

Effects of rearing conditions on the parasitism of *Tetrastichus brontispae* on its pupal host *Octodonta nipae*

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Abstract *Octodonta nipae* (Maulik) (Coleoptera: Chrysomelidae) is currently a serious invasive pest of palm plants in southern China. Although previous studies reported that *Tetrastichus brontispae* Ferriere (Hymenoptera: Eulophidae) could successfully parasitize the pupae of *O. nipae*, little is known of the potential for *T. brontispae* to control this pest. To understand the interaction between *T. brontispae* and *O. nipae*, the effects of parasitoid feeding regimes, host age and density, parasitoid age and density, and cold storage on the parasitism of *T. brontispae* were investigated. Our results indicate that a supply of either glucose or sucrose increased the longevity and fecundity of *T. brontispae*. The parasitoid preferred one-day-old host pupae and its fecundity increased with increasing host or parasitoid female density. Extended cold storage negatively affected the

parasitism of *T. brontispae*. These results demonstrate how to efficiently raise *T. brontispae* and will contribute to the effective management of *O. nipae*.

Keywords Biological control · Cold storage · Invasive insect pest · *Octodonta nipae* · Pupal parasitoid · *Tetrastichus brontispae*

Introduction

The nipa palm hispid beetle, *Octodonta nipae* (Maulik) (Coleoptera: Chrysomelidae), is native to Malaysia and is currently a serious invasive pest of palm plants in southern China (Hou and Weng 2010). This beetle was first discovered in 2001 in Hainan Province, China, and was later found in other southern provinces of China, such as Fujian, Guangdong and Guangxi (Hou and Weng 2010; Liang et al. 2005; Sun et al. 2003; Wu et al. 2007). This beetle was also first reported on the island of Cyprus in 2009 (Vassiliou et al. 2011). The *O. nipae* beetle is gregarious, and the larvae and adults can be found on one palm. Females produce 116.8 eggs on average, and the life expectancy of this organism reaches 114.9 days at 25 °C (Hou and Weng 2010). The larvae and adults attack young palm leaf fronds (Family: Arecaceae), resulting in the decline of the health of infested palms (Vassiliou et al. 2011). Sustained attack may lead to leaf shrivel, curl, and even death, which causes devastating damage to ornamental palms in China. Currently, the

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primary management strategy for this pest is traditional chemical control with broad-spectrum insecticides (Vassiliou et al. 2011). However, certain behaviors of *O. nipae*, such as feeding inside tightly folded immature leaves of the palms, together with the high stems of plants make chemical control ineffective (Hou and Weng 2010; Wu et al. 2007). In addition, the application of pesticides negatively impacts human health, non-target insects, including natural enemies and pollinators, and the environment through greenhouse gas emissions (Biondi et al. 2012; Heimpel et al. 2013; Weisenburger 1993). Therefore, the development of alternative and effective management strategies to control *O. nipae* is urgent and crucial.

Tetrastichus brontispae Ferriere (Hymenoptera: Eulophidae), a gregarious and koinobiont endoparasitoid, is originally from Java, Indonesia, and has been introduced into many countries, such as Celebes, Tahiti, Taiwan, and the Solomon Islands, to successfully control the coconut hispine beetle *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae) (Chen et al. 2010; Chiu et al. 1985; Lever 1969; Lu et al. 2006; Nguyen et al. 2012; Stapley 1973; Xin et al. 2009). Moreover, the potential effectiveness of *T. brontispae* as a natural enemy of *B. longissima* has been thoroughly evaluated (Chen et al. 2010; Nguyen et al. 2012). Howard et al. (2001) reported that *T. brontispae* also parasitized the larvae and pupae of *O. nipae*. Huang et al. (2007) also documented that *T. brontispae* could parasitize the pupae of *O. nipae* and that the parasitism rate of *T. brontispae* for the pupae was 82.25%. However, information regarding the interaction between *T. brontispae* and *O. nipae* is relatively scarce.

An important component of a biological control program is the mass production of biological control agents, which can be affected by many factors. In this study, we determined the effects of *O. nipae* and *T. brontispae* density and age on the life history of *T. brontispae*. For many biological control programs, low production costs of the biological control agents and the timing of their release play critical roles in their success (Colinet and Boivin 2011). An extended shelf-life not only reduces production costs but also increases the flexibility of release time (McDonald and Kok 1990; Venkatesan et al. 2000). Therefore, we also investigated the effects of *O. nipae* and *T. brontispae* cold storage on the life history of *T. brontispae*.

Materials and methods

Insects

Octodonta nipae larvae and adults were fed the central leaves of Fortune's windmill palm, *Trachycarpus fortunei* (Hook), collected from unsprayed trees on the campus of Fujian Agriculture and Forestry University, China and maintained at 25 ± 1 °C with 85 ± 5 % RH and a 12:12 L:D photoperiod as described previously by Hou and Weng (2010). *Tetrastichus brontispae* were originally collected from the mummified pupae of *B. longissima* provided by the Chinese Academy of Tropical Agricultural Sciences in 2008. They were then cultured with one-day-old *O. nipae* pupae as hosts (newly exuviated pupae) in our laboratory for three consecutive generations prior to the experiments. The adult wasps were fed on a streak of 10 % sucrose solution in plastic containers (105×60 mm, diameter×height) and maintained in groups at 25 ± 1 °C, 85 ± 5 % RH, and a photoperiod of 12:12 L:D. Newly emerged and mated *T. brontispae* females and one-day-old naïve *O. nipae* pupae were used unless otherwise specified.

Effects of food sources on adult longevity and parasitism of *T. brontispae*

Thirty *T. brontispae* females were placed in containers and fed with one of the following food regimes (treatments): (1) 10 % honey solution, (2) 10 % glucose solution, (3) 10 % sucrose solution, (4) water, and (5) nothing (control). The food sources were replaced daily, and the survival of *T. brontispae* was checked at an 8 h interval until death. Each treatment was repeated three times.

Five *O. nipae* pupae were exposed to one female *T. brontispae* subjected to one of the five food sources described above for 24 h. The *O. nipae* pupae were subsequently placed individually into a plastic tube (2 ml) until either a *T. brontispae* or *O. nipae* adult emerged. A new set of five *O. nipae* pupae were introduced daily until the death of the *T. brontispae* female. The *T. brontispae* offspring emergence rate, the number of emerged offspring per host, and the F1 female sex ratio were recorded. Each treatment was repeated 30 times. The emergence rate was calculated as the number of mummified pupae from which adult *T. brontispae* emerged divided by the total number of

mummified pupae (mummification was defined as when the hosts became hard and dark after parasitism by *T. brontispae*).

Effects of *O. nipae* age and density on the parasitism of *T. brontispae*

Because six- to seven-days-old *O. nipae* pupae are unsuitable for parasitism by *T. brontispae* (data not shown), one- to five-days-old (each age denotes a treatment, five treatments in total) *O. nipae* pupae were tested for their suitability for parasitism. One female *T. brontispae* was allowed to attack three *O. nipae* pupae (three *O. nipae* pupae are optimal for parasitism, see details in the host density experiments) of the same age in a plastic container for 24 h. The *O. nipae* pupae were then individually placed into a plastic tube (2 ml) until either a *T. brontispae* or *O. nipae* adult emerged. The parasitism rate, the number of emerged offspring per host, and the F1 female sex ratio were recorded. Each treatment was repeated 30 times. The parasitism rate was calculated as the number of mummified pupae divided by the total number of pupae, which were three.

Because the previous experiment demonstrated that one-day-old *O. nipae* pupae were the most suitable for parasitism by *T. brontispae*, one-day-old *O. nipae* pupae were used in this experiment. The *O. nipae* densities (treatments) tested were one, two, three, four, and five one-day-old *O. nipae* pupae. The experimental protocol was identical to the previous experiments. Each *O. nipae* density measurement was repeated 30 times.

Effects of *T. brontispae* female age and density on their parasitism efficiency

Single female *T. brontispae* of different ages (treatments: one- to five-days-old; a newly emerged wasp was considered one-day-old) were placed individually with a 10 % sucrose solution into a plastic container and allowed to attack three *O. nipae* pupae for 24 h. To evaluate the effect of *T. brontispae* density on parasitism, one, two, three, four, or five *T. brontispae* females were allowed to attack one *O. nipae* pupa for 24 h. The pupae were then placed into a plastic tube (2 ml) individually until either a *T. brontispae* or *O. nipae* adult emerged. The parasitism rate, the number of emerged offspring per host, and the F1 female sex ratio were recorded. Each age or density measurement was repeated 30 times.

Daily fecundity of *T. brontispae*

To evaluate the daily fecundity of *T. brontispae* over its lifetime, a *T. brontispae* female was allowed to attack three *O. nipae* pupae. The *O. nipae* pupae were replaced with a new set of three pupae at a 24 h interval for up to 120 h, because the experiment evaluating the effects of food sources on adult longevity in the present study showed that the maximum longevity of a *T. brontispae* female supplied with sucrose is approximately 142 h. The replaced *O. nipae* pupae were placed individually into a plastic tube (2 ml) until either a *T. brontispae* or *O. nipae* adult emerged. The parasitism rate per day, the number of emerged offspring per host per day, and the F1 female sex ratio per day were recorded. The experiment was repeated 30 times.

Effects of cold storage on the parasitism of *T. brontispae*

The temperature of approximately 13 ± 1 °C was the lower threshold for *T. brontispae* (Huang et al. 2008) and was used for the following experiments. Single female *T. brontispae* was maintained at 13 ± 1 °C for 2, 5, 8, 11 or 14 days (treatments) in a plastic container in a climate chamber with 10 % sucrose solution. *Tetrastichus brontispae* females were then transferred to another climate chamber at 25 ± 1 °C and allowed to attack three hosts for 24 h. Control *T. brontispae* females were treated identically but maintained at 25 ± 1 °C. The parasitism rate, the number of emerged offspring per host, and the F1-female sex ratio were recorded. Each treatment was repeated 30 times.

Three *O. nipae* pupae were maintained at 13 ± 1 °C for 5, 8, 11, 14 or 17 days (treatments) in a plastic container in a climate chamber. The pupae were then moved into another chamber at 25 ± 1 °C and exposed to one female *T. brontispae* for 24 h. Hosts maintained at 25 ± 1 °C during the entire process were used as the control. The same parameters as in the above experiment were recorded. Each treatment was repeated 30 times.

A single *O. nipae* pupa was exposed to one female *T. brontispae* for 24 h. The pupae were maintained at 25 ± 1 °C in a climate chamber for 14 days, at which the *T. brontispae* offspring was in the pupal stage and the *O. nipae* pupae denoted mummified pupae, before being subjected to cold storage at 13 ± 1 °C for 7, 14 or 21 days (treatments). Normal temperature (25 ± 1 °C) was resumed after the cold treatment. The *T. brontispae*

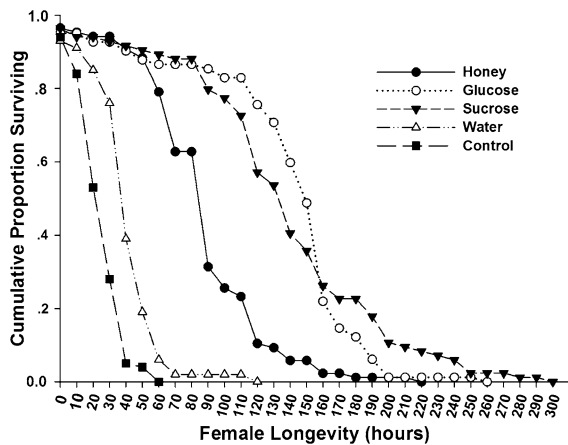


Fig. 1 Effects of food sources on the adult wasp longevity of *T. brontispae*. The Kaplan–Meier survival analysis was used to determine the female longevity among different food sources

Table 1 Effects of food sources on the parasitism (Mean \pm SE) of *T. brontispae*

Food sources	Emergence rate (%)	Number of emerged offspring per host	Female sex ratio (%) (percentage of female progeny)
10 % honey	97.2 \pm 5.9a	31.6 \pm 2.1ab	82.4 \pm 6.1ab
10 % glucose	98.9 \pm 5.6a	38.7 \pm 2.5a	83.8 \pm 1.8ab
10 % sucrose	99.1 \pm 8.1a	42.6 \pm 4.2a	79.2 \pm 3.0ab
Water	89.7 \pm 7.4a	29.0 \pm 2.9b	87.8 \pm 1.7a
Control	77.4 \pm 6.7b	25.2 \pm 1.8b	78.2 \pm 2.5b

The means followed by different lower-case letters within the same column denote significant differences at $\alpha = 0.05$

progeny (F1 generation) emerged from the mummified pupae were allowed to attack a new set of three one-day-old *O. nipae* pupae for 24 h. The number of *T. brontispae* offspring per host from the cold-treated *O. nipae* (F1 offspring), the parasitism rate of the F1 offspring, the number of *T. brontispae* offspring emerged from *O. nipae* parasitized by the F1 offspring (F2 offspring) per host, and the female sex ratio of the F2 offspring were recorded. The *O. nipae* pupae maintained at 25 ± 1 °C during the entire process were used as the control. Each treatment was repeated 30 times.

Data analyses

All statistical analyses were performed with SPSS 17.0 for windows (SPSS Inc., Chicago, IL, USA) with

a significance level of $\alpha = 0.05$. The data of the parasitism rate, emergence rate, number of emerged offspring per host, and female sex ratio were analyzed by one-way ANOVA. Fisher's least significant difference (LSD) was used for multiple mean comparisons. The Kaplan–Meier survival analysis with Mantel–Cox log-rank test was used to determine the female longevity differences depending on different food sources.

Results

Effects of food sources on adult longevity and parasitism of *T. brontispae*

Honey, glucose, sucrose or water obviously prolonged *T. brontispae* female longevity compared to the control ($\chi^2 = 459.86$, $df = 4$, $P < 0.0001$; Fig. 1). Female fed glucose or sucrose had the longest mean longevity (141.78 ± 5.68 and 141.93 ± 6.69 h, respectively), followed by honey (92.81 ± 4.00 h) and water (46.8 ± 2.16 h).

Glucose or sucrose significantly increased the numbers of emerged offspring per host, which were 1.69- and 1.53-times than that of the control ($F_{4,145} = 2.486$, $P < 0.05$; Table 1), respectively. Honey and water showed no effect on this parameter (Table 1). The emergence rate and female sex ratio did not differ among the food sources ($F_{4,145} = 1.237$ and 1.057 , respectively, $P > 0.05$).

Effects of host age and density on parasitism of *T. brontispae*

Tetrastichus brontispae females could successfully colonize one- to five-days-old *O. nipae* pupae (Table 2). However, the parasitism rate and number of emerged offspring per host varied significantly with *O. nipae* age ($F_{4,145} = 9.497$ and 7.930 , respectively, $P < 0.05$). The age of the *O. nipae* did not affect the F1-female sex ratio ($F_{4,145} = 1.261$, $P > 0.05$).

The parasitism rate decreased with an increase in *O. nipae* density ($F_{4,145} = 22.303$, $P < 0.05$; Table 2). The parasitism rate at highest *O. nipae* density (female parasitoid:host = 1:5) was 33.3 % of the parasitism rate at the lowest *O. nipae* density (female parasitoid:host = 1:1). Host densities of less than three pupae increased the number of emerged offspring per

Table 2 Effect of host age and density on the parasitism (mean \pm SE) of *T. brontispae*

Items	Host age		Host density (female parasitoid:host)							
	Day 1	Day 2	Day 3	Day 4	Day 5	1:1	1:2	1:3	1:4	1:5
Parasitism rate (%)	83.3 \pm 7.5a	76.7 \pm 8.7ab	63.4 \pm 3.4b	46.7 \pm 5.5c	33.3 \pm 0.0c	100.0 \pm 0.0a	83.3 \pm 8.3b	59.3 \pm 8.3b	50.0 \pm 7.4c	35.6 \pm 2.9d
Number of offspring per host	25.2 \pm 1.2a	23.0 \pm 2.5a	21.1 \pm 1.8a	16.6 \pm 1.9b	11.2 \pm 0.8c	15.0 \pm 1.3b	18.8 \pm 2.3ab	21.4 \pm 2.8a	21.3 \pm 2.8a	20.5 \pm 1.2ab
Female sex ratio (% female progeny)	81.9 \pm 3.1a	82.2 \pm 2.8a	84.5 \pm 4.6a	86.5 \pm 3.8a	90.6 \pm 1.4a	89.9 \pm 3.2a	88.7 \pm 2.7a	87.1 \pm 2.9a	83.5 \pm 2.2a	86.9 \pm 3.3a

The means followed by different lower-case letters in the same row denote significant differences between the host ages or host density at $\alpha = 0.05$

host with increasing density. Whereas this parameter did not vary significantly with *O. nipae* density when the densities were greater than three pupae. The host density did not affect the F1-female sex ratio ($F_{4,145} = 0.951, P > 0.05$).

Effects of *T. brontispae* female age and density on parasitism

The age of the *T. brontispae* female did not significantly affect the parasitism rate, number of emerged offspring per host, or F1-female sex ratio ($F_{4,145} = 0.369, 0.862$ and 2.058 , respectively, $P > 0.05$; Table 3). The addition of three *T. brontispae* females significantly increased the number of emerged offspring per single host ($F_{4,145} = 7.953, P < 0.05$; Table 3). The increased *T. brontispae* female density decreased the F1-female sex ratio ($F_{4,145} = 39.165, P < 0.05$; Table 3).

Fecundity of *T. brontispae*

The number of emerged *T. brontispae* offspring per host per day (Fig. 2a) and the parasitism rate per day (Fig. 2b), which are indices of daily fecundity, decreased with the increased age of *T. brontispae* females ($F_{4,145} = 48.448$ and 3.174 , respectively, $P < 0.05$). The maximum number of emerged offspring per host per day and the parasitism rate per day occurred when the *T. brontispae* females were one-day-old with a *per capita* 22 wasps emerging and a 60 % parasitism rate. Whereas the average number of offspring and parasitism rate of *O. nipae* were 3.7 ± 0.8 wasps and 36.6 ± 3.3 %, respectively, for five-days-old females. The F1-female sex ratio per day did not vary with the age of the *T. brontispae* females ($F_{4,145} = 0.729, P > 0.05$; Fig. 2c).

Effect of cold storage on the parasitism of *T. brontispae*

Cold storage of the *T. brontispae* females for longer than two days negatively affected the number of emerged offspring ($F_{5,174} = 7.937, P < 0.05$; Fig. 3a). *Tetrastichus brontispae* females stored at 13 ± 1 °C for eight days or longer had lower parasitism rates compared to the control ($F_{5,174} = 3.830, P < 0.05$; Fig. 3b). Cold storage of *T. brontispae* females did not influence the F1-female sex ratio ($F_{5,174} = 0.579, P > 0.05$; Fig. 3c).

Table 3 Effect of parasitoid female age and density on the parasitism (mean \pm SE) of *T. brontispae*

Parasitoid attributes	Parasitoid female age					Parasitoid female density (female parasitoid:host)				
	Day 1	Day 2	Day 3	Day 4	Day 5	1:1	2:1	3:1	4:1	5:1
Parasitism rate (%)	73.3 \pm 8.3a	70.0 \pm 7.8a	72.0 \pm 7.8a	73.4 \pm 4.4a	63.0 \pm 6.7a	100	100	100	100	100
Number of emerged offspring per host	22.9 \pm 1.8a	23.4 \pm 2.9a	28.3 \pm 2.5a	25.1 \pm 2.2a	23.0 \pm 3.2a	19.3 \pm 1.4c	25.1 \pm 1.4c	31.7 \pm 2.5bc	31.7 \pm 2.7ab	35.0 \pm 1.4a
Female sex ratio (%) (percentage of female progeny)	81.3 \pm 3.3ab	85.4 \pm 2.4a	73.0 \pm 4.3b	79.3 \pm 2.5ab	82.5 \pm 3.3ab	82.9 \pm 3.4a	77.2 \pm 2.2a	74.7 \pm 3.6ab	67.4 \pm 4.1b	34.8 \pm 2.3c

The means followed by different lower-case letters within the same row denote significant differences between the parasitoid female ages or parasitoid female density at $\alpha = 0.05$

Cold storage of naïve one-day-old *O. nipae* pupae had a significant effect on the number of emerged offspring per host, with naïve one-day-old pupae stored at 13 ± 1 °C for 11 days or longer having fewer emerged offspring than the control ($F_{5,174} = 3.727$, $P < 0.05$; Fig. 4a). Neither the parasitism rate (Fig. 4b) nor the F1-female sex ratio (Fig. 4c) of *T. brontispae* was significantly affected by the cold storage of naïve one-day-old *O. nipae* pupae ($F_{5,174} = 0.858$ and 0.485 , respectively, $P > 0.05$).

Cold storage of mummified *O. nipae* pupae for seven days or longer significantly decreased the number of F1-emerged offspring per host ($F_{3,116} = 11.787$, $P < 0.05$), and no F1-emerged offspring were observed when the mummified *O. nipae* pupae were maintained at 13 ± 1 °C for 21 days (Fig. 5a). Cold storage of the mummified *O. nipae* pupae also significantly decreased both the number of F2-emerged offspring per host ($F_{3,116} = 7.048$, $P < 0.05$; Fig. 5b) and the parasitism rate of the female progeny ($F_{3,116} = 10.475$, $P < 0.05$; Fig. 5c). Compared to the control, the parasitism rate and the number of F2-emerged offspring per host of the *T. brontispae* that parasitized the mummified *O. nipae* pupae maintained at 13 ± 1 °C for seven days or longer decreased by 36.7 and 19.3 %, respectively. The female sex ratio of the F2 *T. brontispae* was increasingly female biased as the length of cold storage of the mummified *O. nipae* pupae increased to 14 days ($F_{3,116} = 17.908$, $P < 0.05$; Fig. 5d).

Discussion

It is well known that different sugars vary in their nutritional quality for parasitoids, with some having a positive effect and others having little or even negative effects on the parasitoids (Harvey et al. 2012). Several studies have demonstrated that sugar sources considerably prolong the lifespan and fecundity of a parasitoid (Desouhant et al. 2010; Harvey et al. 2012; Wäckers 2001). Sugar sources replenish the energetic and nutritional stores of parasitoids (Narváez et al. 2012). In the present study, both glucose and sucrose were beneficial to the longevity and fecundity of *T. brontispae* compared to water or no supplemental food supply. Honey is primarily composed of sugars but contains some vitamins and minerals (Gheldof et al. 2002; Martos et al. 2000). Honey is widely used as a

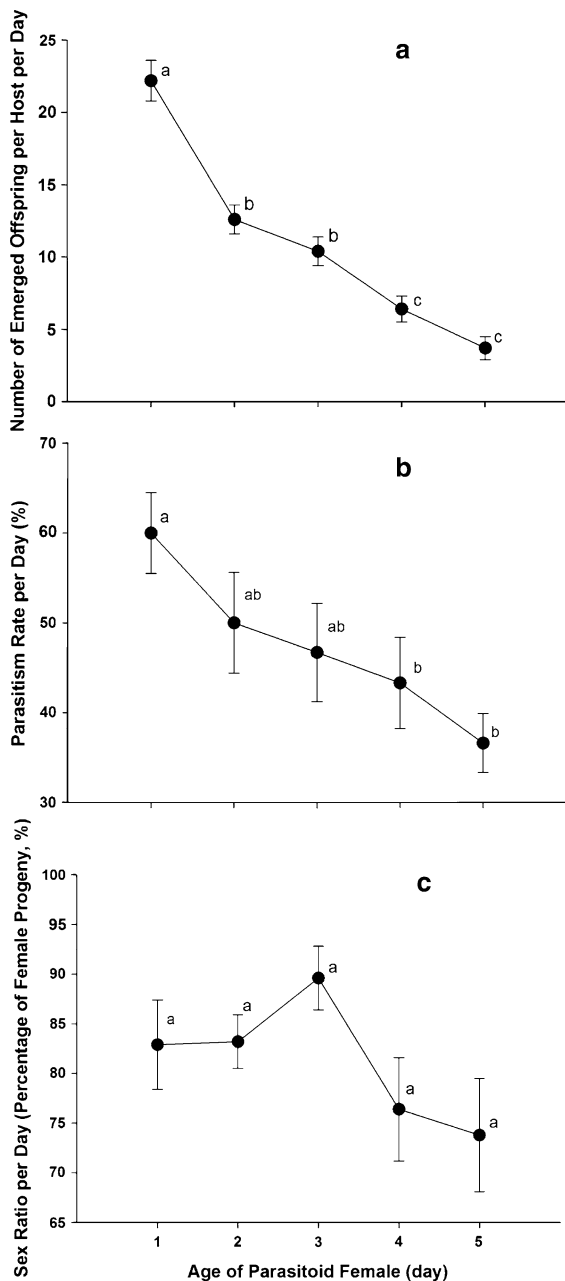


Fig. 2 Daily parasitism over the lifetime of *T. brontispae* on *O. nipae* with a host pupae density of three per day. **a** Number of emerged offspring per host per day. **b** Parasitism rate per day. **c** Sex ratio (percentage of female progeny) per day. The three one-day-old hosts and sucrose were renewed every day for five days. The mean \pm SE represents 30 independent replications. The different lower-case letters beside the curves denote significant differences at $\alpha = 0.05$

substitute food source for sucrose in many laboratories. As a supplemental food source, honey increased *T. brontispae* longevity but did not influence the fecundity of *T. brontispae* on *O. nipae* compared to water. This is consistent with Nguyen et al. (2012), who found that water alone was enough to increase the fecundity because of the oviposition frequency of the parasitoid. However, our result is contradictory to that of Chen et al. (2010), who reported that honey increased both the longevity and fecundity of *T. brontispae* on *B. longissima* compared to water. Given that *T. brontispae* was reared on different hosts, the distinction may be due to the adaptation of *T. brontispae* to different pupal hosts.

Tetrastichus brontispae successfully attacked and developed in one- to five-days-old *O. nipae* pupae. However, one- to three-days-old *O. nipae* pupae were more suitable for the production of *T. brontispae* than four- and five-days-old pupae based on the parasitism rate and the number of offspring. This is consistent with previous reports of *T. brontispae* parasitism on *B. longissima* (Chen et al. 2010; Nguyen et al. 2012). Host quality, which is correlated with the amount of resources available inside the host, affects the fitness of parasitoid progeny (Godfray 1994). Additionally, the fat body of insects is particularly related to the storage of nutrients and intermediary metabolism (Locke 1998). Therefore, we hypothesize that the decreased suitability of the older pupae is attributed to a decrease in fat reserves. The ready acceptance of one- to three-days-old *O. nipae* by *T. brontispae* broadens the time window of release of this parasitoid into the field and may increase the success of *O. nipae* population suppression. The female biased *T. brontispae* offspring, regardless of the *O. nipae* pupal age, may further enhance the success of biological control programs involving *T. brontispae*.

The foraging efficiency of many parasitoids may also be related to the age of the female parasitoids. Therefore, understanding the most effective foraging stages contributes to a successful biological control program (Ayvaz et al. 2008). The progeny per host and parasitism rate of *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae) on *Plutella xylostella* (Lepidoptera: Plutellidae) significantly decreased with increasing *O. sokolowskii* female age, and the ideal

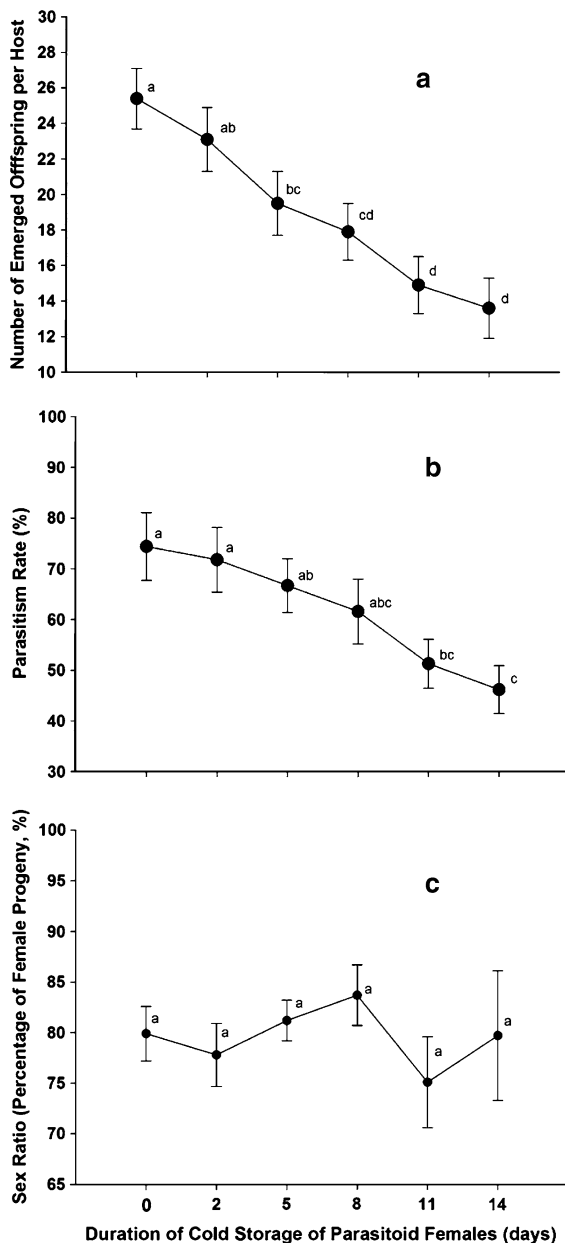


Fig. 3 The effect of cold storage of parasitoid females on the parasitism of *T. brontispae* against *O. nipae*. **a** Number of emerged offspring per host. **b** Parasitism rate. **c** Sex ratio (percentage of female progeny). Single newly emerged mated female wasps were maintained at 13 ± 1 °C for 2, 5, 8, 11 or 14 days in a climate chamber and later transferred to another climate chamber at 25 ± 1 °C. The mean \pm SE represents 30 independent replications. The different lower-case letters beside the curves denote significant differences at $\alpha = 0.05$

ages for parasitism ranged from 24 to 120 h (Sow et al. 2013). The parasitism rates of *T. brontispae* on *B. longissima* were not affected by the age of *T.*

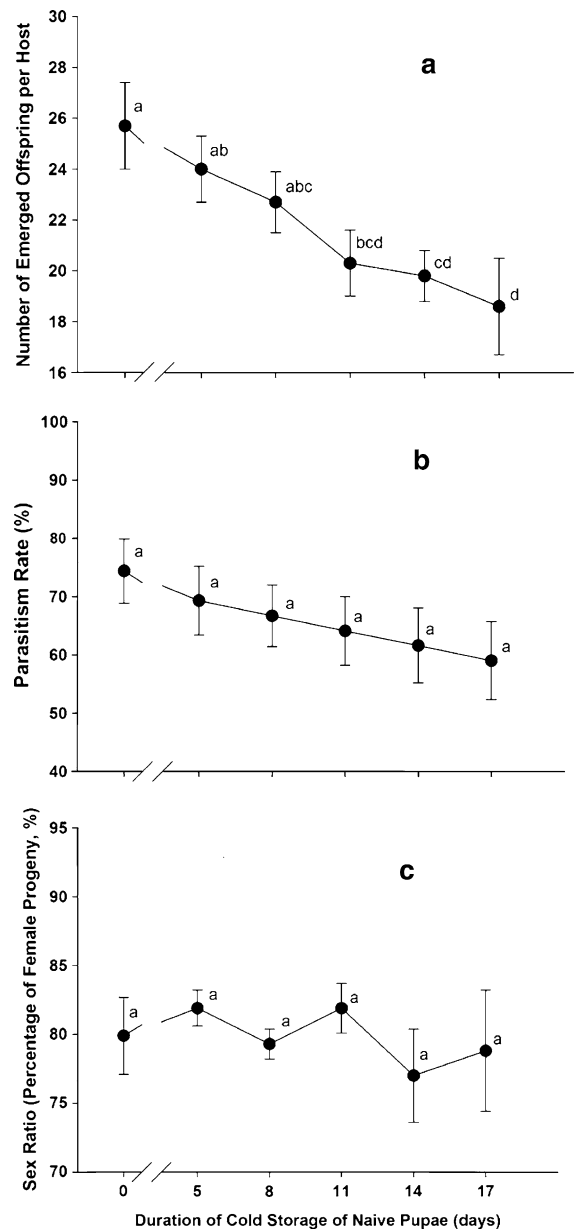


Fig. 4 The effect of cold storage of naïve pupae on the parasitism of *T. brontispae* against *O. nipae*. **a** Number of emerged offspring per host. **b** Parasitism rate. **c** Sex ratio (percentage of female progeny). Three naïve one-day-old hosts were maintained at 13 ± 1 °C for 5, 8, 11, 14 or 17 days in a climate chamber and later moved to another chamber at 25 ± 1 °C. The mean \pm SE represents 30 independent replications. The different lower-case letters beside the curves denote significant differences at $\alpha = 0.05$

brontispae as long as females were less than ten-days-old (Nguyen et al. 2012). Our study revealed that the parasitism potential of *T. brontispae* against *O.*

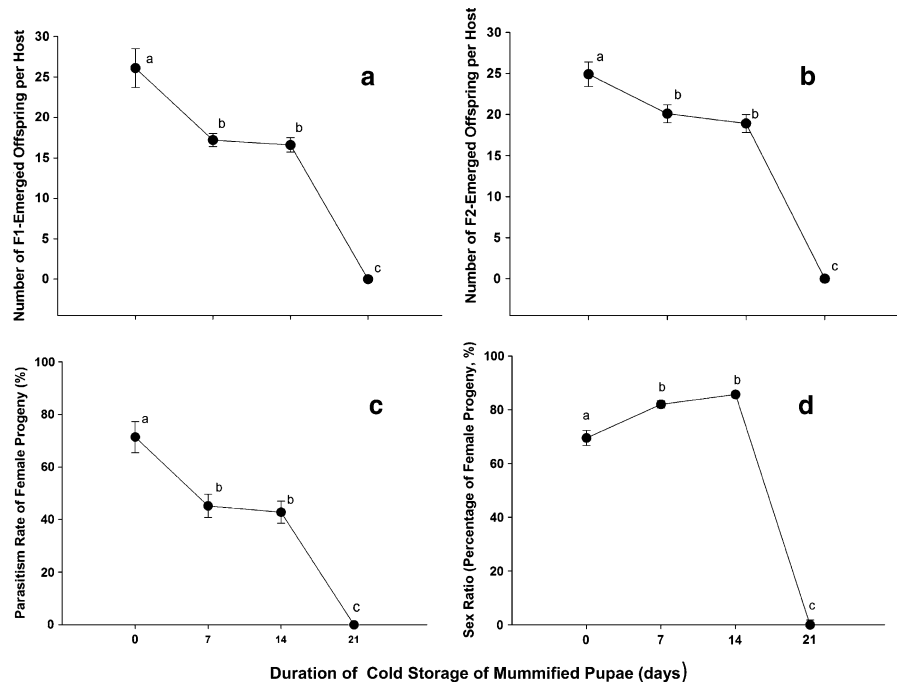


Fig. 5 The effect of cold storage of mummified pupae on the parasitism of *T. brontispae* against *O. nipae*. **a** Number of F1-emerged offspring per host. **b** Number of F2-emerged offspring per host. **c** Parasitism rate of female progeny. **d** F2- sex ratio (percentage of female progeny). The parasitized host was maintained at 25 ± 1 °C in a climate chamber for 14 days at

which the mummified stage was reached. Subsequently, the mummified pupae was moved into another chamber at 13 ± 1 °C for 7, 14 or 21 days, and then maintained at normal temperature 25 ± 1 °C. The mean \pm SE represents 30 independent replications. The different lower-case letters beside the curves denote significant differences at $\alpha = 0.05$

nipae was not affected by its age for as long as 120 h. Parasitoid species can be classified as either proovigenic, in which all or nearly all eggs mature prior to the onset of oviposition, or synovigenic, in which the eggs continue to mature during their entire reproductive life (Flanders 1950; Jervis et al. 2001). The observation that the daily fecundity (parasitism rate per day and number of emerged offspring per day) of *T. brontispae* decreased as the females aged may be attributed to the initial egg load and egg immaturity. Additionally, females were short-lived (the maximum longevity of females fed sucrose is 142 h). Therefore, we speculate that *T. brontispae* is proovigenic. However, this identification should be further validated by specific egg-load analyses. Based on the daily fecundity and effects of female age on parasitism, naïve mated females would be the most suitable for augmentative releases in the field.

The *T. brontispae* progeny per host was positively affected by the parasitoid female density and was maximal at a density of four females. This may be attributed to the gregariousness and superparasitism

behaviors of *T. brontispae*. These behaviors could also adversely affect parasitoid fitness if they result in smaller-sized offspring or shorter adult longevity (Silva-Torres et al. 2009). However, under circumstances of host scarcity, superparasitism can be favorable for *T. brontispae*. In addition, the progeny production per host was also positively affected by the host pupae density, and reached a plateau at a density of three hosts. An increase in *Pteromalus cerealellae* (Hymenoptera: Pteromalidae) progeny with increasing cowpea weevil *Callosbruchus maculatus* (Coleoptera: Bruchidae) density (up to 22 weevils) has been documented (Mbata et al. 2005). The effect of host pupae and parasitoid female densities on the parasitism of *T. brontispae* on *O. nipae* may have implications for augmentative releases in the context of host or parasitoid scarcity.

Cold storage may directly affect key biological attributes of parasitoids (Colinet and Boivin 2011; Lins et al. 2013), and the sensitivity to cold storage may also vary depending on the rearing diet (Ayvaz and Karabörklü 2008; Liu et al. 2007). The extent of

the negative effects may be proportional to the duration of cold storage (Colinet and Boivin 2011). In the present study, the extended cold storage of the naïve pupae, mummified pupae, or parasitoids, negatively affected *T. brontispae* parasitism. The F1-female sex ratio (percentage of female progeny) was marginally affected by cold storage. However, the exposure of mummified pupae to cold temperatures resulted in more female-biased progeny, although many cold-treated parasitoids produce male-dominant offspring (Colinet and Boivin 2011). The present study revealed that the parasitoid females could be stored at cold temperatures for two days and that naïve one-day-old pupae could be stored for eight days without any loss of quality. The cold storage of mummified pupae for seven days resulted in lower parasitism. Therefore, the cold storage of naïve one-day-old pupae appears to be a more suitable method for facilitating the production of *T. brontispae* on *O. nipae*. Huang et al. (2008) reported similar results for *T. brontispae* parasitism on *B. longissima*. In addition to the parasitism rate and female sex ratio, cold storage has profound effects on many other fitness parameters of parasitoids, such as the development time, lifespan and mortality (Colinet and Boivin 2011). Future research will focus on the mechanism by which cold storage affects these fitness parameters for *T. brontispae*.

In summary, we report the biological traits, including parasitism rate, number of emerged offspring per host and F1-female sex ratio, of *T. brontispae* on its host *O. nipae* pupae in the laboratory. Our results indicate that *T. brontispae* is a promising biological control agent for *O. nipae* and also shed light on methods to efficiently raise *T. brontispae* in the laboratory for the management of *O. nipae* in palm plants. However, before using this parasitoid in biocontrol programs against *O. nipae*, studies under field conditions are necessary to assess whether this type of biological control potentialities also occurs in the field. Therefore, further investigations should be conducted to understand the parasitoid field foraging activity in relation to the *O. nipae*-infested palm attractiveness to *T. brontispae* (Lewis and Martin 1990; Steidle and van Loon 2003), the potential for the simultaneous activity of this parasitoid with other biological control agents that attack other host instars (Chailleux et al. 2013), and the potential susceptibility of *T. brontispae* to the negative effects of the pesticide used in palm trees (Biondi et al. 2013).

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