Suitability of Bemisia tabaci (Hemiptera: Aleyrodidae) biotype-B and Myzus persicae (Hemiptera: Aphididae) as prey for the ladybird beetle, Serangium japonicum (Coleoptera: Coccinellidae)

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Key words. Hemiptera, Aleyrodidae, Bemisia tabaci, cotton whitefly, Aphididae, Myzus persicae, green peach aphid, Coleoptera, Coccinellidae, Serangium japonicum, prey suitability, biological control

Abstract. The ladybird, Serangium japonicum (Coleoptera: Coccinellidae), is one of the most important predators of whiteflies in China, however, the suitability of different kinds of prey and nutritional requirements of this predator are poorly studied. The objectives of the present study were to investigate the pre-imaginal development, percentage survival of immatures, adult longevity and fecundity and percentage of eggs hatching when fed on Myzus persicae (Sulzer) (Hemiptera: Aleyrodidae) biotype-B (i.e., eggs and nymphs) and green peach aphid Myzus persicae (Sulzer) (Hemiptera: Aphididae) in order to quantify their relative suitability as prey under laboratory conditions of 25 ± 1°C, 50 ± 10% RH and a photoperiod of 16L : 8D. The results of this study indicate that the total developmental period (from egg to adult) was shorter when they were fed on B. tabaci (18.29 ± 0.13 days) than on M. persicae (19.85 ± 0.22 days). The percentage survival of the immatures, growth index, development rate, adult fresh weight, fecundity and percentage of eggs hatching were higher and the pre-oviposition period was significantly shorter when S. japonicum were fed on B. tabaci than on M. persicae. In contrast, there was no significant difference in their longevity and oviposition period when fed on B. tabaci and M. persicae. The results indicate that as a diet for S. japonicum M. persicae is inferior to B. tabaci. This is the first record of S. japonicum being able to complete its development feeding exclusively on M. persicae. Since B. tabaci and M. persicae often simultaneously occur in vegetable fields, these results indicate that it is likely that S. japonicum could be utilized to control mixed populations of aphids and whiteflies, and further our understanding of ladybird population dynamics in the field in relation to the availability of different species of prey.

1. INTRODUCTION

The cotton whitefly, Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae), occurs worldwide in tropical and subtropical climates as well as in protected agricultural systems in temperate regions. It is an important pest of more than 600 species of plants of numerous field and vegetable crops in many parts of the world (Naranjo et al., 2010). Whiteflies cause serious damage by feeding, producing honeydew and the resultant sooty mould, and disease transmission (Stansly & Natwick, 2010). Although known as a sporadic pest of cotton in China for many years, Bemisia tabaci Biotype-B did not become an important pest in northern China until 2000 (Wu et al., 2002), and is now widely distributed across China (Hu et al., 2011). Many attempts have been made to control B. tabaci. Because of its high reproductive rate, many generations per year and ability to rapidly develop resistance to insecticides, whiteflies are difficult to control using conventional means (Castle et al., 2010). Thus, biological control becomes the primary method for suppressing whitefly populations in agroecosystems (Arno et al., 2010).

The green peach aphid, Myzus persicae (Sulzer) (Hemiptera: Aphididae), is a highly polyphagous insect pest, which feeds on more than 400 species of plants belonging to over 50 families including agricultural crops (e.g., potato, sugar beet and tobacco), horticultural crops (e.g., plants of Brassicaceae, Solanaceae and Cucurbitaceae) and stone fruit (e.g., peach, apricot and cherry) around the world. It causes enormous economic losses because it reduces crop yields by sucking plant sap, transmits over 100 plant viruses and secretes honeydew (Blackman & Eastop, 2000; Kim et al., 2005). Currently, control of M. persicae is mostly based on insecticides, and high levels of insecticide resistance are recorded in many parts of the world (Li et al., 2005).
2016). Effective and environmentally sound management of this whitefly and aphid is needed, and biological control using natural enemies is an attractive option.

The ladybird, *Serangium japonicum* Chapin (Coleoptera: Coccinellidae), is an important predator of whiteflies and scale insects (Ren et al., 2009). It occurs in China and Japan, where *S. japonicum* is potentially a good biological control agent of *B. tabaci* in various crop systems in China, especially vegetables and cotton (Ren et al., 2001). *Serangium japonicum* larvae and adults feed on the immature stages of *B. tabaci* and can effectively suppress its population growth (Li et al., 2015). However, the nutritional ecology, prey preference and food requirements for development and reproduction of *S. japonicum* are not well studied.

Food quality is one of the most important factors influencing the vigour of predators because not all species of prey are equally suitable as food. These influences range from slight effects such as longer developmental times, or a decrease in percentage larval survival to dramatic effects such as toxicity of certain species of prey resulting in high mortality (Obrycki & Kring, 1998). Given that there may be a number of alternative prey available to some predatory insects in an agroecosystem, preference for and the suitability of prey for development are important in determining the potential of predators as biological control agents of a specific pest, which may determine the success or failure of predators in biological control systems (Thompson, 1999). Furthermore, many predatory insects are opportunistic predators in the field and feed on a wide range of species of prey and plant products, but this behaviour may not clearly indicate prey suitability and predator nutritional requirements (Strohmeyer et al., 1998).

In developing an effective IPM program for controlling *B. tabaci* it would be advantageous to maximize the effectiveness of the chosen natural enemies. During a preliminary investigation, we found that *S. japonicum* frequently fed not only whiteflies, but also on the green peach aphid in vegetable fields in northwestern China. This prompted us to speculate that *S. japonicum* could prey on both whiteflies and aphids. Since *S. japonicum* is known as a promising predator of whiteflies, we therefore hypothesized that different species of prey might vary in their suitability as prey for *S. japonicum*. The objective of this study was to determine whether *B. tabaci* and *M. persicae* were equally suitable for the development, survival, longevity and fecundity of *S. japonicum*.

2. MATERIALS AND METHODS

2.1. Insects

*Serangium japonicum* adults were originally collected from the experimental farm of the Northwest A&F University (Yangling, Shaanxi, China; 34°17′09.35″ N and 108°04′17.12′′ E). They were maintained in the laboratory on potted cabbage (*Brassica oleracea* L.) infested with immature *B. tabaci*. All cultures were kept in nylon mesh covered cages (60 × 60 × 60 cm) in a greenhouse maintained at 20–30°C under a natural light regime.

*Myzus persicae* used as prey for the ladybird was cultured on sweet pepper (*Capsicum annuum* L.) plants in the laboratory for 3 years. *Bemisia tabaci* B biotype was collected from tomato (*Solanum lycopersicum* L.) in a greenhouse in Yangling, Shaanxi, China (34°17′37.01″ N and 108°01′03.34″ E). They were reared on cabbages in mesh covered cages (60 × 60 × 60 cm) in the same greenhouse. The whiteflies were identified as *B. tabaci* B-biotype by RAPD-PCR using the mitochondrial COI gene (Wu et al., 2002). To produced mixed ages of *B. tabaci* (eggs and nymphs), cabbage plants were exposed to *B. tabaci* adults in the stock culture cages for 48 h, and then incubated in growth chambers under the same conditions as the ladybirds and aphids. To avoid food adaptation, populations of the adult beetles were established in the same greenhouse and supplied with a mixture of *B. tabaci* and *M. persicae* on their respective host plants in mesh covered cages (60 × 60 × 60 cm) for several generations before they were used in this study.

2.2. Pre-adult development and immature survival

Effect of prey species on the pre-adult development and immature survival of *S. japonicum* was evaluated. Leaves with beetle eggs (<2 h old, about eighty eggs) were excised from plants and placed in two plastic boxes (length × width × depth = 8 × 6 × 3 cm, covered with fine muslin cloth for ventilation) until they hatched. A piece of filter paper was placed at the bottom of the boxes, and a few drops of water were added as needed to maintain humidity. The boxes with the insects were kept in a climatic chamber (25 ± 1°C, 50 ± 10% RH under a photoperiod of 16L : 8D). The *S. japonicum* eggs were inspected carefully every 6 h and the number that hatched were recorded. Newly hatched first instars of *S. japonicum* (<6 h old) were individually transferred to Petri dishes (9.0 cm in diameter and 3.0 cm in height) using a soft camel hair brush and placed in the close vicinity of the prey. A piece of filter paper was placed at the bottom of the Petri dish and a few drops of water were added as needed to maintain humidity. Our preliminary test showed that the first instar larvae of *S. japonicum* did not feed on the adult aphids, so only first instar nymphs of *M. persicae* (about 20–30) were fed to first instar larvae of *S. japonicum*. A mixed age group of aphids (about 60–80) were selected for testing after the first instar ladybirds moulted to the second instar. The sweet pepper leaf with aphids was replaced every 12 h to avoid microbial contamination.

To provide whitefly prey, a cabbage leaf infested with mixed ages of *B. tabaci* was put into a Petri dish (9.0 cm in diameter and 3.0 cm in height). One first instar larva of *S. japonicum* (<6 h old) was added to the arena. Afterwards, each *S. japonicum* larva was transferred daily to a new arena containing fresh prey.

All experiments were conducted in climatic chambers at 25 ± 1°C. 50 ± 10% RH under a photoperiod of 16L : 8D. Forty newly hatched *S. japonicum* larvae (<6 h old) were reared on each category of prey. *S. japonicum* larvae were checked for molting or death every 6 h. The duration of development of each immature stage of *S. japonicum* was recorded and the initial body mass of newly-emerged adults was determined by weighing them individually on an electronic balance (Mettler-Toledo XS64, Greifensee, Switzerland) with a precision of 0.1 mg.

Percentage immature survival, adult emergence and development rate (1/developmental period) were recorded using the following formulae, and the growth index is that proposed by Dubey et al. (1981). Survival was calculated as: Percentage survival (%) = Number surviving in an assessed instar / Number surviving in the previous instar × 100. Growth index was calculated as: Growth index = Percentage pupation / Developmental time of larva × 100. Percentage puation (%) = Number of pupae / Number of fourth instar larvae × 100. Percentage adult emergence (%) = Number of adults emerged / Number of pupae × 100. Percentage
survival to the adult stage (%) = Number of adults that emerged/Initial number of first instar larvae × 100.

2.3. Longevity and reproductive performance

To evaluate the longevity and reproductive performance of *S. japonicum*, newly emerged adults were sexed and paired. A total of fifteen randomly selected pairs were used. Each pair was kept in a Petri dish (9.0 cm in diameter and 3.0 cm in height). A piece of filter paper was placed at the bottom of the Petri dish and a few drops of water added as needed to maintain humidity. Petri dishes were placed in climatic chambers with the same settings as above. Fresh leaf disks with *B. tabaci* or *M. persicae* were provided daily as a source of food. Each couple was observed daily to record longevity, pre-oviposition period, oviposition period and fecundity (number of eggs laid) until all beetles died.

2.4. Data analysis

Duration of development of immature stages, developmental rate, longevity, pre-oviposition period, oviposition period, fecundity and adult body weight of *S. japonicum* were analyzed using one-way analysis of variance (ANOVA); percentage survival was analyzed using the survival analysis (Log Rank test). The experimental data were analyzed using the statistical package SPSS 10.0 (2000; Chicago, IL, USA).

3. RESULTS

3.1. Development and survival of immature *S. japonicum*

The duration of development of the eggs, third instar and fourth instar larvae of *S. japonicum* were shorter when they fed on *B. tabaci* than on *M. persicae* (Table 1). When larvae of *S. japonicum* fed on *B. tabaci*, the developmental time of the pupa increased by 17.1% compared to those fed on *M. persicae* (Table 1). The total developmental period (from egg to adult) was significantly shorter when they fed on *B. tabaci* than on *M. persicae* (Table 1). The body mass of newly emerged adults differed significantly after feeding on *M. persicae* compared to *B. tabaci*, and was higher when fed on *B. tabaci* (Table 1). The percentage survival of first, second, third and fourth instar larvae and the pupal stage did not vary significantly between *S. japonicum* larvae fed on *B. tabaci* and *M. persicae* ($\chi^2 = 1.459, P = 0.227$) (Fig. 1). The percentage of larvae reaching the adult stage was much higher (95%) when fed on *B. tabaci* than on *M. persicae* (67.5%) ($P_{0.05} = 9.928, P < 0.005$).

Table 1. Duration of development (days) of the different immature stages and adult body mass of *Serangium japonicum* fed on *Myzus persicae* and *Bemisia tabaci*.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Duration of development, days ± SE</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Bemisia tabaci</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg</td>
<td>3.83 ± 0.01</td>
<td>1.79</td>
<td>652.552</td>
<td>0.0001</td>
</tr>
<tr>
<td>First instar</td>
<td>2.16 ± 0.06</td>
<td>1.67</td>
<td>0.935</td>
<td>0.337</td>
</tr>
<tr>
<td>Second instar</td>
<td>1.77 ± 0.03</td>
<td>1.64</td>
<td>1.546</td>
<td>0.218</td>
</tr>
<tr>
<td>Third instar</td>
<td>1.52 ± 0.06</td>
<td>1.62</td>
<td>59.652</td>
<td>0.0001</td>
</tr>
<tr>
<td>Fourth instar</td>
<td>4.18 ± 0.04</td>
<td>1.62</td>
<td>4.714</td>
<td>0.034</td>
</tr>
<tr>
<td>Pupa</td>
<td>4.83 ± 0.06</td>
<td>1.62</td>
<td>60.053</td>
<td>0.0001</td>
</tr>
<tr>
<td>Egg–adult</td>
<td>18.29 ± 0.13</td>
<td>1.61</td>
<td>33.030</td>
<td>0.0001</td>
</tr>
<tr>
<td>Adult body weight (mg)</td>
<td>1.53 ± 0.02</td>
<td>1.61</td>
<td>6.065</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td><em>Myzus persicae</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg</td>
<td>4.94 ± 0.04</td>
<td>1.79</td>
<td>652.552</td>
<td>0.0001</td>
</tr>
<tr>
<td>First instar</td>
<td>2.33 ± 0.09</td>
<td>1.67</td>
<td>0.935</td>
<td>0.337</td>
</tr>
<tr>
<td>Second instar</td>
<td>1.93 ± 0.12</td>
<td>1.64</td>
<td>1.546</td>
<td>0.218</td>
</tr>
<tr>
<td>Third instar</td>
<td>2.08 ± 0.04</td>
<td>1.62</td>
<td>59.652</td>
<td>0.0001</td>
</tr>
<tr>
<td>Fourth instar</td>
<td>4.43 ± 0.13</td>
<td>1.62</td>
<td>4.714</td>
<td>0.034</td>
</tr>
<tr>
<td>Pupa</td>
<td>4.14 ± 0.08</td>
<td>1.62</td>
<td>60.053</td>
<td>0.0001</td>
</tr>
<tr>
<td>Egg–adult</td>
<td>19.85 ± 0.22</td>
<td>1.61</td>
<td>33.030</td>
<td>0.0001</td>
</tr>
<tr>
<td>Adult body weight (mg)</td>
<td>1.43 ± 0.03</td>
<td>1.61</td>
<td>6.065</td>
<td>0.017</td>
</tr>
</tbody>
</table>

Fig. 1. Percentage survival of the immature stages of *Serangium japonicum* fed on *Myzus persicae* and *Bemisia tabaci*.

3.2. Reproductive potential and longevity on different species of prey

The growth index of *S. japonicum* was higher when fed on *B. tabaci* than on *M. persicae* (Table 2). The developmental rate of the ladybirds was similar on the two species of prey (Table 2). When *S. japonicum* fed on *B. tabaci*, the preoviposition period markedly decreased by 55.5% compared to when fed on *M. persicae* (Table 2), but the percentage of eggs that hatched significantly increased (Table 2). The oviposition period and longevity did not differ significantly when *S. japonicum* was fed on *B. tabaci* or *M. persicae*, although they were shorter when the ladybird fed on *B. tabaci* (Table 2). The fecundity of the ladybird adults fed *B. tabaci* was significantly increased by 59.3% compared to on *M. persicae* (Table 2).

Over most of the oviposition period, daily fecundity of *S. japonicum* fed on *B. tabaci* was higher than on *M. persicae* (Fig. 2). Daily fecundity of the ladybird was more than 10 eggs per female in the periods 14 to 19 days, 27 to 29 days and 33 to 36 days after the onset of oviposition. When fed on *B. tabaci* a maximum daily fecundity of 46 eggs was recorded on day 17. Mean total fecundity per female was 294.5 eggs when fed on *B. tabaci* (Table 2) and a single female laid 489 eggs. When *S. japonicum* fed on *M. persicae*, the daily fecundity was a relatively constant (around 5 eggs) for 53 days (Fig. 2) and the maximum daily fecundity recorded was 17 eggs on day 42. Mean fecundity per female was 184.9 eggs and a single female laid 372 eggs.
The percentage survival of immatures of are suitable prey for the development of Our study indicates that both survival and reproductive capacity of coccinellid predators. Hodek (1996) categorized prey into essential, alternative and rejected based on their effect on the developmental rate, survival and reproductive capacity of coccinellids (Thompson, 1999). There may be different levels of suitability of individual prey. Osman & Selman (1993) report that although A. fabae is a suitable prey for the lacewing C. carnea it results in this predator producing smaller cocoons and having a lower fecundity. The fertility of Propylea japonica reared on Ephestia kuehniella eggs is significantly lower than when reared on A. pisum, though both are regarded as essential prey (Hamasaki & Matsui, 2006). These studies reveal that suppression of growth and developmental rate of larvae is likely to affect the size and maturation of adults, which in turn may result in reduced reproductive success or survival. More interestingly, we found that when S. japonicum fed on M. persicae, the preoviposition period was significantly prolonged but the oviposition period and longevity were not significantly different. When adults of S. japonicum are fed on the less nutritious food (i.e., M. persicae) it may affect the development of their testes and sperm and/or oocytes and ovarian development that could result in the extension in pre-oviposition period. On the other hand, these females could allocate more resources to longevity at the cost of a reduction in reproduction so as to maintain population development. Further investigations are needed to elucidate the physiological mechanisms that underlie the effect of feeding on less nutritious prey on the subsequent fitness and life history characteristics of S. japonicum.

S. japonicum has considerable potential as a biocontrol agent of B. tabaci in China (Lin et al., 2008). However, the general biology of this ladybird is not well studied. To date, most information on S. japonicum is limited to its compatibility with entomopathogenic fungi (Fatiha et al., 2008; Hu et al., 2009) or insecticides (He et al., 2012; Li et al., 2015; Yao et al., 2015). Only two studies assess the development, survival and reproduction of this ladybird when fed on B. tabaci on different host plants, which resulted in the longevity and fecundity of the females differing significantly (Jing et al., 2003; Yao et al., 2011). In general, host plants on which prey feed affect the nutrition of the prey, and in turn, affect the development and survival of the predator (Giles et al., 2002). Therefore, it is necessary to evaluate the effects of different species and cultivars of host plants on the development, survival and reproduction of S. japonicum in order to improve the efficiency of the control of whiteflies. Currently more than 40 species of coccinellid are described as predators of B. tabaci; however, recent work has only focused on Serangium parcesetosum and Delphastus catalaunae. Detailed evaluation and utilization of most ladybeetles is lacking (Arno et al., 2010). We think this is due to the poor knowledge of their food ecology

Table 2. Growth and reproductive parameters of Serangium japonicum fed on Myzus persicae and Bemisia tabaci.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Bemisia tabaci</th>
<th>Myzus persicae</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth index</td>
<td>10.31</td>
<td>9.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Developmental rate (day⁻¹)</td>
<td>0.069 ± 0.001</td>
<td>0.067 ± 0.001</td>
<td>1.61</td>
<td>2.056</td>
<td>0.157</td>
</tr>
<tr>
<td>Preoviposition period (days)</td>
<td>9.3 ± 0.3</td>
<td>20.9 ± 1.2</td>
<td>1.29</td>
<td>84.599</td>
<td>0.001</td>
</tr>
<tr>
<td>Oviposition period (days)</td>
<td>61.4 ± 5.2</td>
<td>71.2 ± 2.5</td>
<td>1.29</td>
<td>2.944</td>
<td>0.097</td>
</tr>
<tr>
<td>Longevity (days)</td>
<td>79.8 ± 3.9</td>
<td>89.3 ± 3.8</td>
<td>1.59</td>
<td>3.078</td>
<td>0.085</td>
</tr>
<tr>
<td>Fecundity</td>
<td>294.5 ± 38.5</td>
<td>184.9 ± 26.5</td>
<td>1.29</td>
<td>5.510</td>
<td>0.026</td>
</tr>
<tr>
<td>Percentage of eggs that hatched (%)</td>
<td>96.7 ± 0.8</td>
<td>72.9 ± 3.1</td>
<td>1.29</td>
<td>59.294</td>
<td>0.001</td>
</tr>
</tbody>
</table>
and behaviour. In the present study, we firstly found that \textit{S. japonicum} can complete its development and growth when fed exclusively on \textit{M. persicae}, although this aphid is not the best quality prey for this predator. In northern China, both \textit{B. tabaci} and \textit{M. persicae} often coexist in the field and on greenhouse grown vegetables, though their relative abundance varies over time (Zhang et al., 2007). Since \textit{S. japonicum} is an effective predator of \textit{B. tabaci} (Ren et al., 2001), the possibility of utilizing a single natural enemy to control mixed populations of aphids and whiteflies may reduce costs when \textit{S. japonicum} is used for whitefly control in greenhouses. In addition, our results demonstrate that \textit{S. japonicum} can complete its life history for whitfly control in greenhouses. Therefore, this ladybeetle can survive throughout the season in the absence of whiteflies. Mixed diets consisting of several species of prey (e.g. different species of aphids) and other non-prey food (e.g. nectar or pollen) are known to favour the development and survival of \textit{Chrysoperla rufilabris} (Legaspi et al., 1994), \textit{Chrysoperla carnea} (Patt et al., 2003) and \textit{Coccinella septempunctata} (Hauge et al., 1998; Nielsen et al., 2002), but the effects of mixed diets consisting of \textit{B. tabaci} and aphids or other alternative food on the demographic parameters of predatory ladybirds have not been evaluated. Therefore, further investigation of the effects of mixed \textit{B. tabaci} and aphid diets on \textit{S. japonicum} would be valuable in terms of using ladybirds as biological control agents of whiteflies and/or aphids and whether the presence of both prey enhance or reduce the biocontrol of each of them.

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