

## RESEARCH

## Effect of Temperatures and Cold Storage on Performance of *Tetrastichus brontispae* (Hymenoptera: Eulophidae), a Parasitoid of *Brontispa longissima* (Coleoptera: Chrysomelidae)

Kui Liu,<sup>1,2</sup> Buli Fu,<sup>1</sup> Jiangrong Lin,<sup>3</sup> Yueguan Fu,<sup>1</sup> Zhengqiang Peng,<sup>1</sup> and Qi'an Jin<sup>1</sup>

<sup>1</sup>Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Sciences, 571101, China

<sup>2</sup>Corresponding author, e-mail: lk0750@163.com

<sup>3</sup>College of Animal sciences, Agricultural University of South China, Guangzhou 520000, China

**Subject Editor:** Paul Ode

J. Insect Sci. 14(257): 2014; DOI: 10.1093/jisesa/ieu119

**ABSTRACT.** Laboratory studies were conducted to determine the effect of temperature and cold storage on the performance of *Tetrastichus brontispae* (Ferriere) (Hymenoptera: Eulophidae), one of the major endoparasitoids against coconut hispine beetle, *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae). The results revealed that *T. brontispae* could successfully parasitize host pupae under all seven tested temperatures, but no adult emergence was observed at 32°C. It was also revealed that temperatures between 24 and 26°C appeared to be the optimum temperatures for parasitism, as these temperatures resulted in the most parasitized pupae and a significantly higher emergence rate and progeny production. These measurements significantly declined at 20, 30, and 32°C. This study confirmed developmental periods of parasitoid progeny decreased as the temperature increased, and sex ratio of this female-biased parasitoid was not affected by rearing temperatures. More importantly, this study indicated that cold storage of parasitized pupae could extend up to 30 d at 10°C, and a longer storage period had a significant adverse effect on mean adult emergence and parasitism performance. Ten days might be the optimum cold-storage period at 10°C, as parasitism performance, emergence rate, and progeny production at this storage period were similar to the control of 26°C. Furthermore, the developmental period, emergence rate, and sex ratio of progeny that emerged from cold-stored parasitized pupae were not influenced by storage periods, whereas parasitism performance of progeny decreased as storage period increased. This study suggests that about 24–26°C would be the optimal temperature for mass production and release of *T. brontispae* for biological control of *B. longissima*. These results also provide novel findings that a period of 10 d at 10°C may be more suitable and acceptable for ideal cold storage of parasitized pupae of *T. brontispae*.

**Key Words:** biological control, development, parasitism, parasitoid, progeny

The coconut hispine beetle, *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae), which is believed to be native to an area including Indonesia and Papua New Guinea, is one of the most serious economical invasive pests of Palmae plants (Chiu and Chien 1985, Waterhouse 1987). Most recently, the beetle has been accidentally introduced into Southeast Asia including Thailand, Vietnam, Philippines, and Hainan Province in China (Liebregts and Chapman 2004, Lu et al. 2008, Chen et al. 2010, Ichiki et al. 2011, Nguyen et al. 2012). The pest causes heavy damage and large losses on both the coconut industry and the tropical tourism industry, as both larvae and adults feed on the tissues of unopened leaves of coconut palms, resulting in brown leaves and decreased fruit production (Waterhouse 1987; Nakamura et al. 2006, 2008; Rethinam and Singh 2007; Lu et al. 2008). Chemical control of *B. longissima* is deemed undesirable and unpractical, and pesticide application to tall coconut trees poses great risks for applicators because they must climb up to the crown of the tree without protective clothing. Furthermore, application of broad-spectrum pesticides is likely to result in the emergence of insecticide resistant populations, and the residue of pesticides causes health risks to people, domestic and wild animals, and the environment. Augmentative biological control programs have been initiated as parts of integrated pest management programs during the last few years mainly because they are cost-effective means of sustainable management of exotic pests in productive systems. Importation biological control has been conducted mainly using two specialist parasitoids, *Asecodes hispinarum* (Boucek) (Hymenoptera: Eulophidae) and *Tetrastichus brontispae* (Ferriere) (Hymenoptera: Eulophidae), against larval and pupal stage of *B. longissima* (Waterhouse 1987, Voegelé 1989, Lu et al. 2008). Successful classical biological control of *B. longissima* was reported in several countries

(Chiu and Chien 1985, Voegelé 1989). Several recent studies confirmed that the above two parasitoids were capable of controlling *B. longissima* because of their high parasitism performances under laboratory conditions (Halfpapp 2001, Lu et al. 2008, Chen et al. 2010, Nguyen et al. 2012). Thus, environmentally friendly biological control techniques seem to be feasible. *T. brontispae* is one of the vital parasitoids of *B. longissima* and has been successfully introduced to several countries to control the beetle, its success springing from its ability to locate hosts successfully, and its specialization for parasitizing. Details on its biology associated with environment variable factors have been well documented in recent years (Ma et al. 2006, Zhou et al. 2006, Tang et al. 2009, Chen et al. 2010). Temperature is one of the vital factors affecting the development, survival, reproduction, and distribution of such parasitoids, and the development rate, fecundity, and longevity of *T. brontispae* were affected by different temperatures (Chen et al. 2010, Nguyen et al. 2012). Previous studies have offered useful information for mass production of the parasitoid in laboratory before mass release. However, basic knowledge on parasitism performance of parents and progeny of *T. brontispae* related to different temperatures is needed, and knowledge of the effect of temperature must be correctly evaluated before mass rearing and release of the parasitoid wasp in biocontrol programs. One critical issue in the mass rearing of parasitoid wasps is storage of them before application. It is desirable to store sufficient parasitoids to meet a fluctuating demand because field requirements can vary (Bradley et al. 2004, Tezze and Botto 2004). When climatic and environment conditions are suitable or favorable, storage is needed to ensure the production of a large number of parasitoids for mass release as needed. Cold storage can permit a cost-effective production schedule, providing a means to conserve biological control agents

when not immediately needed (Pitcher et al. 2002, Ayvaz et al. 2008). Most studies of cold storage have been focused on *Trichogramma* species (Jalali and Singh 1992, Boivin 1994, Kumar et al. 2005) due to its value in biological control programs against many Lepidoptera pests. Cold storage of parasitoids may induce diapause or quiescence, which is a physiological adaptation of insects to survive under extreme environmental conditions (Ozder and Saglam 2004, Kumar et al. 2005, Ayvaz et al. 2008), resulting in slowed or halted development that resumes once favorable conditions exist. Diapause slows the metabolism of organisms, but the metabolic rate returns to normal upon the return of suitable conditions. Many previous studies indicated that appropriate conditions to store parasitoids varied with species and should be studied in detail for each species separately because all parasitoid species did not have a similar potential to be cold stored prior to their use in biocontrol (Foerster et al. 2004, Tezze and Botto 2004, Bayram et al. 2005, Kumar et al. 2005, Ayvaz et al. 2008). Little is known about the effects of cold storage on the performance of *T. brontispae*, so it is practical to estimate the biology and parasitism performance after cold storage of *T. brontispae* in the laboratory, which would give an accurate prediction for their performance outdoors.

Therefore, in an effort to increase basic knowledge of the effects of extrinsic factors on the performance of parents and progeny of *T. brontispae*, as well as to predict the success of parasitism in the field, experiments were conducted on the effects of temperature and cold storage on the performance of *T. brontispae*. The main objectives of this study were to determine the effects of rearing temperatures on the parasitism capacity and development and the survival of parasitoid progeny. More importantly, to determine the optimal conditions for cold storage, the effects of a range of cold-storage periods on the subsequent parasitism performance of *T. brontispae* were also investigated.

## Materials and Methods

**Host Insect Cultures.** *B. longissima* used in the study were collected from Sanya (18° 15' N, 109° 30' E), Hainan, China, in 2012. Larvae and adults were introduced into plastic containers (17 by 11 by 5 cm, length by width by height) with a screened window in the lid and reared continuously with fresh coconut leaves under standard laboratory conditions in a climate cabinet (KBWF720, WTB-BINDER, Germany) (26 ± 0.5°C, 65 ± 5% RH, and a photoperiod of 12:12 (L:D) h). Both males and females were kept together to ensure mating. A piece of tissue paper soaked in a 10% honey solution was fixed on the wall of the box as additional food. Newly emerged pupae of the same generation were selected as hosts for maintaining parasitoids and were used for the experimentation.

**Parasitoid Insect Cultures.** The colony of *T. brontispae* was originally obtained from Taiwan in 2012 and was maintained using young pupae of *B. longissima*. Adult parasitoids of similar age were maintained in groups and fed with 10% honey solution in Petri dishes (6.0 by 2.5 mm, diameter by height). For mass rearing of parasitoids, ~200 1-d-old pupae from the host culture were provided in each box together with 400 adult wasps. Then boxes were left until adult wasps died. To segregate parasitoids for continuous mass rearing and experiments, parasitized pupae were removed from the culture and placed individually in tubes (1 cm in diameter) under the same condition as described above. These procedures were repeated everyday.

**Effect of Temperature on Performance of Females and Progeny of *T. Brontispae*.** Prior to the experiment, five *B. longissima* pupae were randomly selected (<2-h old), placed in groups, and were transferred into plastic tubes (7.5 cm in height, 1 cm in diameter) as prey. A cotton ball soaked in 15% honey and water solution was provided in the bottom of the tube as food. Newly emerged male and female parasitoids were placed together for a period of 24 h to permit mating. One randomly selected female parasitoid wasp was introduced into the tubes to parasitize the pupae. The tubes were kept at seven different constant temperatures (treatments), 20, 22, 24, 26, 28, 30, and 32 ± 0.5°C, and maintained in climate cabinets at similar conditions as above, respectively. Thirty

female parasitoid wasps (replicates) were tested under each temperature. After a period of 24 h for parasitism, new groups of five young pupae (<2-h old) were transferred into the tubes with the previous female wasp for continued parasitism until the parasitoid wasps were dead. Newly parasitized pupae (progeny) were removed and placed in a new tube to incubate under the same temperature. After the 5th day of emerged progeny, the parasitized pupae were dissected to confirm parasitism status. Based on the above procedures, the number of parasitized pupae, and female and male progeny were counted separately under different temperatures. Female survival, emergence rate, sex ratio, and developmental period of parents and progeny were recorded and calculated to assess parasitism performance related to different temperatures.

Through the observation of previous and present experiment, we distinguished the parasitized pupae from nonparasitized on the changes of the color. For parasitized pupae, once the host pupae were parasitized then the color deeply changes to black-brown (5-d old) with increasing the time. However, this would not be observed for nonparasitized pupae, they were still keep pale yellow or *B. longissima* would emerge out after the 5th day, but the parasitized pupae maintain the pupae shape after the 5th day. The number of parasitized pupae contained two portions. One was the cumulative number of pupae which once normal parasitoid wasps emerged out in the 5 d of emergence period before dissection. Another was nonemerged parasitoid wasp (means dead wasp or failure of development from egg to adult) when parasitized pupae were dissected to confirm parasitism status after the 5th day of emerged progeny. Thus, total parasitoid progeny wasps were the sum number of normal emerged parasitoid wasps plus dead wasp. Based on above, the emergence rate was the percentage of normal emerged parasitoid wasps in total parasitoid progeny wasps.

The data on developmental time under the constant temperatures, 20, 22, 24, 26, and 28°C were used for establishing the temperature-developmental rate regression equation. The developmental threshold ( $T_0$ ) and the effective accumulative temperature ( $K$ ) were calculated as following:  $T_0 = [\sum V^2 \sum T - \sum V \sum VT] / [n \sum V^2 - (\sum V)^2]$ ,  $K = [n \sum VT - \sum V \sum T] / [n \sum V^2 - (\sum V)^2]$ , where  $T$  indicates average temperature of development,  $V$  represents growth rate, and  $n$  is the number of treatments.

**Effect of Cold Storage on Biological Performance of *T. Brontispae*.** Prior to the experiment, ~200 1-d-old pupae of *B. longissima* in the same generation were placed together with sufficient adult parasitoids in a plastic box (17 by 11 by 5 cm, length by width by height) under the same standard laboratory conditions at 26°C. After a period of 24 h of parasitism, the 5-d-old parasitized pupae were randomly selected, divided into three groups, and stored in darkness for periods of 10, 20, and 30 d at 10°C in a climate cabinet. When the storage period was finished, the cold-stored pupae were transferred into tubes (7.5 cm in height, 1 cm in diameter) for normal rearing at 26°C. After the 5th day of emerged progeny, parasitized pupae were dissected to confirm parasitism status. Thirty cold-stored pupae were tested at each storage period (treatment), and one group was stored at 26°C as a control treatment. The number of female and male wasps that emerged from cold-stored pupae was counted daily. The emergence rate and the time of development from egg to adult were also recorded under different storage periods.

**Effect of Cold Storage on Parasitism Performance of *T. Brontispae*.** To estimate the effect of cold storage on parasitism performance, young adult *T. brontispae* that had emerged from 10, 20, and 30 d of cold storage as pupae were selected as parasitoids. Males and females were placed together for a period of 24 h to ensure mating, and afterward, one female was randomly selected and placed into a tube (7.5 cm in height, 1 cm in diameter) with five *B. longissima* pupae (<2-h old). A cotton ball soaked with 10% honey solution was provided in the tube as food. The tube was maintained at 26°C in climate cabinets at similar conditions as above. After a period of 24 h for parasitism, new groups of five *B. longissima* pupae (<2-h old) were

transferred into the tube with the previous female wasp for continued parasitism until the wasps were dead. Newly parasitized pupae (progeny) were removed and placed in a new tube to incubate at a temperature of 26°C. Thirty female parasitoid wasps (replicates) that emerged from each of the three cold-storage periods were tested, and parasitoids stored at 26°C were used as a control treatment. The number of parasitized pupae and females and males of progeny were counted and recorded daily, and sex ratios at different treatments were calculated.

**Statistical Analysis.** All statistical tests were performed using the GLIMMIX procedure in SAS version 9.13 (www.sas.com). The effects of temperature on survivorship, number of parasitized pupae, emergence rate, developmental time, and offspring production were analyzed using one-way analysis of variance of Tukey's test. The significant differences between treatments concerning cold-storage periods with control group were determined by Dunnett's test. Differences at a probability level of  $P < 0.05$  were considered significant.

**Results**

**Effects of Temperature on Performance of Female *T. Brontispae*.**

The effects of temperature on survivorship and parasitism of parent *T. brontispae* are summarized in Table 1. Temperature had a highly significant effect on the survivorship of adult *T. brontispae* (Table 1). Higher temperatures were associated with declines in adult survivorship, and the survival of female parasitoids was significantly longer at 20 and 22°C, whereas it declined at 32 and 30°C. Survival difference were not significant among the 24, 26, and 28°C treatments (Table 1).

Temperature had a varied effect on parasitism performance of *T. brontispae*. Female wasps showed the highest parasitism capacity at 26°C. The number of parasitized pupae significantly decreased as the temperature increased to 30 and 32°C, respectively. No significant difference was found on parasitized pupae at 20, 22, 24, and 28°C (Table 1).

**Table 1. Longevity and number of host parasitized of *T. brontispae* under different constant temperatures**

Temperature (°C)	Longevity (d)	No. of host parasitized
20	10.54 ± 0.38 a	3.03 ± 0.32 ab
22	10.07 ± 0.53 a	3.10 ± 0.18 ab
24	6.26 ± 0.28 b	3.67 ± 0.24 ab
26	4.38 ± 0.31 bc	3.93 ± 0.48 a
28	4.47 ± 0.18 bc	2.30 ± 0.17 ab
30	4.32 ± 0.23 c	1.73 ± 0.12 bc
32	3.86 ± 0.21 c	1.10 ± 0.17 c

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 test.

**Table 2. Developmental time and reproduction of *T. brontispae* under different constant temperatures**

Temperature (°C)	Developmental times (d)	% Adult emergence rate	No. of emerged females	Sex ratio (% female)
20	29.32 ± 0.20 a	67.90 ± 7.75 c	20.06 ± 0.62 c	89.71 ± 3.35 a
22	28.56 ± 0.13 a	90.89 ± 5.51 a	26.23 ± 1.34 b	90.35 ± 3.46 a
24	21.30 ± 0.16 b	91.67 ± 4.16 a	32.77 ± 2.30 a	88.29 ± 4.07 a
26	18.61 ± 0.11 c	91.67 ± 6.75 a	33.93 ± 1.68 a	88.60 ± 4.24 a
28	17.23 ± 0.08 c	85.06 ± 5.25 b	18.50 ± 3.37 c	90.55 ± 4.89 a
30	17.18 ± 0.23 c	46.43 ± 6.82 d	9.70 ± 0.61 d	89.81 ± 3.38 a
32	—	—	—	—

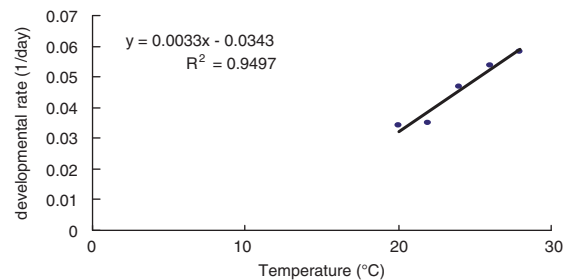
Data are presented as means ± SE. Means in the same column followed by different small letters are significantly different at  $P = 0.05$  level by Tukey's test.

Obtained data on emergence rates emerged from parasitized pupae of *T. brontispae* under different temperatures are displayed in Table 2. Emergence rates at 26, 24, and 22°C were significantly higher than other temperatures, but the differences were not significant among the three temperatures. No parasitoid wasp emerged at 32°C, as the larval stage had died after dissection of such parasitized pupae reared under such temperature.

**Effect of Temperature on Host Suitability for Parasitism of *T. Brontispae*.** Data on the number of wasp progeny are presented in Table 2. The mean number of adult wasps that emerged from parasitized pupae was significantly affected by rearing temperatures. The highest numbers of progeny generated per parent female wasp were at 26 and 24°C, and numbers declined at 20, 22, 28, and 30°C. The sex ratio (% female) was not significantly affected by temperature, as the overall sex ratios of emerged adults were female biased under different temperatures (Table 2).

The effect of temperature on developmental times is presented in Table 2. The mean developmental time of progeny of *T. brontispae* decreased with increasing temperature, and the developmental time from egg to adult was significantly longer at 20 and 22°C than other treatments. The relationship between temperature (20–28°C) and development rate of progeny of *T. brontispae* could be reflected by the regression equation  $V = 0.0033T - 0.0343$  ( $R^2 = 0.9497$ ,  $P = 0.05$ ) (Fig. 1), where  $V$  is developmental rate from egg to adult and  $T$  is the temperature. The developmental threshold was 10.48°C, and the effective accumulative temperature was 303.03 degree days accordingly.

**Effect of Cold Storage on Parasitism of *T. Brontispae*.** The effects of cold storage on emergence rate, developmental time from parasitized pupae to adult, mean number of female progeny emerged from parasitized pupae, and sex ratio are listed in Table 3. Emergence rate was significantly affected by longer storage periods when the parasitized pupae were stored at 10°C. In the control, 90.00 ± 5.77% of adults emerged, but this significantly decreased after 10-, 20-, and 30-d storage periods. A similar decrease was also observed in the number of female progeny that emerged from cold-stored parasitized pupae. The sex ratio (% female) was not significantly influenced by



**Fig. 1.** Regression analysis of progeny of *T. brontispae* development rate at five constant temperatures.

**Table 3. Emergence rate, developmental time, and number of females per parasitized pupae and sex ratio of *T. brontispae* under different cold-storage periods of pupae at 10°C**

Days in cold storage	% Adult emergence rate	Developmental time (d)	No. of females per parasitized pupae	Sex ratio (% female)
0	90.00 ± 5.77 a	18.17 ± 0.03 d	9.77 ± 0.78 a	83.93 ± 4.09 a
10	80.00 ± 5.75 ab	27.88 ± 0.12 c	8.90 ± 0.73 ab	85.52 ± 5.22 a
20	70.00 ± 5.77 b	38.59 ± 0.16 b	6.60 ± 0.90 bc	86.05 ± 5.47 a
30	66.67 ± 6.66 c	48.75 ± 0.18 a	4.40 ± 0.23 c	84.61 ± 4.65 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 Dunnett's test.

cold-storage periods, as the overall sex ratios of emerged adults were female biased.

Cold-storage periods of parasitized pupae at 10°C had a significant effect on development. The developmental duration from parasitized pupae to adult wasp was significantly prolonged with increasing storage periods when compared with the control (Table 3). Storage periods of 20 and 30 d lead to increased developmental time, but the emergence rate and number of progeny were significantly reduced by such storage periods. Parasitized pupae would likely benefit from cold storage for 10 d at 10°C, as no significant difference was found compared with the control.

**Effect of Cold Storage on Survival and Reproduction of *T. brontispae*.** The mean survival period of *T. brontispae* adults that emerged from cold-stored parasitized pupae was significantly influenced by storage period when it parasitized the pupae of *B. longissima*. An increase in storage period resulted in lower survival time than that of the control, but the adult females that emerged from 10 d of cold-stored parasitized pupae at 10°C showed capable survival time compared with the control, and this significantly decreased for those cold stored for 20 and 30 d (Table 4). A similar decreasing trend was also found in the number of parasitized pupae as the storage period increased (Table 4). A storage period of 20 and 30 d negatively affected the number of parasitized pupae. The emergence rate of progeny was not affected by cold storage compared with the control.

Comparisons of the control treatment with any other treatment on the mean number of female progeny, which were produced by parent parasitoid wasps that emerged after cold storage, varied greatly depending on storage period. The number of females that emerged per wasp significantly decreased from the control and 10 d of storage to 20 and 30 d of storage (Table 5). The sex ratio (% female) was not significantly affected by cold-storage period (Table 5), as the overall sex ratios of emerged adults were female biased. No significant difference was observed on developmental time among the treatments (Table 5).

## Discussion

Temperature is one of the major environmental variables affecting the development, reproduction, longevity, and survival of parasitoids, as well as the parasitism performance and progeny production. It can be

**Table 4. Longevity and number of host parasitized of *T. brontispae* under different cold-storage periods of pupae at 10°C**

Days in cold storage	Longevity (d)	No. of host parasitized
0	4.39 ± 0.32 a	3.93 ± 0.48 a
10	4.24 ± 0.38 a	3.93 ± 0.20 a
20	3.42 ± 0.53 b	2.13 ± 0.20 b
30	3.16 ± 0.28 b	1.97 ± 0.19 b

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 Dunnett's test.

**Table 5. Developmental time and reproduction of *T. brontispae* under different cold-storage periods of pupae at 10°C**

Days in cold storage	Developmental times (d)	% Adult emergence rate	No. of emerged females	Sex ratio (% female)
0	18.72 ± 0.15 ab	90.69 ± 6.24 a	33.93 ± 1.68 a	88.60 ± 3.57 a
10	18.86 ± 0.12 ab	91.90 ± 6.78 a	33.97 ± 0.23 a	88.30 ± 3.22 a
20	21.24 ± 0.21 a	88.26 ± 5.57 a	19.20 ± 1.14 b	90.14 ± 3.85 a
30	21.56 ± 0.24 a	89.64 ± 5.45 a	18.37 ± 2.04 b	90.76 ± 4.06 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 Dunnett's test.

seen from this study that survival time of adult *T. brontispae* was negatively related to increasing temperature when the female wasp parasitized pupae of *B. longissima*. Similarly, a reduction in survivorship of *T. brontispae* due to high temperature has been shown by previous studies (Ma et al. 2006, Tang et al. 2009, Chen et al. 2010). Adult *T. brontispae* could survive for  $3.86 \pm 0.21$  d at 32°C in our study (Table 1), suggesting that *T. brontispae* could be a desirable candidate species for mass rearing and release as a biological control agent in hot climates due to its tolerance of higher temperatures. Few parasitized pupae and no emergence were observed at 32°C (Table 2), but the *B. longissima* pupae parasitized by female wasps had died under such temperature.

There are many intrinsic and extrinsic factors that affect parasitism, such as host density, host stages, and parasitoid age (Hentz 1998, Honda 1998, Aung et al. 2010). Chen et al. (2010), and Nguyen et al. (2012) demonstrated that *T. brontispae* successfully attacked and developed in all stages of hosts from the fourth instar larvae to 4-d-old pupae, but the optimum host stage could be young pupae (<1-d old). Our study indicated that 24–26°C was the optimum temperature for parasitism because of the high number of parasitized pupae, the high emergence rate, and large production of progeny (Table 2). This result suggests that *T. brontispae* would be most capable of attacking *B. longissima* if the environment temperature is near 24–26°C when the parasitoid is released in biocontrol programs. The results of our study are consistent with the study of Nguyen et al. (2012), who indicated that temperatures from 22 to 28°C were appropriate for parasitizing *B. longissima* pupae and lower and higher temperatures were not beneficial to parasitization. Furthermore, 32°C might lead to mortality for *T. brontispae* larvae because no progeny of wasp emerged from parasitized pupae at this temperature (Table 2). This result is in accordance with Nguyen et al. (2012), who also demonstrated that wasps of *T. brontispae* could not complete development below 16 and above 31°C. Control efficiency of *T. brontispae* may have been limited in the field environment because the natural temperature may have been too hot or too cold.

In this study, sex ratio of progeny was not significantly different among all temperatures and confirmed *T. brontispae* is a female-biased parasitoid wasp (Table 2), which indicates that this parasitoid has the potential to reduce the population of coconut leaf beetle because of the amount of female parasitoids produced (Chiu and Chien 1985, Jervis and Copland 1996, Tang et al. 2009). The developmental time of progeny significantly declined with increasing the temperatures (Table 2). This result is similar to the results of Ma et al. (2006), but the time was comparatively longer compared with Chen et al. (2010). The difference is most likely due to different host insect cultures. The development threshold of *T. brontispae* progeny in our study was 10.48°C (Fig. 1), indicating that *T. brontispae* may show the potential of overwintering ability to establish its population in Hainan Province where the average temperature is ranging from 16 to 24°C in the coldest months of winter.

For effective mass production, it is necessary to develop effective methods for storing parasitoids without negatively affecting their fitness (Langer and Hance 2000). The capacity to survive at low temperatures can be exploited for parasitoid cold storage before mass release in biological control programs (Hofsvang and Hagvar 1977, Levie et al. 2005). However, there are few studies and little information that record the influence of cold storage on survivorship and parasitism of *T. brontispae*. In this study, development of *T. brontispae* was prolonged with prolonged storage periods. *T. brontispae* successfully emerged from all cold-stored parasitized pupae, but emergence rate and parasitism performance declined as the storage period increased (Tables 3 and 4). The emergence rate was similar to the control after a storage period of 10 d at 10°C but significantly declined after 30 d (Table 3). A reduction in emergence rate due a long period of cold storage was also seen on *Trichogramma* spp. by Jalali and Singh (1992), Foerster et al. (2004), and Rundle et al. (2004), who clearly demonstrated that longer storage times accompanied with lower temperatures

adversely influenced adult emergence. Similarly, the mean number of females that emerged from cold-stored parasitized pupae was also negatively affected by length of storage period of 20 and 30 d (Table 3), possibly it was attributed to the delay and interference of normal metabolic conditions after being cold stored for a prolonged period (Tezze and Botto 2004). Nevertheless, no significant difference was observed between a storage period of 10 d at 10°C and without cold storage at 26°C (Table 3). Thus, the above data showed that parasitized pupae could be safely stored up to 30 d at 10°C, and the ideal storage period was 10 d, which might be a storage technique useful in biological control programs for mass release of *T. brontispae*.

Parasitism performance of emerged parasitoids declined with cold-storage treatments even though all emerged parasitoids parasitized the host pupae. The number of parasitized pupae achieved by emerged parasitoids was not significantly different than the control (26°C) after 10 d storage at 10°C, whereas it was significantly lower after storage periods of 20 and 30 d (Table 4). Reduced parasitism performance of cold-stored parasitoids has been reported for several other egg parasitoids (Pitcher et al. 2002, Rundle et al. 2004, Bayram et al. 2005), possibly because they were not well formed, were too weak, or had other physiological changes. Many factors might be involved in explaining this observation, such as an incomplete development, a decrease in sperm survival or motility, or even death of germ cells during cold storage (Levie et al. 2005). These need further investigation.

Longer storage resulted in a significant decrease of emerged-adult survivorship and number of progeny, except for 10 d in cold storage. The results of our study suggest that there was no adverse effect of emerged parasitoids from cold-stored parasitized pupae on emergence rate and developmental duration from egg to adult of progeny (Table 5). This result can be useful in preserving both the quality of the founder colony of *T. brontispae* and the parasitoid production prior to mass releases.

The proportion of female progeny and the emergence rate of progeny were not significantly modified when the parasitized pupae were stored at 10°C. This demonstrated that storage period had no differential effects on pupal survival depending on the sex, as sex ratios of adult parasitoids were not affected by storage treatments. Data on sex ratio also clearly confirmed that *T. brontispae* was a female-biased parasitoid wasp (Chen et al. 2010), which indicates that this parasitoid has the potential to successfully manage *B. longissima* in the field through mass production and release (Nguyen et al. 2012).

On the basis of results of this study, we conclude that survivorship, development, and parasitism of *T. brontispae* are significantly affected by temperature, and 24–26°C appears to be the optimum temperature for parasitism, as *T. brontispae* had a higher emergence rate and more production of progeny under such temperatures. More importantly, even though cold storage of parasitized pupae can extended up to 30 d at 10°C, emergence rate and parasitism performance were adversely influenced by longer storage periods. Thus, 10 d is the optimum cold-storage period at 10°C. Storage under such conditions may enable production of a large enough quantity of parasitoids for release and maintenance of the quality of the laboratory colony. Further studies on the efficiency of cold-stored *T. brontispae* against coconut leaf beetles in field environments, storage possibilities of host eggs and adults, and more acceptable storage temperatures related to parasitism performance may be needed to develop parasitoid mass production techniques for biological control. The results of this study could allow better understanding of the ecology of *T. brontispae* and improve biological control of *B. longissima*.

#### Acknowledgments

This project supported by Special Fund for Agro-scientific Research in the Public Interest from Ministry of Agriculture of China (201103026). We thank Dr. Dong Q. Zeng and Dr. Liang D. Tang for critical evaluations of this work and for helpful comments on the

manuscript. We also thank two reviewers for their constructive comments and valuable suggestions for this paper.

#### References Cited

- Aung, K.S.D., M. Takagi, and T. Ueno. 2010. Effect of female's age on the progeny production and sex ratio of *Ooencyrtus nezarae*, an egg parasitoid of the bean bug *Riptortus clavatus*. J. Faculty Agric. Kyushu Univ. 55: 83–85.
- Ayvaz, A., E. Karasa, S. Karaborklu, and A. Tuncbilek. 2008. Effects of cold storage, rearing temperature, parasitoid age and irradiation on the performance of *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae). J. Stored Prod. Res. 44: 232–240.
- Bayram, A., H. Ozcan, and S. Kornoson. 2005. Effect of cold storage on the performance of *Telenomus busseolae* Gahan (Hymenoptera: Scelionidae), an egg parasitoid of *Sesamia nonagrioides* (Lefebvre) (Lepidoptera: Noctuidae). Biol. Control 35: 68–77.
- Boivin, G. 1994. Overwintering strategies of egg parasitoids, pp. 219–244. In E. Wajnberg and S. A. Hassan (eds.), Biological control with egg parasitoids. CAB International, Wallingford, United Kingdom.
- Bradley, J. R., L. J. Thomson, and A. A. Hoffmann. 2004. Effects of cold storage on field and laboratory performance of *Trichogramma carverae* (Hymenoptera: Trichogrammatidae) and the response of three *Trichogramma* spp. (*T. carverae*, *T. nr. brassicae*, and *T. funiculatum*) to cold. J. Econ. Entomol. 97: 213–221.
- Chen, Q., Z. Peng, C. Xu, B. Lu, Q. Jin, H. Wen, and F. Wan. 2010. Biological Assessment of *Tetrastichus brontispae*, a pupal parasitoid of coconut leaf beetle, *Brontispa longissima*. Biocontrol Sci. Technol. 20: 283–295.
- Chiu, S. C., and B. H. Chien. 1985. Importation and establishment of *Tetrastichus brontispae*, a parasitoid of the coconut leaf beetle, in Taiwan. Taiwan Agric. Res. Inst. 19: 12–13.
- Foerster, L. A., A. K. Doetzer, and L.C.F. Castro. 2004. Emergence, longevity and fecundity of *Trissolcus basalis* and *Telenomus podisi* after cold storage in the pupal stage. Pesquisa Agropecuaria Brasileira 39: 841–845.
- Halfpapp, K. 2001. Introduction of *Tetrastichus brontispae* for control of *Brontispa longissima* in Australia. In Proceedings of the Sixth Workshop for Tropical Agricultural Entomology, 11–15 May 1998, Darwin, Australia. Department of Primary Industry and Fisheries, Northern Territory of Australia.
- Hentz, M. G. 1998. Development, longevity, and fecundity of *Chelonus* sp. nr. *curvimaaculatus* (Hymenoptera: Braconidae), an egg-larval parasitoid of pink bollworm (Lepidoptera: Gelechiidae). Environ. Entomol. 27: 443–449.
- Hofsvang, T., and E. Hagvar. 1977. Cold storage tolerance and supercooling points of mummies of *Ephedrus cerasicola* Stary and *Aphidius colemani* Viereck (Hym., Aphidiidae). J. Entomol. 24: 1–6.
- Honda T. 1998. Age-related fecundity and learning ability of the egg larval parasitoid *Ascogaster reticulatus* Watanabe (Hymenoptera: Braconidae). Biol. Control 13: 177–181.
- Ichiki, R. T., M. Murata, S. Takano, T. T. Oo, H. T. Nguyen, W. Suasa-Ard, S. Uraichuen, and S. Nakamura. 2011. Artificial diets for rearing the coconut hispine beetle, *Brontispa longissima* (Coleoptera: Chrysomelidae), and their suitability to two specialist parasitoids. Biocontrol Sci. Technol. 21: 1423–1435.
- Jalali, S. K., and S. P. Singh. 1992. Differential response of four *Trichogramma* species to low temperatures for short term storage. Entomophaga 37: 159–165.
- Jervis, M. A., and M.J.W. Copland. 1996. The life cycle in insect natural enemies: practical approaches to their study and evaluation, pp. 63–161. In M. A. Jervis and N.A.C. Kidd (eds.), Chapman & Hall.
- Kumar, P., M. Shenhmar, and K. S. Brar. 2005. Effect of low temperature storage on the efficiency of three species of trichogrammatids. J. Biol. Control 19: 17–21.
- Langer, A., and T. Hance. 2000. Overwintering strategies and cold hardiness of two aphid parasitoids species (Hymenoptera: Braconidae: Aphidiinae). J. Insect Physiol. 46: 671–676.
- Levie, A., P. Veron, and T. Hance. 2005. Consequence of acclimation on survival and reproductive capacities of cold-stored mummies of *Aphidius rhopalosiphii* (Hymenoptera: Aphidiinae). J. Econ. Entomol. 9: 704–708.
- Liebrechts, W., and K. Chapman. 2004. Impact and control of the coconut hispine beetle, *Brontispa longissima* Gestro (Coleoptera: Chrysomelidae), pp. 19–34. In Report of the Expert Consultation on Coconut Beetle Outbreak in APPPC Member Countries. FAO Regional Office for Asia and the Pacific.
- Lu, B., C. Tang, Z. Peng, J. L. Salle, and F. Wan. 2008. Biological assessment in quarantine of *Asecodes hispinarum* Boucek (Hymenoptera: Eulophidae) as an imported biological control agent of *Brontispa longissima* (Gestro) (Coleoptera: Hispididae) in Hainan, China. Biol Control 45: 29–35.

- Ma, Z., X. Zhou, S. Zhao, C. Li, and G. Huang. 2006.** Effect of temperature on developmental time and parasitizing capacity of *Tetrastichus brontispae*. *Chin. J. Trop. Crops* 27: 61–65 (in Chinese).
- Nakamura, S., K. Konishi, and K. Takasu. 2006.** Invasion of the coconut hispine beetle, *Brontispa longissima*: current situation and control measures in Southeast Asia, pp. 1–9. In **Ku, T. Y. and M. Y. Chiang** (eds.), Proceedings of the International Workshop on Development of Database (APASD) for Biological Invasion, vol. 3, Taiwan Agricultural Chemicals and Toxic Substance Research Institute, Taichung, Taiwan, Food and Fertilizer Technology Center (FFTC) for the Asia and Pacific Region, Taipei, Taiwan.
- Nakamura, S., K. Konishi, and K. Takasu. 2008.** The coconut hispine beetle, *Brontispa longissima*: recent invasion to Southeast Asia, *Nogyo Gijutsu. J. Agric. Sci.* 64: 36–41.
- Nguyen, H. T., T. T. Oo, R. T. Ichiki, S. Takanoa, M. Murataa, K. Takasub, K. Konishic, S. Tunkumthongd, N. Chomphookhiawd, and S. Nakamuraa. 2012.** Parasitisation of *Tetrastichus brontispae* (Hymenoptera: Eulophidae), a biological control agent of the coconut hispine beetle *Brontispa longissima* (Coleoptera: Chrysomelidae). *Biocontrol Science and Technology* 8: 955–968.
- Ozder, N., and O. Saglam. 2004.** Effect of short term cold storage on the quality of *Trichogramma brassicae*, *T. cacoeciae*, and *T. evanescens* (Hymenoptera: Trichogrammatidae). *Great Lakes Entomol.* 37: 183–187.
- Pitcher, S. A., M. P. Hoffmann, J. Gardner, M. G. Wright, and T. P. Kuhar. 2002.** Cold storage of *Trichogramma ostrinae* reared on *Sitotroga cerealella* eggs. *Biocontrol* 47: 525–535.
- Rethinam, P., and S. P. Singh. 2007.** Current status of the coconut beetle outbreaks in the Asia-Pacific region, pp. 1–23. In S. Appanah, H. C. Sim, and K. V. Sankaran (eds.), *Developing an Asia-Pacific strategy for forest invasive species: the coconut beetle problem: bridging agriculture and forestry*. Food and Agriculture Organization of the United Nations Regional Office for Asia and the Pacific.
- Rundle, B. J., L. J. Thomson, and A. A. Hoffmann. 2004.** Effects of cold storage on field and laboratory performance of *Trichogramma carverae* (Hymenoptera: Trichogrammatidae) and the response of three *Trichogramma* spp. (*T. carverae*, *T. nr. brassicae*, and *T. funiculatum*) to cold. *J. Econ. Entomol.* 97: 213–221.
- Tang, C., Z. Q. Peng, Q. A. Jin, and H. B. Wen. 2009.** Establishment of laboratory population life table of *Tetrastichus brontispae* (Ferriere). *Acta Ecologica Sinica* 29: 185–191.
- Tezze, A. A., and E. N. Botto. 2004.** Effect of cold storage on the quality of *Trichogramma nerudai* (Hymenoptera: Trichogrammatidae). *Biol. Control* 30: 11–16.
- Voegelé, J. M. 1989.** Biological control of *Brontispa longissima* in Western Samoa: an ecological and economic evaluation. *Agric. Ecosyst. Environ.* 27: 315–329.
- Waterhouse, D. F. 1987.** *Brontispa longissima* (Gestro) in biological control, pp. 134–141. In M. Pressley (ed.), *Pacific prospects*. Inkata Press, Melbourne, Australia.
- Zhou, X., S. Li, G. Huang, Z. Ma, and S. Zhao. 2006.** A study on threshold temperature and effective accumulated temperature for the development of *Tetrastichus brontispae* (Ferriere) (Hymenoptera: Eulophidae). *Nat. Enemies Insects* 28: 8–12.

*Received 26 September 2013; accepted 2 August 2014.*