

## Host Suitability of Different Pupal Ages of Oriental Fruit Fly, *Bactrocera dorsalis*, for the Parasitoid, *Pachycrepoideus vindemmiae*

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**Abstract.**- In this study preferences of the parasitoid *Pachycrepoideus vindemmiae* Rondani, to different ages of oriental fruit fly, *Bactrocera dorsalis* Hendel pupae, number of progenies, development time, adult longevity and sex ratio of the parasitoids have been studied. The results showed that *P. vindemmiae* parasitized pupae of all ages, and the rate of parasitism was high for 3 to 6 days-old pupae. The development time of parasitoid was significantly increased; in 3 to 6 days-old host compared with other age groups. The host mortality decreased with increasing host age. Male progeny developed faster than females, and older host pupae produced a higher proportion of males. Adult longevity of *P. vindemmiae* offspring were not affected by the age of the pupae. The results suggest that 3 to 6 days-old pupae of *B. dorsalis* are suitable host ages for *P. vindemmiae* and can be used for mass rearing *P. vindemmiae* under laboratory conditions for possible use in integrated pest management.

**Key words:** *Pachycrepoideus vindemmiae* Rondani, oriental fruit fly, biological control, reproductive strategies.

### INTRODUCTION

Parasitoids display remarkable inter- and intraspecific variation in their reproductive and associated traits (Jervis *et al.*, 2008). In the life history of typical insect parasitoid, the female lays its eggs within or on the bodies of other insects. The reproductive success of a female can be affected by many factors, such as host species, host age, host nutritional quality, etc. The ability of a parasitoid to recognize its host species at appropriate age allows a female parasitoid to decide whether to accept or reject parasitization and oviposition on the given host. The ability of an ovipositing female to discriminate between different quality hosts is critically important and directly related to the fitness of the offspring (Babendreier and Hoffmeister, 2003). Some Hymenoptera parasitoids can discriminate different age hosts. The ability of a parasitoid to distinguish between different age hosts

can enhance its performance by preventing wastage of eggs, by avoiding loss of hosts due to multiple attacks, and by saving time of laying eggs (van Lenteren, 1981). Host discrimination can be used as an important criterion for evaluation of natural enemies used as biological control agents (Luck, 1990; Zappalà and Hoy, 2004; van Lenteren, 1981). Many factors influence larval fitness and development of immature parasitoids in their hosts, such as host species, host age, host size, host mortality risks, diet at parasitism, and host physiological condition (Dover and Vinson, 1990; Mironidis and Savopoulou-Soultani, 2009; Pennacchio and Strand, 2006; Rotheray and Barbosa, 1984). Among these factors, host age affects host preference and host suitability of parasitoids (Vinson, 1976; Vinson and Iwantsch, 1980). In addition, host age has a greater effect on sex ratio of their progeny (Islam, 1994; Singh and Pandey, 1986; Ueno, 1999).

Oriental fruit fly, *Bactrocera dorsalis* Hendel (Diptera: Tephritidae) is a key fruit pest in India, East-Asia and the Pacific (Clarke *et al.*, 2005). It has very broad host range, relatively wide climatic tolerance, and dispersal capacity (Peterson and

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Denno, 1998). This invasive species has caused significant financial losses in orchard crops. Control of *B. dorsalis* mainly relies on chemical insecticides, bait traps or sterile insect techniques (SIT) for mature adults at present time. These control measures have many disadvantages to human health, non-target beneficial fauna and environment. The classical biological control of invasive tephritid fruit fly pests using hymenopteran parasitoids has been used in Hawaii. More than 30 parasitoid species from Africa, Asia and Australia have successfully controlled major tephritid pests (Purcell, 1998; Wharton, 1989). The egg-pupa parasitoid, *Fopius arisanus* (Sonan) has been successfully used to control two major tephritid pests, the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) and the oriental fruit fly *B. dorsalis*, which are the most successful examples of classical biological control of fruit fly pests in the world (Purcell, 1998; Wharton, 1989). As biological control of insect pests is one of the most cost effective and environmentally sound methods of pest management, there is a renewed interest in obtaining more effective parasitoids for biological control of *B. dorsalis*.

*Pachycrepoideus vindemmiae* (Rondani, 1875) (Hymenoptera: Pteromalidae) is a cosmopolitan dipteran ectoparasitoid of many dipteran species, such as *Bactrocera cucurbitae* (Coquillett), *Ceratitis capitata* Wiedemann, *Cyrtoneurina paraescita* Couri (Marchiori and Silva Filho, 2007), *Megaselia scalaris* (Marchiori and Barbaresco, 2007), *Musca domestica* L., *Sarcodexia lambens* Walker, ect. This parasitoid has been evaluated mainly for the control of house and stable flies (Meyer *et al.*, 1990; Wharton, 1989). It was also introduced to control *Anastrepha* spp. and *C. capitata* in some countries (Ovruski *et al.*, 2000; Purcell, 1998). Wang and Messing (2004) reported that *P. vindemmiae* can parasitize *B. dorsalis*. Our previous investigations have also shown that *P. vindemmiae* can parasitize *B. dorsalis* in China (unpublished). In order to obtain a greater understanding of the host-parasitoid relationships and improve the utilization of *P. vindemmiae* in integrated pest management (IPM) of the fruit flies, favorable host age, development time, sex ratio, proportion of survival and adult longevity, detailed laboratory research are required. The

present study was carried out to determine whether *P. vindemmiae* females are able to discriminate different host ages, thereby avoiding wastage when mass-reared and providing an optimum release time for this parasitoid to control oriental fruit flies in the field.

## MATERIALS AND METHODS

### *Insects: host and parasitoid*

*B. dorsalis* were mass-reared on banana-based artificial diets (50g corn flour, 150g banana, 0.6g sodium benzoate, 30g yeast, 30g sucrose, 30g winding paper, 1.2 ml hydrochloric acid and 300mL water) in the Key Laboratory of Entomology, Ministry of Agriculture, China. Adults were fed on sucrose: yeast (1:1). In order to obtain synchronised cohorts different stages of host pupae for experiments, newly pupated *B. dorsalis* were collected and separated within 24 h, then pupae of different ages (2, 3, 4, 5, 6, 7, and 8 days-old) were kept and used for the parasitizing experiments. Host pupae of the same size were selected for the experiments. Pupae were kept in separate plastic cups according to age groups until being used in the tests.

A laboratory population of *P. vindemmiae* was initially established from collections of adult wasps that can naturally infest the other tephritid fruit flies cultured in the laboratory. For rearing, pupae of the *B. dorsalis* were used as the common host species for parasitoids. Emerged parasitoids were kept in a cage (25×25×30 cm) at a density of 100 to 150 pairs with water and honey provided. About one hundred to two hundred 2-3 days-old *B. dorsalis* puparia on a petri dish (9cm diameter) were put in the cage and parasitized for 48h. For later tests, we used 2 days-old *P. vindemmiae* females that had been housed with males since their emergence in cages with food but no hosts provided.

Colonies were maintained and experiments were conducted in a climate controlled room (24±1°C, RH 65±5%, and 14:10 (L:D) photoperiod).

### *Host preference*

To determine the preference of *B. dorsalis* pupae by *P. vindemmiae* for parasitization, a single

female was introduced into the center of a 500 ml beaker containing 30 host pupae, which were placed along the circumference. A piece of cotton wool saturated with 15% honey solution was placed inside for providing food to the adults. The opening of the bottle was covered with black gauze. After exposure of 24h, the host pupae were placed in 100 ml plastic cups covered with a little humid sand until the wasp emergence. The emerged wasps were kept in a room at 23-25°C. The opening of the plastic cup was covered with a fine nylon mesh (120 meshes). Trials with pupae of each age were replicated 30 times, and 7200 host pupae were used in the tests. Host pupae were observed on daily basis. For each host age class, the numbers of hosts parasitized, unparasitized and host died were recorded.

#### Host suitability

To determine the effects of host age on the development of *P. vindemmia*, 2 days-old mated female parasitoids (without oviposition experience) were exposed to host pupae as described in above tests. Each experiment cage (15×15×20 cm) contained 100 host pupae of a particular age and 6 female parasitoids. After 24h, the exposed pupae were placed individually in 100 ml plastic cups until either flies or wasps emerged. Trials with pupae of each age (1-8 days-old) were replicated 6 times. For each host age class, the development time from egg to adult, body weight, sex ratio and adult longevity were determined.

#### Statistical analysis

The following formulae were used to calculate the percentage of host mortality of *P. vindemmia* to host pupae.

$$\text{Mortality (\%)} = \frac{(\text{No. of pupae tested} - \text{No. of parasitized pupae})}{\text{No. of pupae tested}} \times 100$$

Comparisons of numbers of host pupae parasitized, development time, adult longevity, and female numbers of progeny were made between the parasitoids reared from host pupae of different age classes, by analysis of variance.

## RESULTS

### *Parasitization of host pupae of different ages*

*P. vindemmia* parasitized all ages of *B. dorsalis* pupae, when the host different pupal age classes were exposed separately to female parasitoids. However, the rates of parasitism varied significantly among various host age classes (Table I). The parasitism was higher in intermediate age class (4 days-old) and lowest in the oldest age class (8 days-old), although the differences among 3, 4, 5 and 6 days-old age classes were not significant. Emergence percentage of parasitoids varied among different host ages, the middle age classes (3 to 6 days-old) showed significantly higher emergence than other age classes (1, 2, 7 and 8 days-old) (Table I). Mortality of host decreased gradually with increasing host pupal age (Table I).

**Table I.-** Effects of host pupal stages on parasitism, emergence and mortality of *Bactrocera dorsalis* hosts parasitized by *Pachycrepoideus vindemmia*.

Age of host pupae(day)	Parasitism (%)	Emergence (%)	Mortality (%)
1 <sup>st</sup>	16.44±1.26c	64.42±4.30c	17.46±1.13a
2 <sup>nd</sup>	23.67±1.60b	76.00±2.89b	16.33±1.36a
3 <sup>rd</sup>	42.56±2.40a	89.42±1.14a	15.37±1.31a
4 <sup>th</sup>	46.86±2.04a	92.12±1.42a	11.16±1.34b
5 <sup>th</sup>	45.22±2.21a	91.74±1.48a	11.05±1.54b
6 <sup>th</sup>	46.00±2.32a	90.74±1.96a	9.41±1.37bc
7 <sup>th</sup>	14.22±0.83c	79.94±4.72b	6.39±1.05c
8 <sup>th</sup>	12.58±0.83c	76.50±5.12b	5.94±1.44c

All the numbers of host pupae tested in this experiment is 30 with ca. 30 pupae in each stage. Means followed by same letter in columns (Duncan's test) do not differ statistically (p<0.05)

### *Host suitability*

With observations made once daily, the age of the host parasitized affected the development time, sex ratio and adult longevity of the parasitoid offspring (Table II). The development time of both females and males were longer in young host pupae, which decreased for middle-aged pupae, but increased again when the host pupae were much older. Analyses of variance revealed that females generally took longer to develop than males (P<0.05). The sex ratio of the progeny was a female

**Table II.-** Development duration (Mean  $\pm$ SEM; days), female (%) ratio and adult longevity of parasitoid *Pachycrepoideus vindemmiae* parasitizing *Bactrocera dorsalis* hosts.

Age of host pupae (day)	Development time (day)		Sex ratio (% females)	Adult longevity (day) (n=40)	
	Female	Male		Female	Male
1 <sup>st</sup>	17.94 $\pm$ 0.16ab (n=121)	17.07 $\pm$ 0.16*a (n=83)	79.97	9.95 $\pm$ 0.21a	9.52 $\pm$ 0.21a
2 <sup>nd</sup>	17.71 $\pm$ 0.16b (n=125)	17.04 $\pm$ 0.14*a (n=102)	75.06	10.24 $\pm$ 0.19a	9.77 $\pm$ 0.20a
3 <sup>rd</sup>	16.92 $\pm$ 0.08c (n=139)	16.41 $\pm$ 0.11*b (n=98)	72.23	10.13 $\pm$ 0.21a	9.89 $\pm$ 0.21a
4 <sup>th</sup>	16.94 $\pm$ 0.07c (n=187)	16.33 $\pm$ 0.12*b (n=115)	70.22	10.37 $\pm$ 0.18a	9.97 $\pm$ 0.18a
5 <sup>th</sup>	16.93 $\pm$ 0.08c (n=196)	16.37 $\pm$ 0.11*b (n=116)	68.34	10.62 $\pm$ 0.20a	10.06 $\pm$ 0.21a
6 <sup>th</sup>	17.18 $\pm$ 0.09c (n=193)	16.52 $\pm$ 0.11*b (n=111)	65.14	10.51 $\pm$ 0.18a	10.03 $\pm$ 0.23a
7 <sup>th</sup>	17.98 $\pm$ 0.12ab (n=195)	17.08 $\pm$ 0.15*a (n=119)	63.43	10.33 $\pm$ 0.21a	9.91 $\pm$ 0.21a
8 <sup>th</sup>	18.05 $\pm$ 0.13a (n=170)	17.18 $\pm$ 0.18*a (n=109)	59.03	10.02 $\pm$ 0.19a	9.87 $\pm$ 0.22a

Means followed by same letter in columns (Turkey's test) do not differ statistically ( $p < 0.05$ )

\*Significant difference between female and male ( $p < 0.05$ ) by T-test.

biased. Proportion of females also varied according to pupal age with the highest occurring in the 2 days-old hosts, followed by 1, 3, 4, 5, 6, 7, and 8 days-old hosts (Table II). The stage of the host parasitized had no effect on adult longevity of the parasitoid offspring. Adult longevity was about 10.27 days for female progeny and 9.95 days for male progeny, respectively.

## DISCUSSION

The effects of host pupal age on parasitoids have received less attention when compared with studies on host egg or larvae (Husni *et al.*, 2001; Mackauer and Sequeira, 1993; Vinson and Iwantsch, 1980; Wang and Liu, 2002). The reports available to date indicate that many parasitoids preferentially attack certain age of a given host species, although in most cases their progeny can successfully develop in all host stages. Generally, with increase in age the nutritional quality of the host pupae decrease which should select hosts to maximize its fitness return and foraging efficiency (Charnov and Stephens, 1988). Some studies mentioned that parasitoids preferred younger host pupae for oviposition to get higher nutrition for subsequent development (Hailemichael *et al.*, 1994; Lasota and Kok, 1986; Ueno, 1997). Some studies showed that host pupae of medium age classes were more preferred and more suitable for development (Pfannenstiel *et al.*, 1996). In our preliminary experiments, the results showed that *P. vindemmiae*

females preferred 3 to 5 days-old hosts for oviposition, host pupae of intermediate ages produced the highest parasitic rate. Several factors could influence the rate of parasitism, such as pupal injury caused at the time of oviposition, host nutritional quality, and developmental disruption of the parasitoid larvae. The lower rate of parasitism on young pupae was associated with relatively higher mortality of host pupae, suggesting that young pupae are more susceptible to injury caused during oviposition, including venom injection or sting or both. In contrast, parasitism did not seem to induce mortality in older pupae.

With the increase of age, host's defense system enhances in strength or effectiveness. For better development, parasitoids not only regulate the host's development but also withstand attacks by that defense system (Puttler, 1961; Vinson and Iwantsch, 1980; Harvey, 1996). Our results indicated that the development of *P. vindemmiae* in middle-aged hosts was faster than other aged hosts. The lower parasitic rate on 7 and 8 days-old hosts supports the concept that the host's immune system was strong enough to prevent development of the parasitoid. In contrast, low parasitic rate in 1 and 2 days-old hosts was due to high mortality. Some factors (host size, age of parent parasitoid, and parental sex ratio) can affect sex ratio of the wasp offsprings. Ueno (1997, 1999) reported that the pupal parasitoid of *Itopectis naranyae* male-biased on old host pupae and the parasitoid *Pimpla nipponica* Uchida (Hymenoptera: Ichneumonidae)

laid more female eggs in younger host pupae when choice among hosts of different ages were allowed. Our results showed that sex ratio (proportion female) of *P. vindemmiae*'s offspring declined with pupal ages (Table II).

In tropical and subtropical regions, natural populations of *B. dorsalis* are characterized by broadly overlapping generations. Under these circumstances, *B. dorsalis* pupae of various ages are usually available for parasitizing by parasitoids. Our results showed that *P. vindemmiae* was able to discriminate between host pupae of different ages and was able to choose hosts of the most suitable age for parasitization. To conclude, *P. vindemmiae* parasitized and developed successfully in all host pupal ages, but host pupae of intermediate ages (3 to 6 day-old) produced the highest parasitic rate. The development of *P. vindemmiae* in 3 to 6 day-old hosts was faster than in 1, 2, 7 and 8 day-old hosts. Therefore, when augmentative field release of *P. vindemmiae* is initiated for management of *B. dorsalis*, parasitoids should be released when 4 to 8 day-old hosts are present in the field. This study also provided insight into the understanding of host age preference behavior and parasitoid-host interactions.

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