Effects of Host Age on the Parasitism of Pachycrepoideus vindemmiae (Hymenoptera: Pteromalidae), an Ectoparasitic Pupal Parasitoid of Bactrocera cucurbitae (Diptera: Tephritidae)

Authors: Hai-Yan Zhao, Ling Zeng, Yi-Juan Xu, Yong-Yue Lu, and Guang-Wen Liang

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EFFECTS OF HOST AGE ON THE PARASITISM OF PACHYCREPOIDEUS VINDEMMIAE (HYMENOPTERA: PTEROMALIDAE), AN ECTOPARASITIC PUPAL PARASITOID OF BACTROCERA CUCURBITAE (DIPTERA: TEPHRITIDAE)

HAI-YAN ZHAO, LING ZENG, YI-JUAN XU, YONG-YUE LU* AND GUANG-WEN LIANG*
Department of Entomology, South China Agricultural University. Wushan Road, Guangzhou, P.R. China, 510642

*Corresponding author; E-mail: insectlu@163.com, gwliang@scau.edu.cn

ABSTRACT

Biological control using an ectoparasitic idiobiont (e.g., Pachycrepoideus vindemmiae Rondani) (Hymenoptera: Pteromalidae) can be an effective approach to reduce the economic losses caused by the melon fly, Bactrocera cucurbitae (Coquillett) (Diptera: Tephritidae). In this study, we determined the parasitism of P. vindemmiae on B. cucurbitae pupae of different ages. The results showed that B. cucurbitae pupae at all ages could be successfully parasitized by P. vindemmiae. The higher numbers of pupae parasitized (10.12 to 9.32 pupae/day for no-choice tests and 8.0 to 7.82 pupae/day for choice tests) were achieved when 3 or 4-day old pupae were used as hosts. The selection coefficient revealed that P. vindemmiae preferred 3 or 4-day old pupae as the host. Pachycrepoideus vindemmiae females developed more quickly on 3 or 4-day old hosts than on pupae of other ages. Longevity of parasitoid offspring obtained from 2 and 7-day old melon fly pupae varied from 9.45 to 10.37 days and from 8.97 to 9.45 days for females and males, respectively. The emergence rate and proportion of female offspring of P. vindemmiae were not affected by the age of the pupae. However, our results indicate that the performance and suitability of P. vindemmiae were affected by the age of the host pupa age. Our findings may be useful for the development of biological control programs for B. cucurbitae.

Key Words: melon fruit fly, parasitoid, Bactrocera cucurbitae, Pachycrepoideus vindemmiae, host age, host suitability

RESUMEN

El ectoparásito idiobionte, Pachycrepoideus vindemmiae Rondani (Hymenoptera: Pteromalidae), puede ser un agente efectivo de biocontrol para prevenir pérdidas económicas causadas por la mosca del melón (Bactrocera cucurbitae [Coquillett]; Diptera: Tephritidae). En este estudio, se determinó el parasitismo de pupas de diferentes edades de B. cucurbitae por P. vindemmiae. Los resultados mostraron que las pupas de B. cucurbitae de 2 a 7 días de edad podría ser exitosamente parasitadas por P. vindemmiae. El mayor número de pupas parasitadas (10.12 a 9.32 pupas/día en una prueba de严格的选项), y 8.0 a 7.82 pupas/día en una prueba de selección) se obtuvo cuando se utilizaron pupas de 3 o 4 días de edad como hospedadoras. El coeficiente de selección también reveló que P. vindemmiae prefirió parasitar pupas de 3 o 4 días de edad. Las hembras de Pachycrepoideus vindemmiae mostraron un desarrollo más rápido sobre pupas hospedadoras de 3 o 4 días de edad en comparación con pupas más jóvenes o mayores. La longevidad de los progenies de P. vindemmiae que emergieron de las pupas de 2-7 días de edad varió de 9.44 a 10.64 y de 8.94-9.56 días para las progenies machos y hembras, respectivamente. Ni la tasa de emergencia, ni la proporción de progenies hembras de P. vindemmiae fue afectada por la edad de las pupas hospedadoras. Estos resultados indican que la edad de las pupas hospedadoras afectó el desempeño y la idoneidad de P. vindemmiae como un agente de biocontrol para B. cucurbitae.

Palabras Clave: mosca de melón, parasitoide, Bactrocera cucurbitae, Pachycrepoideus vindemmiae, edad de hospedero, idoneidad de hospedero

The melon fly, Bactrocera cucurbitae (Coquillett) (Diptera: Tephritidae) is an economically important pest of fruits and cucurbitaceous vegetables in many parts of the world (Uchida et al. 1990; Vayssières et al. 2007; Amin et al. 2011), including watermelon (Citrullus lanatus (Thunb.) Matsum. & Nakai; Cucurbitales: Cucurbitaceae), cucumber (Cucumis sativus L.; Cucurbitaceae) and courgette or zucchini (Cucurbita pepo L.; Cucurbitaceae), etc. (Tikkanen et al. 2000). Bactrocera cucurbitae has seriously impeded international trade because of quarantine regulations...
Melon fly integrated management could be achieved using local area management or wide-area management (Dhillon et al. 2005). The local area management technologies include bagging of fruits, field sanitation, protein baits and cue-lure traps, host plant resistance, biological control, and soft insecticides (McQuate et al. 2005). The control programs used for a wide area management approach include sterile insect releases (Hendrichs et al. 2002), protein bait sprays and/or traps, augmentative parasitoid releases, field sanitation, insect transgenesis, and quarantine control techniques in combination with local area management options (Dhillon et al. 2005; Jang et al. 2008; Vargas et al. 2008). Area-wide IPM programs for melon fruit fly established in Hawaii and Seychelles have been successful suppressing fruit flies to below the economic thresholds (Mumford 2004; Dhillon et al. 2005). The augmentative release of biological control agents is one of the important methods to control the melon fly. In Hawaii, the establishment of melon fly has resulted in the introduction of many hymenopteran parasitoids (Wharton 1989; Purcell 1998). The natural enemy complex of melon fly in Hawaii consists of 8 species (Nishida 1955). *Peytalia fletcheri* (Silvestri) (Hymenoptera: Braconidae), *Fopius arisanus* (Hymenoptera: Braconidae) and *Opius fletcheri* Silv. (Hymenoptera: Braconidae) were introduced into Hawaii and are now successfully distributed throughout the major island chain, resulting in substantial reductions of fruit fly populations (Bautista et al. 2000; Bautista et al. 2004; Vargas et al. 2004). Moreover, *F. arisanus* and *Diachasmimorpha longicaudata* (Ashmead) were mass-reared and used in a augmentative release strategy. Because of the successful establishment of egg and larval parasitoids there is an historical lack of data regarding pupal fruit fly parasitoids.

Idiobiont ectoparasitoids usually have a wide host range. *Pachycrepoideus vindemmiae* (Rondani) (Hymenoptera: Pteromalidae) is an ectoparasitic idiobiont that exercises control over a great number of Diptera in the families of Tephritidae, Tachinidae, Sarcophagidae, Muscidae, Calliphoridae and Anthomyiidae (Marchiori & Barbaresco 2007). It is a solitary pupal parasitoid and a biological weapon against economically important tephritid flies including *B. cucurbitae* (Wang et al. 2004), *B. dorsalis* and *Ceratitis capitata* (Wiedemann) and several *Drosophila* (Marchiori et al. 2002, 2003). It is one of the parasitoids most widely used for the biological control of *Musca domestica* (L.) and *Stomoxys calcitrans* (Linnaeus) (Meyer et al. 1990; Petersen et al. 1992). It is also known to be a hyperparasitoid of dipteran and hymenopteran parasitoids, and a superparasitoid and multiparasitoid when ovipositing in a single host (Tormos et al. 2009). As a facultative hyperparasitoid *P. vindemmiae* can attack some parasitic tachnid flies and other tephritid fruit fly parasitoids, such as *Coptera silvestrii* Kieffer (Dipteridae), *Diachasmimorpha fullawayi* (Silvestri), *D. tryoni*, *Psyttalia humilis* Silvestri (all Braconidae), and *Tetrastichus giffardinianus* Silvestri (Eulophidae). These reports are all anecdotal and scattered among the older literature, and detailed information is lacking (Noyes 2002; Wang & Russell 2004). However, Van Alphen & Thunnissen (1983) have reported that *P. vindemmiae* can attack 2 larval parasitoids: *Asobara tabida* Nees and *Leptopilina heterotoma* (Thomson). *Pachycrepoideus vindemmiae* was introduced into Hawaii and several other countries in South America to control *C. capitata* and *Anastrepha* spp. (Purcell 1998; Ovruski et al. 2000), and was mass-released for the control of *C. capitata* in Costa Rica (Ovruski et al. 2000). Almost sixty fly species from over sixty countries have been reported as hosts for *P. vindemmiae* (Wang & Russell 2004). Wang et al. (2004) found that *P. vindemmiae* parasitized *B. cucurbitae* in mainland China, suggesting it could be mass-reared and used for the control of *B. cucurbitae*.

Successful parasitism requires a series of interconnected steps and complex interactions between parasitoids and their hosts, including habitat finding, host location, host acceptance, host suitability and host regulation. Host age has been frequently found to be a major factor affecting host acceptance and host suitability (Vinson & Iwantsch 1980). Many pupal parasitoids prefer to attack certain sizes or ages of a given host, although in most cases their progeny can develop in all host pupal ages (Li et al. 2006). Parasitoids can regulate the timing of their own life history through the mechanism of host selection (McGregor 1996). Hence, before undertaking large-scale field releases of *P. vindemmiae*, it is essential to determine the optimum pupal age that can be most effectively parasitized by *P. vindemmiae* under laboratory conditions. With this information, *P. vindemmiae* can be released at the most effective time for the management of melon fruit flies.

In the present study, we examined the effects of *B. cucurbitae* pupal age on the parasitization, host mortality, development time, sex ratio and the longevity of adult progeny of *P. vindemmiae* under laboratory conditions.

**Materials and Methods**

Parasitoid and Host Cultures

*Bactrocera cucurbitae* were mass-reared on banana-based artificial diets (50 g corn flour, 150 g banana, 0.6 g sodium benzoate, 30 g yeast, 30 g sucrose, 30 g winding paper, 1.2 mL hydrochloric acid and 300 mL water) in the Key laboratory of Entomology of the Ministry of Agriculture, 

...
China. Adults were fed with 1:1 sucrose: yeast. In order to obtain pupae of different ages, newly pupated B. cucurbitae were collected daily from lab colonies and kept separately according to the cohort in the 2, 3, 4, 5, 6 and 7-day groups. These pupae at different developmental stages were used for the parasitism assays. One-day old pupae were not used, as the preliminary experiments demonstrated a high mortality upon parasitization.

A laboratory colony of P. vindemmiae was established from adult wasps that were naturally infesting other tephritid fruit flies cultured in the laboratory. Pupae of B. dorsalis were used as hosts for parasitoid rearing throughout this study. Adults (100 to 150 pairs) were kept in a cage (25 × 25 × 30 cm) provided with water and honey. About one to two hundred 2-3 day old B. dorsalis pupae were placed on a petri dish (9 cm Ø) and introduced in the cage, and wasps were allowed to parasitize pupae for 48 h. All experiments were conducted under the same laboratory conditions at 26 ± 1 °C, 65 ± 5% RH and 14:10 (L:D).

Host Preference Study

No-Choice Tests

To determine the most suitable host age for parasitoid development, B. cucurbitae pupae of six age cohorts (2, 3, 4, 5, 6 and 7-day old) were tested. A single P. vindemmiae female was introduced in a 500 mL glass bottle (HuanQiu GG-17, Jiangdu Glass Company) containing 30 pupae of a given age were placed along the circumference bottom of the bottle. The opening of the bottle was covered with black gauze for ventilation. After 24 h of exposure, the host pupae were individually placed in 100 mL plastic cups with humid sand (2 cm H and 30-40% RH) until emergence of wasps at room temperature. The opening of the plastic cup was covered with a fine nylon mesh (120 thread per inch²) for ventilation. Experiments with each cohort were replicated 25 times; as a control, 300 pupae of each age cohort were kept in plastic cups to estimate mortality in the absence of the parasitoid.

Choice Test

To examine the preference of female wasps for host pupae of different ages, 2-day-old mated females were offered host pupae of different ages in oviposition cages as described before. Each cage contained 6 parasitoids and 180 pupae (30 pupae in each of the six different age cohorts). After a 24 h exposure period, pupae were individualized in 100 mL plastic cups as described before. A total of 28 replicates were performed; as a control, 300 pupae of each age were kept in plastic cups to estimate the mortality in the absence of parasitoid.

Host Suitability Study

To determine the effects of host age on the development of P. vindemmiae, host pupae were exposed to 2-day old mated female parasitoids. Each oviposition cage contained 100 host pupae of a particular age and 15 female parasitoids, with 5 replicates per each pupal age. The exposed pupae were placed individually in 100 mL plastic cup until flies or wasps emerged. When adult parasitoids emerged, they were placed in a glass tube (10 cm L × 2.5 cm Ø) with 5 other parasitoids emerged from the same age group, and provided with a 15% honey solution. The developmental time from egg to adult, sex ratio and adult longevity were registered.

Data Analysis

The following formulas were used to calculate the parasitism selection coefficient and the percentage of host mortality. The selection coefficient (Pi) of each stage in the choice tests was calculated by the following formula (Li et al. 2006):

\[
P_i = R_i / \sum_{i=1}^{m} R_i
\]

Where, P is the relative parasitism index of stage i, R is the number of host pupae parasitized in age state i, and m is the total number of pupal stages tested in a trial (6 ages). The following formula improved from Abbott (1925) was used to correct for control mortality:

\[
\text{Mortality} \% = \left[ \frac{(M_{\text{treatment}} - \text{Parasitism} \%) - M_{\text{control}}}{(100 - M_{\text{control}})} \right] \times 100
\]

One-way ANOVA was used to analyze differences in the number of parasitized melon fly pupae, the parasitoid developmental time, mortality and survival rates in the different host pupal ages (SPSS 16.0 software package, 2008). Percentages were arcsine transformed before analysis but presented here as means ± SE. Tukey's test was conducted to detect differences in the above parameters among the different host pupal ages.

RESULTS

Host Preference Study

No-Choice Tests

The highest number of parasitized pupae that were achieved in 4-day old pupae, and the lowest in 7-day old pupae (Table 1). The number of parasitized pupae did not differ between 3 and
4-day host pupae (Table 1). The host mortality rate decreased with an increase of the pupae age. The age of the host had no effect on the percentage of parasitoid emergence.

Choice Test

When host pupae of different age groups were simultaneously available, *P. vindemmiae* parasitized all ages of *B. cucurbitae* pupae, but the highest numbers of parasitized pupae were recorded in 3 and 4-day old host pupae. The lowest number of parasitized pupae was registered when the host age was 7 days (Table 2). The selection coefficients of *P. vindemmiae* for 3 or 4-day old pupae were significantly higher than any other host age whereas 7-day old pupae had the lowest coefficients. The highest mortality (11.7%) occurred in 4-day old pupae, while the lowest mortality (3.6%) occurred in 7-day old pupae (Table 2). The emergence rate of adult wasps from host pupae of different ages did not differ.

Host Suitability Study

Mean development times from egg to adult emergence were affected significantly by the pupae age (df = 5, 917; $F = 8.087, P < 0.05$ for female and df = 5, 640; $F = 3.556, P < 0.05$ for male). The mean developmental times of *P. vindemmiae* were significantly longer in females that emerged from 6 and 7-day old pupae and males from 7-day old hosts, compared with other host ages. No significant differences were detected in developmental times of the individuals associated with 2, 3, 4 and 5-day old host pupae. The sex ratios for broods were similar between wasps associated with different host ages (Table 3). However, the highest proportion of females occurred in the 3-day old pupae, followed by 5, 2, 6, 4 and 7-day old pupae, respectively (Table 3).

The age of the host significantly affected the longevity of *P. vindemmiae* adult females. *Vindemmiae* females that emerged from 3-day old host (10.4 days) lived longer than those emerging from hosts of other ages while females from 7-day old hosts (9.5 days) had a shorter life. The adult longevity (9-10 days) of males was not affected by the host age.

**DISCUSSION**

Different hosts, different host instars or different host pupal ages may have different nutritional qualities (Harvey et al. 2000; Harvey & Strand 2002). The offspring of hymenopteran parasitoids rely on the host for all their nutritional needs. Most parasitoids have the ability to deter-

| Table 1. Parasitism, parasitoid emergence, and corrected mortality recorded from *Bactrocera cucurbitae* pupae parasitized at different ages by *Pachyceroideus vindemmiae* under no-choice conditions. |
|---|---|---|---|
| Age of host pupae (days) | Parasitism (%) | Corrected mortality (%) | Parasitoid emergence (%) |
| 2 d old | 22.00 ± 2.26 b | 15.90 ± 1.50 a | 97.56 ± 1.13 a |
| 3 d old | 31.07 ± 2.73 a | 12.41 ± 1.12 b | 96.95 ± 1.21 a |
| 4 d old | 33.73 ± 1.99 a | 9.90 ± 1.16 b | 97.69 ± 1.31 a |
| 5 d old | 27.20 ± 2.88 b | 4.97 ± 0.85 c | 97.45 ± 1.28 a |
| 6 d old | 26.80 ± 3.04 b | 4.23 ± 0.93 c | 98.42 ± 1.13 a |
| 7 d old | 11.60 ± 1.92 c | 2.78 ± 0.84 c | 100 ± 0.00 a |

Note: Numbers of host pupae and female wasps involved at each host age were 180 and 6, respectively. Means in each column followed by same letter (Duncan’s test) do not differ statistically \((P \geq 0.05)\).

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| Table 2. Parasitism, corrected mortality (%), selection coefficient and parasitoid emergence rate (%) of *Bactrocera cucurbitae* pupae of different ages when the host pupae were exposed simultaneously to 2-day-old *Pachyceroideus vindemmiae* females. |
|---|---|---|---|
| Age of host pupae (days) | Parasitism (%) | Corrected mortality (%) | Selection Coefficient\((P_i)\) | Parasitoid emergence (%) |
| 2 d old | 12.73 ± 1.89 c | 8.01 ± 2.32 ab | 0.114 ± 0.018 c | 98.81 ± 1.19 a |
| 3 d old | 26.07 ± 2.17 a | 8.27 ± 1.67 ab | 0.242 ± 0.018 a | 97.48 ± 1.11 a |
| 4 d old | 26.67 ± 2.58 a | 11.71 ± 1.73 a | 0.243 ± 0.019 a | 98.11 ± 1.14 a |
| 5 d old | 18.81 ± 1.83 b | 10.00 ± 2.50 ab | 0.185 ± 0.019 b | 97.85 ± 1.50 a |
| 6 d old | 19.29 ± 1.73 b | 5.23 ± 1.17 ab | 0.179 ± 0.02 b | 98.56 ± 1.04 a |
| 7 d old | 4.05 ± 1.08 d | 3.64 ± 1.18 b | 0.036 ± 0.008 d | 100 ± 0.00 a |

Note: Numbers of host pupae and female wasps involved at each host age were 180 and 6, respectively. Means in each column not followed by the same letter are significantly different at 5% level as determined by one-way ANOVA.
Table 3. Effect of Bactrocera cucurbitae pupal age on the developmental time, female progeny ratio, body size and adult longevity of Pachycrepoideus vindemmiae (n = number of replicates).

<table>
<thead>
<tr>
<th>Age of host pupae (days)</th>
<th>Development time in days from egg to adult emergence</th>
<th>Longevity in days (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female (n)</td>
<td>male (n)</td>
</tr>
<tr>
<td>2 d old</td>
<td>17.31 ± 0.10 b (n = 142)</td>
<td>16.70 ± 0.08 a (n = 1 26)</td>
</tr>
<tr>
<td>3 d old</td>
<td>17.22 ± 0.09 b (n = 165)</td>
<td>16.71 ± 0.09 a (n = 131)</td>
</tr>
<tr>
<td>4 d old</td>
<td>17.09 ± 0.12 b (n = 146)</td>
<td>16.75 ± 0.11 a (n = 120)</td>
</tr>
<tr>
<td>5 d old</td>
<td>17.34 ± 0.09 b (n = 142)</td>
<td>16.79 ± 0.10 a (n = 98)</td>
</tr>
<tr>
<td>6 d old</td>
<td>17.91 ± 0.10 a (n = 144)</td>
<td>17.02 ± 0.089 b (n = 99)</td>
</tr>
<tr>
<td>7 d old</td>
<td>17.74 ± 0.21 a (n = 84)</td>
<td>17.31 ± 0.19 b (n = 72)</td>
</tr>
</tbody>
</table>

Note: Values (means ± SE) within each column followed by the same letter are not significantly different (Duncan’s test P > 0.05).

mine host quality during oviposition (Husni et al. 2001; Wang & Liu 2002; Chinwada et al. 2003; Li et al. 2006; Roriz et al. 2006). Younger host pupae generally offer nutrition of higher quality for subsequent development, so they are usually preferred for oviposition by parasitoids (Takagi 1986; Olo 1992; Ueno 1997; Husni et al. 2001). However, some parasitoids prefer host pupae of medium age for their development (Pfannenstiel et al. 1996; Colinet et al. 2005).

We showed here that P. vindemmiae can successfully develop on pupae of all ages tested, but female parasitoids prefer pupae at medium ages (3 and 4 day old hosts) in both choice and no-choice tests. This suggested that there were some cues that allowed the female wasps to discriminate between the hosts of different ages. We speculate that 3 or 4-day old pupae may provide better nutritional quality than pupae of other ages. Other parasitoid species, i.e., Diadromus collaris (Wang & Liu 2002), Diadegma mollipla (Nofemela 2008) and Microplitis mediator (Li et al. 2006), also showed a preference for hosts of a particular age.

Both physiological and morphological changes in the host are related to development at a particular age, which varies and results in variation in its acceptability to the parasitoid and its suitability for the progeny of the parasitoid (Henry et al., 2005) Food quality is one of the most important factors, as pupae of different ages may not have the same nutritional quality. During the pupal development internal tissues undergo histolysis, histogenesis and differentiation to form adult internal organs and sclerotized appendages. Thus, older pupae may contain fewer resources for the developing parasitoids (Chapman 1971). Oviposition decisions (i.e., host selection and sex allocation) of female parasitoids are expected to correspond with host quality, as the fitness of their offspring is dependent on the amount and quality of resources provided by a single host (Stephens & Krebs, 1986). Theories predict that the female wasp adjusts the sex ratio of her offspring according to environmental conditions or host quality. Hu et al. (2012) reported that the percentage of males among the offspring of P. vindemmiae decreased as the female’s age increased, but increased again later. Females with poor food quality produced more male offspring. In our study, we found the lowest proportion of parasitoid females in 7-day old hosts.

Based on our results, it is preferable to use 3 or 4-day old B. cucurbitae to mass rear P. vindemmiae to optimize parasitism, parasitoid development and survival. In China, the area of the protected culture and greenhouse vegetable planting has developed rapidly in recent years. These particular planting suffered from infestations of melon flies. The melon fly always exists as a predominant species. There are no reports of successful use of natural enemies against the melon fruit fly in China, and parasitism of B. cucurbitae by native parasitoid species could be insignificant. In south china, B. cucurbitae is characterized by broadly overlapping generations (Ou et al. 2008). In the fields, various ages of B. cucurbitae pupae are usually available to be attacked by pupal parasitoids, such as P. vindemmiae during most of a year. The parasitoids can discriminate the different ages of host pupae, and choose the most...
suitable host ages for parasitization, and this offers an apparent advantage for the survival of the parasitoid population. To our knowledge, this is the first report showing that P. vindemiae can develop on B. cucurbitae pupae of all ages.

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