i>B. tabaci</i>	<i>B. tabaci</i>	P
Prey for male		<i>T. urticae</i>	<i>B. tabaci</i>	<i>T. urticae</i>	<i>B. tabaci</i>	
Response variables	Preoviposition duration (days)	0.9±0.1b	2.3±0.2a	3.3±0.4a	3.6±0.7a	<0.001
	Oviposition duration (days)	16.1±1.5a	11.0±1.0b	5.6±0.9c	5.6±1.1c	<0.001
	Cumulative fecundity (eggs per female)	30.3±2.79a	15.7±2.6b	4.1±0.5c	3.4±0.9c	<0.001
	Offspring hatching rate (%)	80.8±1.9a	72.8±5.4a	38.9±7.0b	22.9±11.2b	<0.001
	Prop. female offspring	0.61±0.02a	0.40±0.06b	0.32±0.10bc	0.20±0.07c	0.001

Means ± SE followed by different lowercase letters in the same row were significantly different at p < 0.05 across the treatments, compared using Tukey-HSD.

## Discussion

In this study, we assessed the suitability of *T. urticae* and *B. tabaci*, as prey for the predator *A. tsugawai*. Results of our prey stage preference experiments suggested that the predator prefers the larva to egg and protonymph stages of *T. urticae*, while it prefers eggs to the first instar of *B. tabaci*. When the two most preferred stages are provided together, the predator seemed to show no preference. This observation is similar to previous observations by Zhang *et al.* (2015) studying preference of the phytoseiid *A. orientalis* to spider mites and whiteflies. Previous studies showed that preference of generalist predators are tradeoffs among multiple factors, including nutrition quality, prey quantity, and the ease to detect and access, etc. Sometimes nutritionally inferior prey might be more preferred than nutritionally superior prey (Eubanks & Denno 2000; Chailleux *et al.* 2014).

Generalist predators with random selection suggested predation potential even to pest with lower nutrition quality.

*Amblyseius tsugawai* is the first indigenous phytoseiid species of China that showed positive intrinsic rate of population increase when fed solely with *B. tabaci* as prey. In addition, comparing to *A. orientalis* that used to be found and applied in orchards (Yang *et al.* 1987; Zhang *et al.* 1992), this species might be better adapted to herbaceous plants. Surveys conducted in orchards and woody plants always showed low densities of *A. tsugawai* (Kishimoto 2002; Toyoshima 2003; Toyoshima and Amano 2006; Toyoshima *et al.* 2008; Sonoda *et al.* 2012; Wari *et al.* 2014; Ishii *et al.* 2018). In contrast, *A. tsugawai* appeared to be a dominant species in soybean and other beans (Gotoh *et al.* 2007; Gotoh & Mori 2011). Funayama & Sonoda (2014) reported higher densities of *A. tsugawai* on *Plantago asiatica* L. in apple orchards than on apple trees. The colony used in the present study was established from individuals collected in weeds near wheat fields. McMurtry *et al.* (2013) categorized generalist predatory phytoseiids into 5 groups based on microhabitat and morphology, including predators living on dicotyledonous plants or on monocotyledonous plants. We think it is also valuable to identify whether a predator is more adapted to woody or herbaceous plants, and to further verify our hypothesis here that predators preferring herbaceous host plants might better adapt to greenhouse environment.

However, the reproduction of *A. tsugawai* when eating *B. tabaci* is still very low, being only 14.6% comparing to that when using *T. urticae* as prey. This result suggested low chance for population establishment. Poor establishment is always a big problem for augmentative biological control. Besides multiple releases, natural enemy population can also be enhanced by providing additional resources, such as alternative food, prey, hosts, oviposition sites or shelters (Messelink *et al.* 2014). Previous studies showed that when *A. swirskii* and *A. limonicus* were used to control whiteflies in greenhouses, their chances for population establishment and control efficiency can be improved through providing pollen as alternative food (Nomikou *et al.* 2010; Lee & Zhang 2018; Kishimoto 2015). It was reported that each *A. tsugawai* female laid more than 20 eggs in 10 days when fed with tea pollen (Kishimoto 2015); this fecundity is comparable to what we achieved when *A. tsugawai* was reared on *T. urticae* (ca. 1.72 eggs per day). It is reasonable to assume that supplying alternative food, such as pollen, to *A. tsugawai* in greenhouses would be helpful to improve its population establishment and to achieve longer efficient control of *B. tabaci*.

In the present study, we also estimated impact of prey species on *A. tsugawai* reproduction. Reproduction and offspring sex allocation are generally considered to be regulated precisely by the female (Nagelkerke & Sabelis, 1998; Walzer & Schausberger, 2015). In the present study, when only the male was under poor nutritional condition (reared on *B. tabaci*), *A. tsugawai* fecundity was reduced by 48.2%, and male biased offspring sex ratio was observed. Although this fecundity and proportion of female offspring are higher than when the female was under poor nutritional, the impact of male status on reproduction should not be neglected. An interesting question is how males impact reproduction? The most possible situation is that they just produced fewer sperms, but there are also possibilities they can deliver signals of environmental pressure in other ways. In one of our recent studies, we counted number of sperms per spermatophore in *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) at different timings of mating (Jiang *et al.* 2019). Similar observations will be carried out on more phytoseiid species under different levels of environmental pressure to further clarify the role played by males in reproductive regulation.

Overall, although *A. tsugawai* is still not an ideal natural enemy for the control of greenhouse whiteflies, it appears to be the best domestic phytoseiid predator of *B. tabaci* in China at present. Field evaluations should be performed to further estimate its biological control potential, especially with strategies to enhance its population establishment and long term control efficiency, such as providing supplementary food.

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