Neotrop Entomol (2016) 45:420–426
DOI 10.1007/s13744-016-0389-7

BIOLOGICAL CONTROL

Parasitism Performance of *Tetrastichus brontispae* Ferriere over the Coconut Hispine Beetle, *Brontispa longissima* (Gestro)

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Keywords
Biological control, choice test, host, parasitoid

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Edited by Tiago Cardoso Costa Lima – Embrapa

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Received 28 September 2014 and accepted 2 March 2016
Published online: 5 April 2016

Abstract

In this study, the effect of host density, host, and parasitoid ages in choice and no-choice tests on the parasitism performance of *Tetrastichus brontispae* Ferriere, one of the major parasitoid of *Brontispa longissima* (Gestro), was investigated in the laboratory. The results revealed that an increased host density resulted in no increased parasitism of *B. longissima* by *T. brontispae*; the optimal host density was three host pupae per parasitoid when considering the costs for mass rearing. Moreover, parasitoid age was quite crucial for effective parasitism and affected the emergence rate. Although 2-h to 4-day-old parasitoids successfully parasitized the host pupae, younger parasitoids (within 2-day-old) presented higher parasitism capacity than older parasitoids. More importantly, both choice and no-choice tests confirmed that all host stages tested from 2-h to 4-day-old were suitable for *T. brontispae* parasitization, although 2-h to 2-day-old hosts were preferred. We also demonstrated that sex ratio, emergence rate, and egg to adult developmental time were not influenced by host density, parasitoid, and host age in both choice and no-choice tests. Our data will allow for more accurate prediction and interpretation on the parasitization by *T. brontispae*, supporting mass-production initiatives and mass release in programs of *B. longissima*.

Introduction

The coconut hispine beetle, *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae), is native to Indonesia and Papua New Guinea and is one of the most invasive and economically threatening insects in the region, where it is a major pest on *Palmae* plants (Chiu & Chien 1985, Waterhouse 1987). The species was accidentally introduced into Southeast Asia and has since dispersed throughout the area, including the countries of Thailand, Vietnam, and the Philippines, and the Hainan Province of China (Chiu & Chien 1985, Waterhouse 1987, Nakamura *et al* 2006, 2008, Lu *et al* 2008, Chen *et al* 2010, Liu *et al* 2014). This particular beetle damages plants mainly by feeding on their leaves, resulting in losses to the coconut and tropical tourism industry (Nakamura *et al* 2006, 2008, Lu *et al* 2008, Chen *et al* 2010, Liu *et al* 2014). Therefore, the beetle's invasion and infestations have raised great concern internationally, and the species was listed in the Global Invasive Species Database in 2010 (Nguyen *et al* 2012, Liu *et al* 2014). In China, the pest was first discovered in Haikou (Hainan Province) in 2002, after which it quickly spread throughout the island to become the most serious threat to the tourism and coconut industry of Hainan.

In order to control *B. longissima*, chemical pesticides have been frequently applied in most of the countries where the pest is located. However, insecticides are always deemed undesirable and impracticable, primarily because the individuals who apply it face great risk when scaling tall coconut trees and because its application is highly likely to lead to insect resistance (Nakamura *et al* 2008). Previously, an entomopathogenic fungus was used for controlling *B. longissima* on young coconut seedlings (Nakamura *et al* 2006), but its application was difficult and impracticable in areas with tall coconut plants.

Considering the cost-benefit and sustainability of biological control, two promising biological control agents—*Asecodes hispinarum* Boucek (Hymenoptera: Eulophidae) and *Tetrastichus brontispae* Ferrière (Hymenoptera: Eulophidae)—have been introduced to control *B. longissima* (Waterhouse 1987, Voegele 1989, Lu *et al* 2008). Previous studies have demonstrated that these two parasitoids were capable of controlling *B. longissima* because of their high host specificity (Lu *et al* 2008, Chen *et al* 2010, Ichiki *et al* 2011, Nguyen *et al* 2012, Liu *et al* 2014). Successful biological control of *B. longissima* was achieved in the countries of Samoa, Sulawesi, Tahiti, the Solomon Islands, and Taiwan (Chiu & Chien 1985, Voegele 1989, Nguyen *et al* 2012). Hence, environmentally friendly biological control techniques seem to be feasible and reasonable.

Tetrastichus brontispae is one of the most promising endoparasitoids of *B. longissima* and has been successfully introduced into several countries, primarily because of its ability to locate hosts (i.e., parasitization specificity) (Chen *et al* 2010, Ichiki *et al* 2011, Nguyen *et al* 2012). Existing details on the biological traits of *T. brontispae* associated with different factors have been well-documented in recent years (Ma *et al* 2006, Zhou *et al* 2006, Tang *et al* 2009, Chen *et al* 2010, Ichiki *et al* 2011, Nguyen *et al* 2012, Liu *et al* 2014), providing useful information for its mass rearing in the laboratory before mass release.

Determining the characteristics of the parasitoid-host relationship is also critical and essential for biological control, and such works have been widely conducted on other parasitoid wasps (Duan & Messing 1999, Hill & Hoy 2003, Haye *et al* 2005, Sheppard *et al* 2005, Ayvaz *et al* 2008). In this regard, Chen *et al* (2010) reported how temperature, relative humidity, photoperiod, and food sources affect the parasitism rate of *T. brontispae*; Nguyen *et al* (2012) collected limited data to describe the effect of host age and parasitoid age on parasitization by *T. brontispae*. In addition, Tang *et al* (2014a, b) studied the parasitism of *T. brontispae* on another host, *Octodonta nipae*. Our recently published works mainly investigated the biological characteristics and parasitism of *T. brontispae* on *B. longissima* in relation to different temperatures and cold storage (Liu *et al* 2014). However, little information exists on the particular characteristics of parasitism of *T. brontispae* on *B. longissima*, and no study has examined how host density and choice and no-choice tests influence the parasitism performance of *T. brontispae.*

Parasitism specificity testing within the confines of the controlled conditions of a laboratory is the most common way to obtain data on the parasitoid-host relationship; results may then be extrapolated to the natural environment. Hence, the main objective of this study was to investigate the effect of host density and host and parasitoid age on the characteristics of the parasitoid-host relationship in relation to *T. brontispae* parasitism on *B. longissima*. Furthermore, in an effort to improve the basic knowledge and accurate assessment of parasitism specificity, choice and non-choice tests were conducted to determine the parasitism performance of *T. brontispae* on *B. longissima*.

Material and Methods

Host insect cultures

Adult *B. longissima* were originally collected in 2010 from Haikou (Hainan Province, China; 20°1′N, 110°19′E) and later reared in a laboratory to establish colonies. Rearing conditions consisted of a temperature of 26 ± 1°C, 75 ± 5% RH, and a 12-h light and 12 h of scotophase. Approximately 300 *B. longissima* adults were used for establishing the initial cultures. Males and females were kept together, to allow for mating, in plastic containers (17 length × 11 width × 5 height, in cm) with a screened window in the lid. Feral individuals were added to the colonies twice a year to maintain genetic diversity. *B. longissima* were continuously reared on fresh coconut leaves, and a piece of tissue paper soaked in a 10% honey solution (weight/volume) was fixed on the wall of the boxes. When offspring larvae in the same generation emerged, they were transferred into other boxes. Afterwards, newly emerged pupae were collected daily for maintaining the parasitoids and for use in the experiment.

Parasitoid insect cultures

The colony of *T. brontispae* was originally obtained from Taiwan in 2012 and was maintained using 48-h-old host pupae. Both males and females were kept together in Petri dishes (6.0 mm in diameter, 2.5 mm in height), fed with 10% honey solution soaked tissue paper, and maintained at the laboratory conditions as described above. For mass rearing, approximately 200 one-day-old pupae were placed in each box together with 400 adult wasps. The boxes were removed after the adult wasps died. The newly emerged adults were collected daily and used in the experiments.

Effects of host density on parasitism

One-day-old female and male parasitoid wasps were kept together for 24 h to ensure mating. Next, mated female parasitoid were randomly selected and one was introduced per tube (2 cm in diameter), with a 10% honey solution soaked piece of tissue paper. Two-hour-old *B. longissima* host pupae were then selected and divided into six groups (treatments) based on tested host densities of 2, 3, 4, 5, 6, and 7 individuals. For each treatment, host pupae were placed into the tubes together with the female parasitoid and maintained in the cabinets at conditions similar to those described above. After 24 h of parasitism activity, each female wasp was removed from the tubes. After the fifth day, the pupae were dissected to confirm parasitism status, and number of parasitized pupae counted and recorded. Fifty replicates were tested for each host density treatment. Based on observations from previous experiments and the present experiment, we could distinguish the parasitized pupae from the non-parasitized by color changes. Once parasitized, the color of the cuticle changes to a deep black-brown (5-day-old pupae), which gets darker with time. In contrast, the non-parasitized pupae remain pale yellow until emergence.

Effects of parasitoid age on parasitism

Female and male parasitoids emerged the same day and were collected and kept together for 24 h to permit mating. Afterwards, 1-, 2-, 3-, and 4-day-old female parasitoids were randomly selected and introduced into tubes (2 cm in diameter, 0.5 cm in length) with a 10% honey solution provided. Fifty female parasitoid wasps replicates were tested for each parasitoid age. Two-hour-old *B. longissima* pupae in groups of five were placed in each tube with one female parasitoid. After 24 h of parasitism activity, new groups of five host pupae were again transferred into the tubes with the original female parasitoid for continued parasitism. These procedures were repeated until the parasitoid wasps died. After the fifth day, the progeny emerged, the pupae were dissected to confirm parasitism status, and the number of parasitized pupae was counted. To determine the effect of parasitoid age on the performance of *T. brontispae*, 30 parasitized pupae (30 replicates) for each parasitoid age were transferred into new tubes and maintained under the same laboratory conditions. After emergence, the number of adult parasitoids was counted, and the sex ratio (male/female), the emergence rate, and the egg to adult development time were determined.

Choice and no-choice tests

Choice and no-choice tests were conducted to determine *T. brontispae* host preference and performance. Before the tests, *B. longissima* pupae were randomly selected from five host age classes: 2-h, and 1-, 2-, 3-, and 4-day-old. For the choice test, we used one round container (50.5 mm in diameter, 5.5 mm in height) with five small enclosed areas (45 mm in diameter) each with an open door. Figure 1 is a diagram of the choice test equipment used. A group of ten pupae from each of the five host age groups was transferred into the small rooms with a 10% honey solution soaked piece of tissue paper. After that, one mated female parasitoid was placed in

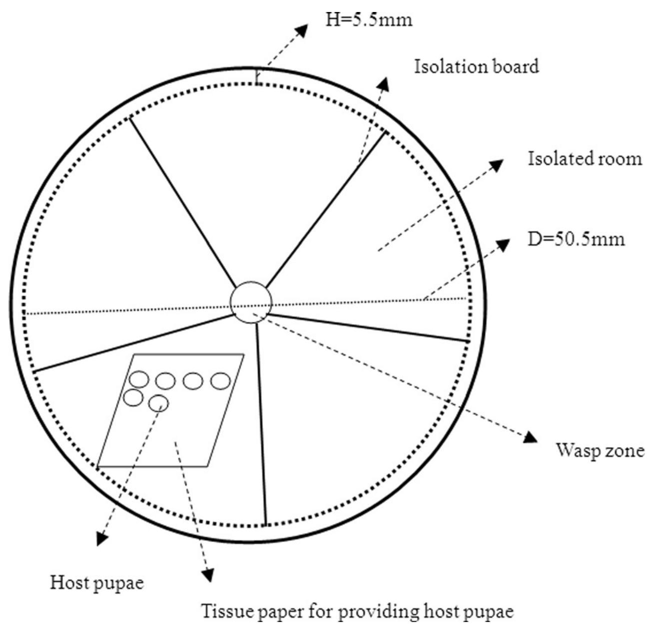

Fig 1 The diagram of the choice test equipment used for determining host preference of *Tetrastichus brontispae* over *Brontispa longissima*.

the central position of the box, and the box was maintained in a climate cabinet under the conditions described above. For the no-choice tests, five tubes (2 cm in diameter, 0.5 cm in length) were used in which ten *B. longissima* pupae for of each of the five host ages were selected and paired with one mated female parasitoid and later transferred into each tube with the already-established ten host pupae.

For both the choice and no-choice tests, 50 female parasitoid wasps (replicates) were tested for each host age. All female parasitoids were removed after 24 h of parasitism activity. After the fifth day, the progeny emerged and the parasitized pupae were dissected to confirm parasitism status, the parasitized pupae were counted, and the parasitism rate was calculated. Furthermore, 30 parasitized pupae for each host age for both tests were removed and placed in another new tube. Once adults emerged, we recorded the developmental time from egg to adult, the number of emerged females and males, the sex ratio, and the emergence rate.

Data analysis

For the choice tests, a choice index (SI) was used to assess parasitism preferences. SI was calculated using the following equation: $\mathrm{SI} = R_i / \sum_{i=1}^{m} R_i$, where R_i is the percentage of parasitized pupae in all tested pupae for each treatment, and m is the number of different host categories used in the test.

The experimental design was completely randomized and balanced. The data presented as percentages were normalized using a logarithmic transformation. All statistical tests were performed using the GLIMMIX procedure in SAS version 9.13 (www.sas.com). Data were subjected to an analysis of variance for determination of differences between means by using one-way ANOVA and the Tukey's HSD test. Differences in treatments at a probability level of $p < 0.05$ were considered to be significant.

Results

Effects of host density

The effect of host density on parasitism of *T. brontispae* is shown in Fig 2. A similar number of parasitized pupae were observed when the host pupae density was 3, 4, 5, and 7, whereas the number significantly declined when the host density was 2 and 6 ($p < 0.05$, Tukey's test).

Effects of parasitoid age

Generally, the parasitism performance of *T. brontispae* was highly influenced by wasp age, and the parasitism capacity decreased with an increase in parasitoid age. Younger parasitoids (1- to 2-day-old) parasitized a similar number of host pupae and had a higher parasitism than older females ($p < 0.05$, Tukey's test, Table 1). Parasitoid age also influenced the adult emergence rate and offspring production, which were higher in younger wasps. However, the sex ratio and developmental time from egg to adult were not affected by parasitoid age, ranging from 84.7 to 87.3% and 18.0 to 18.6 days, respectively (Table 1).

Choice tests and the effects of host age

There was female *T. brontispae* parasitization of pupae in each host age group (Table 2). The parasitoids selected relatively more 2-h-, 1-day-, and 2-day-old host pupae to parasitize. However, for 3- and 4-day-old host pupae, parasitism declined to an average of 0.23 and 0.10 pupae, respectively, when they were exposed to the parasitoids together with the other treatment in the choice test (Table 2). The above results were also observed with respect to the parasitism rate of *T. brontispae* in consideration of the tested host stages of *B. longissima* pupae (Table 2). Taken together, all tests indicated that *T. brontispae* had a greater preference for parasitization of younger *B. longissima* pupae; thus, the 2-h to 2-day-old pupae had higher parasitism than the 3- and 4-day-old pupae. However, the sex ratio, the emergence rate, and the developmental time were not influenced by the host stages ($p > 0.05$, Tukey's test).

No-choice tests and the effects of host age

In the no-choice tests, a similar number of 2-h and 3-day-old parasitized pupae were found, ranging from an average of 2.0 and 2.4 pupae, respectively, whereas the number significantly declined to only 0.6 pupae for the 4-day-old pupae treatment (Table 3). These same results were also observed for the rate of *T. brontispae* parasitism for the tested host stages of *B. longissima* pupae (Table 3). However, the host stages did not affect the parasitoid sex ratio, the emergence rate, or the developmental time of offspring, as there were no differences among the treatments ($p > 0.05$, Tukey's test) (Table 3).

Discussion

In biological control programs, the most significant challenges are using an effective parasitoid rearing technique and the parasitism specificity tests in the laboratory. The present study of the characteristics of the parasitoid-host relationship of *T. brontispae* parasitism on *B. longissima* is essential for promoting an understanding of the successful mass rearing of *T. brontispae*. This research may also be used to accurately interpret and provide predictions regarding these characteristics in relation to natural settings, before the actual release.

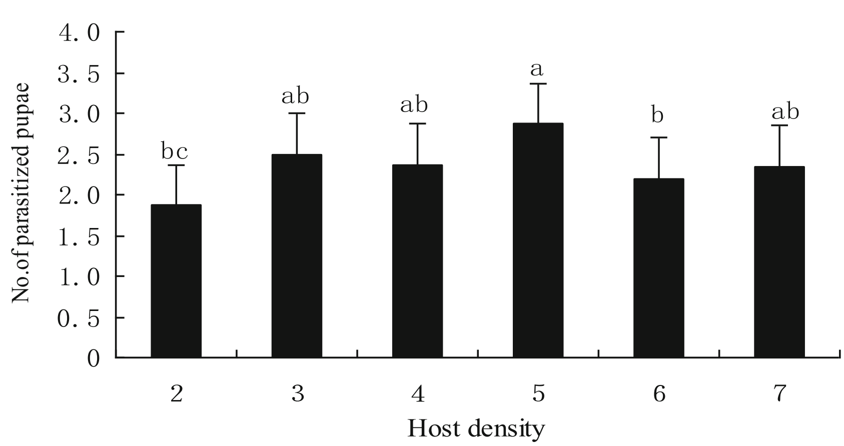

Fig 2 Number of parasitized pupae under different host densities (2 to 7 in of *Brontispa longissima* pupae) at 26 ± 1°C and 75 ± 5% RH under 12:12-L/D photoperiod in the laboratory conditions. Data are presented as means ± SE. For each set, *bars having the same letter* are not significantly different at $p = 0.05$ by Tukey's HSD test.

Table 1 Parasitism performance and reproduction of 1-, 2-, 3-, and 4-day-old *Tetrastichus brontispae* on *Brontispa longissima* under controlled laboratory conditions (26 ± 1°C; 75 ± 5% RH; 12:12-L/D photoperiod).

Parasitoid age	No. of parasitized pupae	No. of emerged female	Sex ratio (% female)	% Adult emergence rate	Development time (days)
1-day-old	2.9 ± 0.33 a	28.7 ± 3.48 a	84.8 ± 4.22 a	91.2 ± 6.08 a	18.6 ± 0.11 a
2-day-old	2.0 ± 0.18 a	24.9 ± 1.28 a	86.1 ± 4.16 a	90.9 ± 6.15 a	18.3 ± 0.11 a
3-day-old	0.8 ± 0.16 b	8.7 ± 0.95 b	84.8 ± 4.55 a	84.6 ± 5.87 b	18.5 ± 0.12 a
4-day-old	0.2 ± 0.17 b	7.2 ± 1.62 b	87.3 ± 5.02 a	81.2 ± 5.24 bc	18.1 ± 0.11 a

Data are presented as means ± SE. Means in the same column followed by different small letters are significantly different at $p < 0.05$ level by Tukey's HSD test.

Successful biological control of *B. longissima* by mass release of *T. brontispae* has been reported in several countries (Voegele 1989, Chiu & Chien 1985, Nakamura *et al* 2006); however, knowledge on host specificity of the parasitoid needs to be identified and improved. Our previous study demonstrated that *T. brontispae* parasitism is largely influenced by temperature and the cold storage period (Liu *et al* 2014); this study increases the amount of information on the characteristics of *T. brontispae* parasitism on *B. longissima*.

In the current study, more parasitized pupae were observed when there were five host pupae exposed to one parasitoid (Fig 2). Moreover, no statistically significant differences were observed with densities of 3, 4, 5, or 7 host pupae per parasitoid (Fig 2). Therefore, considering the costs for mass rearing in biological control programs, we recommend three host pupae per parasitoid. In contrast, Chen *et al* (2010) demonstrated that *T. brontispae* oviposition frequency became higher as host density increased because the parasitoid would have had more opportunities to encounter a host. Tang *et al* (2014a, b) also demonstrated that the fecundity of *T. brontispae* increased as the host density of *O. nipae* increased. Furthermore, the current study revealed that the number of parasitized pupae declined with low host density, especially when the host density was only two pupae. These results are consistent with the results of Godfray (1994) and Quicke (1997), who reported that other parasitoid species avoided previously parasitized hosts. It is possible that superparasitism occurs with lower host density (Ichiki *et al* 2011, Nguyen *et al* 2012), but this behavior requires further study. Another likely reason is that the capacity for parasitism might be restricted because of few opportunities to encounter a host in a lower host density environment.

The female parasitoid age is rather important for successful parasitism (Aung *et al* 2010), and Amalin *et al* (2005) showed the importance of parasitoid age to parasitism using a field release experiment. Research of parasitism in relation to parasitoid age has been widely documented for other species (Hentz 1998, Honda *et al* 1998, Amalin *et al* 2005, Ayvaz *et al* 2008). In this study, younger parasitoids (1- to 2-day-old) had a higher parasitism performance than older parasitoids (3- to 4-day-old). Our results are consistent with those of Nguyen *et al* (2012), who also detected a decrease in *T. brontispae* parasitism on *B. longissima* in older parasitoids. Similarly, younger *T. brontispae* were also more effective at attacking the host *O. nipae* (Tang *et al* 2014a, b). However, Pitcher *et al* (2002) demonstrated that parasitism efficiency of another parasitoid species was higher for older parasitoids than for younger ones, primarily owing to the higher searching speed, activity rate, and oviposition frequency of the former. Meanwhile, Ayvaz *et al* (2008) reported that middled-aged *Trichogramma evanescens* had a higher parasitism rate than younger and older individuals.

The current study reveals that host age also played an important role in the parasitism of *T. brontispae*. Both choice and no-choice tests confirmed that *T. brontispae* has a strong preference for parasitization of younger *B. longissima* pupae (from 2-h to 2-day-old) than older pupae (Tables 2 and 3). This result is consistent with Chen *et al* (2010) and Nguyen

Table 2 Parasitism and reproduction of *Tetrastichus brontispae* on different stages (2-h to 4-day-old) of *Brontispa longissima* pupae in choice experiments under controlled laboratory conditions (26 ± 1°C; 75 ± 5% RH; 12:12-L/D photoperiod).

Host age	No. of parasitized pupae	Parasitism rate (%)	Choice indexes	Sex ratio (% female)	% Adult emergence rate	Development time (days)
2-h-old	0.8 ± 0.14 a	15.3 ± 2.83 a	0.28 ± 0.06 a	85.3 ± 4.25 a	89.9 ± 6.02 a	18.7 ± 0.12 a
1-day-old	0.9 ± 0.14 a	18.0 ± 2.77 a	0.29 ± 0.04 a	84.5 ± 4.09 a	88.2 ± 5.94 a	18.6 ± 0.11 a
2-day-old	0.8 ± 0.18 a	17.3 ± 3.68 a	0.29 ± 0.06 a	86.7 ± 5.21 a	88.7 ± 5.48 a	18.3 ± 0.13 a
3-day-old	0.2 ± 0.10 b	4.8 ± 2.08 b	0.07 ± 0.03 b	87.7 ± 5.06 a	84.4 ± 5.31 a	18.9 ± 0.11 a
4-day-old	0.1 ± 0.06 c	2.0 ± 1.11 c	0.03 ± 0.01 c	84.9 ± 4.54 a	81.3 ± 5.23 ab	18.9 ± 0.13 a

Data are presented as means ± SE. Means in the same column followed by different small letters are significantly different at $p < 0.05$ level by Tukey's HSD test.

Table 3 Parasitism and reproduction of *Tetrastichus brontispae* on different stages (2-h to 4-day-old) of *Brontispa longissima* pupae in non-choice experiments under controlled laboratory conditions (26 ± 1°C; 75 ± 5% RH; 12:12-L/D photoperiod).

Host ages	No. of parasitized pupae	Parasitism rate (%)	Sex ratio (% female)	% Adult emergence rate	Development time (days)
2-h-old	2.2 ± 0.17 a	44.1 ± 3.49 a	86.7 ± 4.02 a	90.2 ± 6.03 a	18.5 ± 0.11 a
1-day-old	2.0 ± 0.21 a	40.7 ± 4.23 a	84.9 ± 4.05 a	90.1 ± 6.14 a	18.5 ± 0.12 a
2-day-old	2.3 ± 0.17 a	46.0 ± 3.48 a	85.3 ± 4.26 a	90.0 ± 6.25 a	18.2 ± 0.12 a
3-day-old	2.4 ± 0.18 a	48.0 ± 3.66 a	87.0 ± 4.41 a	89.3 ± 5.74 a	18.8 ± 0.10 a
4-day-old	0.6 ± 0.13 b	12.0 ± 2.64 b	86.3 ± 5.27 a	88.5 ± 5.62 a	18.7 ± 0.12 a

Data are presented as means ± SE. Means in the same column followed by different small letters are significantly different at $p < 0.05$ level by Tukey's HSD test.

et al (2012), who demonstrated that the optimum host age in *B. longissima* for parasitism was 1-day-old pupae. Recently, Tang *et al* (2014a, b) reported that *T. brontispae* also preferred 1-day-old *O. nipae* pupae, rather than older pupae. The primary reason why *T. brontispae* prefer younger pupae is that older pupae are less suitable for oviposition, primarily because it is more difficult for the parasitoid to penetrate the hard cuticle of older pupae. In addition, it is possible that older pupae are more capable of generating a physical or physiological defense response to parasitism, or perhaps they only provide adequate nourishment for parasitoid development for a limited time. Such immune responses should be explored following the example of Tang *et al* (2014a, b), who investigated the effects of *T. brontispae* parasitization on the immune system of the host, *O. nipae*. Nevertheless, some studies obtained opposite results on other parasitoids, such as one on the parasitism of *Lariophagus distinguendus* (Foerster) which was shown to positively correlated with the host age of *Sitophilus oryzae* (Line) (Van den Assem 1971). Kitt & Keller (1998) also indicated a decrease in the suitability of younger hosts, which they suggested was most likely due to their smaller size.

Conducting both choice and no-choice tests resulted in a more accurate assessment of host range, host preference, and parasitism performance of parasitoids (Ayvaz *et al* 2008, Mansfield & Mills 2004, Murray et al 2010). The results of the current study show that both choice and no-choice tests perform the same in terms of determining the preferences of *T. brontispae* in relation to the range of host ages. The role and the value of using choice versus no-choice host specificity tests for other parasitoids have been debated previously (Van Driesche & Murray 2004, Withers & Mansfield 2005, Murray et al 2010). In nature, parasitoids that cannot locate their preferred hosts may disperse, rather than accept, lower quality hosts, yet hosts across the widest possible range of quality may be accepted in no-choice tests. However, this implies the risk of false positives with the tests because of severely overestimating the potential range in host quality, whereas in nature parasitoids may reject lower quality hosts (Goldson & Phillips 1990, Withers & Browne 2004); this may explain a higher parasitism rate in no-choice tests compared to choice tests (Tables 2 and 3). If so, choice tests arguably are useful for modeling the host selection processes including the location of host habitat, host attack, and host suitability (Kitt & Keller 1998, Withers & Mansfield 2005) because they provide a reasonable opportunity to display host preferences. However, choice tests must also be viewed with caution, specifically in regards to whether parasitoids do in fact "choose" among the different host ages under natural conditions. Most parasitoids are unlikely to encounter more than one host age at a time in nature. Otherwise, Edwards (1999) and Barratt (2004) reported that a parasitoid accepts or rejects encountered hosts based on stimuli present at the time, and the previous experience and physiological state of the parasitoid.

In summary, results of the present study lead to the conclusion that a host density of three host pupae per parasitoid is ideal when the costs for mass rearing are also considered. Parasitoid age was critical for successful parasitism, with younger *T. brontispae* performing better than older *T. brontispae*. Both choice and no-choice tests confirmed that the parasitoid had a stronger preference to parasitize the 2-h to 2-day-old host pupae. Taken together, the results of the present study have increased knowledge of the parasitoid-host relationship of *T. brontispae* on *B. longissima*.

Acknowledgments This work was supported by the Special Fund for Agro-scientific Research in the Public Interest of China (No. 200903026-5).We would like to thank Zeng DQ Professor for critical evaluations of this work and for helpful comments on the English writing. We would also thank reviewers for their constructive comments and valuable suggestions for this paper.

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