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RESEARCH ARTICLE

Parasitisation of *Tetrastichus brontispae* (Hymenoptera: Eulophidae), a biological control agent of the coconut hispine beetle *Brontispa longissima* (Coleoptera: Chrysomelidae)

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The gregarious endoparasitoids *Tetrastichus brontispae* Ferrière is one of the important natural enemies of the coconut hispine beetle *Brontispa longissima* (Gestro), a serious invasive pest on coconut palm plants (*Cocos nucifera* L.) in Southeast Asia. Development at different temperatures, effect of host and female ages, effect of food and oviposition frequency and superparasitism were investigated in the laboratory. Females were allowed only one attack against one host in all experiments. The wasp developed in a host between 19 and 30°C, whilst no wasp completed its immature development at 16 and 31°C. Host and female ages affected parasitisation. Parasitoid emergence was high on day 0 and day 1 pupal hosts, and younger females produced more offspring than older females. The longevity of the female was affected not only by food supply, but also by oviposition frequency. The female survived longer when oviposition frequency was low. However, the total number of hosts parasitised by the female during her lifetime did not differ at different oviposition frequencies. In superparasitism, although the percentage of adult emergence and body size of offspring decreased with an increasing number of attacks per host, a host parasitised by up to four females could produce parasitoid offspring.

Keywords: *Tetrastichus brontispae*; larval parasitoid; ecology; biological control; coconut hispine beetle; invasive insect pest

Introduction

The coconut hispine beetle, *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae) is a serious insect pest threat to coconut palm plants (Waterhouse 1987). Recently, it was accidentally introduced into Vietnam, Thailand and other countries in Southeast Asia (Liebregts and Chapman 2004; Nakamura, Konishi and Takasu 2006; Rethinam and Singh 2007). Its larvae and adults feed on the tissues of unopened leaves. Infestation with the beetle turns leaves brown and decreases fruit production. Sustained heavy attack may kill trees (Kalshoven 1981; Waterhouse 1987).

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Tetrastichus brontispae Ferrière (Hymenoptera: Eulophidae) was originally reported from Java, Indonesia (Ferrière 1933) and was introduced into some countries to control the beetle (Chen et al. 2010). Successful classical biological control of *B. longissima* was reported in four regions such as Celebes (Lever 1969), Tahiti, the Solomon Islands (Stapley 1973) and Taiwan (Chiu, Lai, Chen, Chen and Shiau 1985). However, there is little information on the biology and ecology of *T. brontispae*. Although Chen et al. (2010) described developmental time, parasitism rate, sex ratio and longevity of *T. brontipae*, their data were based on experiments using multiple females kept with multiple hosts for 24 h. They provided useful information for producing wasps on a large scale. Nevertheless, in gregarious parasitoids when superparasitism occurs or one female oviposits more than once into a single host, the number of offspring produced per host, offspring body size or sex ratio would be different from those when one female attacks a host only once (e.g. van Alphen and Visser 1990; Jervis and Copland 1996). Therefore, to better understand the reproductive strategy of this species, we conducted experiments using a single female wasp attacking a host beetle once. The following studies were carried out to investigate the biology of *T. brontispae* including development time at different temperatures, the effect of host and female ages, the effect of oviposition frequency and food and the effect of superparasitism.

Materials and methods

Insects

Brontispa longissima used in this study were collected in September 2009 on Ishigaki Island, Okinawa Prefecture, Japan. Since fresh leaves of coconut plants are difficult to obtain in Japan, where we conducted all experiments, we kept the beetle colony on fresh leaves of the narrow leaf cattail *Typha angustifolia* L. instead. This alternative host plant promotes normal development of the larvae and reproduction of the adults (Winotai, Sindhusake and Morakote 2007; Yamashita, Winotai and Takasu 2008). Larvae and adults were maintained separately on fresh leaves in plastic containers ($155 \times 115 \times 50$ mm) with a mesh window in the lid.

Tetrastichus brontispae was obtained from Taiwan in September 2009 and maintained using day 0 (defined as on the day of moulting to the current stage) or day 1 (defined as 1 day after moulting to the current stage) pupae of *B. longissima* as hosts. Adult wasps were maintained in groups and fed a 10% honey solution in Petri dishes (60×25 mm). Presumably mated wasps between 12 and 24 h after emergence and host pupae on day 0 and day 1 were used unless otherwise specified.

We defined mummification as a condition where the parasitised hosts become hard and dark brown in colour.

Different temperatures on biological parameters of T. brontispae

The effects of seven constant temperatures (16, 19, 22, 25, 28, 30 and 31°C) [60–70% RH, 12L: 12D] on the biological parameters of *T. brontispa*e were investigated with their hosts in climatic chambers. Day 0 or day 1 pupae were exposed to 10 newly emerged and mated *T. brontispae* females in a Petri dish for 30 min at 25°C (60–70% RH, 12L: 12D). Soon after oviposition began, the wasp and host were transferred to

another Petri dish until the wasp finished oviposition. We defined this event as a single attack. Parasitised hosts were removed and incubated under one of seven constant temperatures until wasp or adult host emergence. After emergence, we counted the number of dead wasps within the host. If female wasps did not attack the host during a 30 min interval, the host was discarded. Data on the percentage of parasitism, development time, adult emergence, sex ratio and forewing length of the offspring were recorded.

We calculated the effect of temperature on the development rate, which was described by a linear regression equation as: $y = b^*X + a$, where y is development rate (1/development time), X is temperature and a and b are constants. The lower thermal threshold (T_0) and thermal constant (K) were estimated as: $T_0 = -a/b$ and $K = 1/b$ (Campbell, Frazer, Gilbert, Gutierrez and Makauer 1974).

Percentages of parasitism were calculated as follows: % mummification = (number of mummies/number of hosts attacked) × 100; % adult emergence = (number of mummies producing adult wasps/number of hosts attacked) × 100. We determined the sex of newly emerging adult wasps and measured the body size of adult wasps under a stereomicroscope.

Host age acceptance and suitability of T. brontispae

We did not include 3rd larval instar hosts in this experiment, because all were dead after 2 or 3 days of oviposition. We used 4th instar larva (4 or 5 days after the 3rd moult), prepupa (2 days after a host larva stopped food consumption and became inactive), day 0, day 1, day 2, day 3 or day 4 pupa as a host. A host was exposed to 10 newly emerged and mated *T. brontispae* females in a Petri dish for up to 30 min for one attack. All procedures were conducted following the same methods previously described for *T. brontispae*.

Female age on parasitisation of T. brontispae

To assess the effect of female age on parasitisation and biological parameters, we prepared six groups of females. Newly emerged females after mating were individually kept in Petri dishes, in which a piece of paper was soaked in a 10% honey solution as a food source. Ten females of day 1, 3, 6, 10, 15 or 21 were placed together in a Petri dish. One host at day 0 or day 1 pupa was exposed in the Petri dish. Soon after a female inserted her ovipositor into a host, the female and host were carefully transferred into another Petri dish. After transferring the ovipositing female, we added another female of the same age with the rest of the females to continue the experiment. All procedures were conducted as previously described for *T. brontispae*.

Food and oviposition frequency on parasitisation and adult longevity of T. brontispae

Egg maturation in an ovary

To observe ovigeny and the number of mature eggs in an ovary after emergence, newly emerged, mated *T. brontispae* females were provided with a 10% honey solution, water or nothing. Each female was kept individually in a Petri dish.

We dissected females on day 0 (after 3 h of feeding), day 1 or day 2 and recorded the number of mature eggs in an ovary. Each observation was repeated 14–15 times.

Effect of food on the longevity of female T. brontispae *(this experiment referred as 'no-host' hereafter)*

Newly emerged, mated *T. brontispae* females were provided with a 10% honey solution, water or nothing. Each female was kept individually in a Petri dish and its survival was recorded daily until the female died. Each observation was repeated 32–35 times.

Effect of a single host per day on longevity and parasitisation of T. brontispae *females (this experiment referred as 'one-host' hereafter)*

Experiments were conducted as previously described, but one day 0 or day 1 pupal host was provided to the female each day until she died. After the female finished oviposition, the host was carefully transferred into a Petri dish until adult wasps or a host emerged. When a female did not attack a host for 2 h, it was recorded as no parasitisation on that day. The percentage of mummification, number of emerging offspring, sex ratio, body size and development time of the immature stage of offspring (from oviposition until offspring emergence) were recorded. Each observation was repeated 14–21 times.

Effect of more than one host per day on longevity and parasitisation of T. brontispae *females (this experiment referred as 'three-host' hereafter)*

In a preliminary experiment, as the females never attacked more than three hosts per day, the maximum number of hosts provided to the wasp was set as 3 per day. Experiments were conducted as previously described, with multiple hosts per day given to a female. After oviposition, another host was provided to the female until the female died. When the female did not attack a host for 2 h, the host was discarded. Each treatment was replicated 21–23 times.

Superparasitism

To assess the effect of conspecific superparasitism on the development of the wasp and host mortality, one day 0 or day 1 pupa was exposed to 10 newly emerged and mated *T. brontispae* females in a Petri dish. Soon after a female inserted her ovipositor into a host, both wasp and host were carefully transferred into another Petri dish and kept there until the wasp finished oviposition. After the female was finished ovipositing, the parasitised hosts were continuously exposed to the females and attacked 1, 2, 3 or 4 times by different naive female individuals. Thereafter, parasitised hosts were kept individually in a Petri dish until the parasitoid offspring or the beetle emerged. The percentage of mummification, number of emerging offspring, sex ratio, body size and development time of the immature stage of offspring (from oviposition until offspring emergence) were recorded. All the hosts used in the experiment were between 10.0 and 11.5 mg in fresh weight.

All procedures were conducted at 25°C, 60–70% RH and photoperiod of 12L: 12D unless otherwise specified.

Statistical analysis

We compared percentages of mummification, adult wasp emergence, dead hosts, beetle emergence, sex ratio (% females) and host without attack by wasps among different conditions using Ryan's multiple-range test for proportions following a χ^2 test (Ryan 1960). We used the Tukey–Kramer honestly significant difference (HSD) test following a one-way ANOVA to compare the development time, the number of wasps that emerged per host, the number of offspring dead per host, their forewing lengths and longevity among different conditions. A *t*-test was used to compare differences in the forewing lengths between males and females.

Results

Different temperatures on biological parameters of T. brontispae

Although the percentage of mummification was not significantly different among the treated temperatures, wasps did not emerge at 16 and 31°C (ANOVA, $P > 0.05$; Table 1). Wasp emergence was low and the number of offspring dead within a host was high at 19 and 30°C. Female body size at 19°C was significantly smaller than that at other temperatures (Tukey–Kramer HSD test following ANOVA, $P < 0.05$). Development time decreased when temperatures increased. The lower thermal thresholds for oviposition to mummification and mummification to adult emergence were 9.9 and 12.1°C, respectively. The thermal constants for those were 119.1 and 178.6 degree-days, respectively.

Host age acceptance and suitability of T. brontispae

Parasitoid females attacked hosts at all stages provided in the experiment (Table 2). However, percentages of mummification and adult wasp emergence varied among host stages, and were highest for day 1 and day 0 pupae. Host mortality without wasp emergence was significantly higher in 4th instar larva (Ryan's multiple-range test for proportions following a χ^2 test, $P < 0.05$). For day 4 pupae, 50% of attacked hosts emerged as adults without parasitisation. The number of emerging wasps, body size of offspring and sex ratio per host were not significantly different among different host stages (ANOVA, $P > 0.05$).

Female age on parasitisation of T. brontispae

The percentages of mummification and adult emergence decreased with increasing female age (Ryan's multiple-range test for proportions following a χ^2 test, $P < 0.05$; Table 3). They were significantly different between day 1–10 and day 15–21. Although there were no significant differences in sex ratio and the number of offspring emergence per host from day 1 to day 15, significantly fewer offspring were produced by females at day 21 than the rest of female ages (Tukey–Kramer HSD test following ANOVA, $P < 0.05$). The developmental time from oviposition to adult emergence was not

Table 1. Effect of temperature on *Tetrastichus brontispae*.

	Temperature (°C)						
	16	19	22	25	28	30	31
N	30	38	36	34	30	38	35
Mummification (%)[1]	56.7a	68.4a	75.0a	82.4a	76.7a	68.4a	65.7a
Adult emergence (%)[1]	0b	13.2b	69.4a	79.4a	70.0a	7.9b	0b
Host dead (%)[1]	90.0a	78.9a	30.6b	20.6b	30.0b	76.3a	85.7a
Beetle emergence (%)[1]	10.0a	7.9a	0a	0a	0a	15.8a	14.3a
Developmental time (days)[2]							
Egg to mummification	25.1 ± 0.3a	14.1 ± 0.4b	9.6 ± 0.1c	6.9 ± 0.1d	6.0 ± 0.2e	6.7 ± 0.3de	6.1 ± 0.1e
Mummification to adult emergence	–	31.4 ± 1.2a	18.6 ± 0.2b	13.1 ± 0.2c	11.6 ± 0.2d	13.0 ± 2.0cd	–
No. of offspring emergence/host[2]	–	6.0 ± 1.4b	12.9 ± 0.7a	14.2 ± 0.6a	12.6 ± 0.9a	11.7 ± 0.3ab	–
No. of offspring dead/host (including 0 individual)[2]	5.3 ± 0.8b	9.5 ± 0.9a	0.6 ± 0.4c	0.3 ± 0.2c	1.3 ± 0.5c	7.9 ± 1.1ab	8.9 ± 0.9a
Sex ratio (% female)[2]	–	95.0a	83.3ab	86.8b	83.1b	82.6ab	–
Forewing length (mm)[2,3]							
Female	–	0.90 ± 0.01dA	1.08 ± 0.01aA	1.03 ± 0.01bA	1.00 ± 0.00cA	0.98 ± 0.01cA	–
Male	–	0.81 ± 0.01cB	0.94 ± 0.01abB	0.96 ± 0.01aB	0.90 ± 0.01cB	0.90 ± 0.02bcB	–

[1]Values followed by the same letter(s) within the same row do not differ significantly (Ryan's multiple-range test for proportions after χ^2 test, $P > 0.05$).
[2]Mean ± SE. Values followed by the same lower-case letter(s) within the same row are not significantly different (Tukey–Kramer HSD test following ANOVA, $P > 0.05$).
[3]Mean ± SE. Values followed by the same capital letter within the same column are not significantly different (*t*-test, $P > 0.05$).

Table 2. Host age acceptance and suitability of *Tetrastichus brontispae*.

	Host age on developmental stage						
	4th instar	Prepupa	Day 0	Day 1	Day 2	Day 3	Day 4
N	33	31	31	31	30	31	30
Mummification (%)[1]	36.4a	74.2bc	87.1b	90.3b	76.7bc	58.1c	43.3c
Adult emergence (%)[1]	33.3a	64.5ab	83.9b	83.9b	70.0ab	54.8ab	40.0a
Host dead (%)[1]	66.7a	29.0b	6.5b	9.7b	13.3b	16.1b	10.0b
Beetle emergence (%)[1]	0a	6.5ab	9.7ab	6.5ab	16.7ab	29.0bc	50.0c
Developmental time (days)[2]	19.3 ± 0.4bc	18.4 ± 0.2c	18.7 ± 0.2bc	18.5 ± 0.1c	18.9 ± 0.2bc	19.5 ± 0.2b	20.6 ± 0.3a
No. of offspring emergence/host[2]	15.5 ± 0.9a	15.6 ± 0.8a	14.2 ± 0.6a	14.5 ± 0.6a	13.9 ± 0.8a	14.9 ± 0.8a	13.8 ± 1.1a
No. of offspring dead/host (including 0 individual)[2]	6.0 ± 1.3a	1.2 ± 0.5b	1.1 ± 0.6b	0.5 ± 0.3b	1.0 ± 0.4b	1.3 ± 0.6b	0.9 ± 0.4b
Sex ratio (% female)[2]	85.5a	84.2a	82.8a	81.1a	80.5a	84.8a	84.9a
Forewing length (mm)[2,3]							
Female	0.98 ± 0.00bA	1.02 ± 0.00aA	0.99 ± 0.00bA	0.99 ± 0.00bA	0.99 ± 0.01bA	0.95 ± 0.01cA	0.93 ± 0.01cA
Male	0.89 ± 0.01aB	0.91 ± 0.01aB	0.91 ± 0.01aB	0.90 ± 0.01aB	0.90 ± 0.01aB	0.89 ± 0.01aB	0.88 ± 0.01aB

[1]Values followed by the same letter(s) within the same row do not differ significantly (Ryan's multiple-range test for proportions after χ^2 test, $P > 0.05$).
[2]Mean ± SE. Values followed by the same lower-case letter(s) within the same row are not significantly different (Tukey–Kramer HSD test following ANOVA, $P > 0.05$).
[3]Mean ± SE. Values followed by the same capital letter within the same column are not significantly different (*t*-test, $P > 0.05$).

Table 3. Effect of female age on parasitisation of *Tetrastichus brontispae*.

	Female age after emergence					
	Day 1	Day 3	Day 6	Day 10	Day 15	Day 21
N	30	32	29	30	30	32
Mummification (%)[1]	90.0a	90.6a	65.5ab	63.3ab	43.3b	40.6b
Adult emergence (%)[1]	90.0a	81.3a	65.5a	63.3a	26.7b	25.0b
Host dead (%)[1]	6.7a	9.4a	10.3a	16.7a	26.7a	13.3a
Beetle emergence (%)[1]	0.0a	0.0a	3.4a	6.7a	13.3a	9.4a
Host without being attacked by wasp (%)[1]	3.3b	9.4ab	20.7ab	13.3ab	33.3a	34.4a
Developmental time (days)[2]	18.7 ± 0.1c	19.0 ± 0.2c	19.0 ± 0.1c	19.9 ± 0.3b	20.1 ± 0.1ab	21.1 ± 0.4a
No. of offspring emergence/host[2]	15.7 ± 0.6a	18.0 ± 0.8a	17.3 ± 0.8a	15.9 ± 1.0a	14.3 ± 2.2a	8.1 ± 1.5b
No. of offspring dead/host (including 0 individual)[2]	0.1 ± 0.1a	1.4 ± 0.7a	1.0 ± 0.9a	1.4 ± 0.7a	2.2 ± 1.2a	2.2 ± 0.8a
Sex ratio (% female)[2]	85.2a	83.4a	82.2a	73.1a	78.9a	67.5a
Forewing length (mm)[2,3]						
Female	1.02 ± 0.00bA	1.01 ± 0.00bcA	0.99 ± 0.00dA	1.02 ± 0.00bcA	1.01 ± 0.00cA	1.05 ± 0.01aA
Male	0.91 ± 0.00bB	0.91 ± 0.01bB	0.87 ± 0.01cB	0.92 ± 0.00bB	0.90 ± 0.01bB	0.97 ± 0.01aB

[1]Values followed by the same letter(s) within the same row do not differ significantly (Ryan's multiple-range test for proportions after χ^2 test, $P > 0.05$).
[2]Mean ± SE. Values followed by the same lower-case letter(s) within the same row are not significantly different (Tukey–Kramer HSD test following ANOVA, $P > 0.05$).
[3]Mean ± SE. Values followed by the same capital letter within the same column are not significantly different (*t*-test, $P > 0.05$).

significantly different between females at day 1 and day 6, but was significantly longer for the remaining ages (Tukey–Kramer HSD test following ANOVA, $P < 0.05$). The body size of offspring did not differ among the female age groups (ANOVA, $P > 0.05$).

***Food and oviposition frequency on parasitisation and adult longevity of* T. brontispae**

Females on day 0 already had an average of 30.9 (nothing provided) to 36.2 eggs (water provided) in an ovary for all food conditions. The number of eggs in the ovary slightly increased from day 0 to day 1, but they were not significantly different, except between day 0 and day 1 when no food was provided (Tukey–Kramer HSD test following ANOVA, $P < 0.05$; Figure 1). The wasp successfully attacked and developed in *B. longissima* under all food conditions when hosts were provided (Figure 2A). However, female longevity was affected not only by the food supply, but also by oviposition frequency. When honey was given, longevity tended to be longer than that when water or no food was provided (Figure 2A). At the same time, longevity decreased when oviposition frequency increased. The longevity at no-host (17.9 ± 1.3 days) became shorter at one-host (14.0 ± 1.0 days) and abruptly shortened at three-host (3.3 ± 0.3 days) (Tukey–Kramer HSD test following ANOVA, $P < 0.05$; Figure 2A). Regarding the total number of hosts parasitised by a female during her lifetime, there was no significant difference between one-host (3.8 ± 0.3) and three-host (3.0 ± 0.3) treatments when honey was provided (Figure 2B). There was also no difference between treatment provisions of honey (3.0 ± 0.3) and water (3.0 ± 0.2) at three-host levels, but a significant difference was found between one-host and

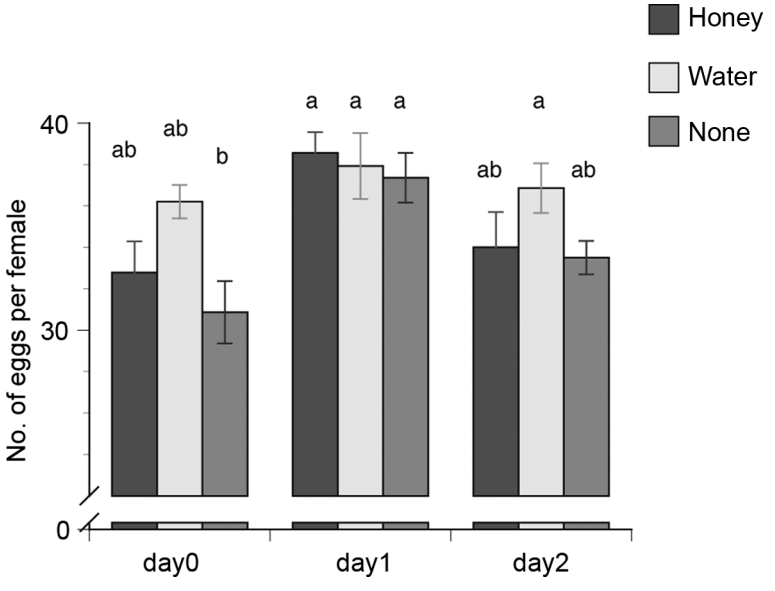

Figure 1. Mean number of matured eggs in an ovary/female. Newly emerged and mated females were provided with a 10% honey solution, water, or nothing as a food supply condition. In each condition, female wasps were kept individually and dissected on day 0, 1, or 2. Mean $\pm$ SE. Bars followed by the same letter(s) are not significantly different among all treatments (Tukey's HSD test following ANOVA, $P > 0.05$).

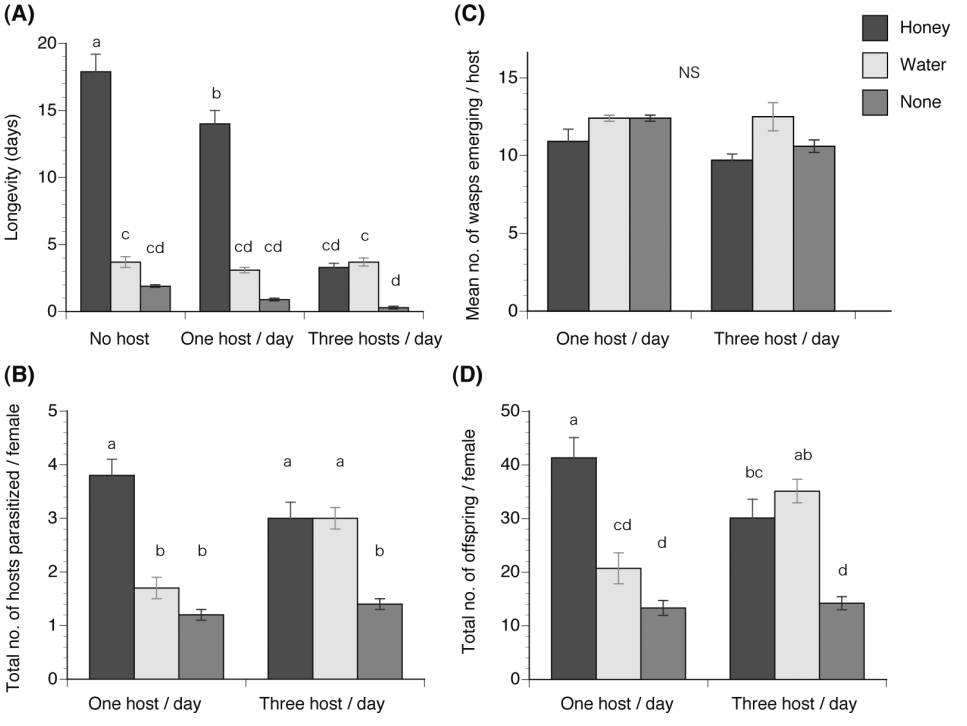

Figure 2. Female longevity and parasitisation of *Tetrastichus brontispae* at different food conditions and host-encountering rate. (A) longevity, (B) total number of hosts parasitised per female during her lifetime, (C) mean number of wasps emerging per host, (D) total number of offspring produced per female during her lifetime. Mean $\pm$ SE. Bars followed by the same letter(s) are not significantly different among all treatments (Tukey's HSD test following ANOVA, $P > 0.05$). NS shows no significant difference (ANOVA, $P > 0.05$).

three-host when water was provided (1.7 ± 0.2, 3.0 ± 0.2, respectively) (Tukey–Kramer HSD test following ANOVA, $P < 0.05$; Figure 2B). The mean number of wasps emerging per host was not significantly different among all treatments (ANOVA, $P > 0.05$; Figure 2C). The sex ratio of all treatments was similar, and female biased: 85.2–88.6% (ANOVA, $P > 0.05$; showing no data). The total number of offspring produced by a female during her lifetime was significantly different among the three types of food supply (Tukey–Kramer HSD test following ANOVA, $P < 0.05$; Figure 2D). Nevertheless, those provided honey produced fewer offspring when oviposition frequency increased from one-host (41.3 ± 3.8) to three-hosts (30.1 ± 3.5) (Tukey–Kramer HSD test following ANOVA, $P < 0.05$; Figure 2D). On the other hand, those provided water increased their number of offspring when oviposition frequency increased from one-host (20.7 ± 2.9) to three-host (35.1 ± 2.2) (Tukey–Kramer HSD test following ANOVA, $P < 0.05$; Figure 2D).

Superparasitism

The percentages of mummification and adult emergence decreased when the number of oviposition per host increased (Ryan's multiple-range test for proportions

following a χ^2 test, $P < 0.05$; Table 4). In contrast, the number of emerging wasps per host and percentage of host dead increased as the number of oviposition per host increased (Tukey–Kramer HSD test following ANOVA, $P < 0.05$). Developmental time of immature stages of offspring and sex ratios were not significantly different among the treatments (ANOVA, $P > 0.05$). The body size of offspring differed significantly among treatments and there was a negative correlation between the number of ovipositions and the body size (Tukey–Kramer HSD test following ANOVA, $P < 0.05$).

Discussion

The present study indicated that temperatures from 22 to 28°C (60–70% RH) were appropriate for immature development and parasitisation of *T. brontispae* (Table 1). Results were consistent with Chen et al. (2010) showing that suitable temperatures for this wasp were between 20 and 28°C (65–95% RH). Our results also revealed that no wasps completed development at 16 and 31°C. *Tetrastichus brontispae* successfully attacked and developed in all stages of hosts between 4th instar larvae and day 4 pupae, but the optimum host stages for parasitisation could be day 0 and day 1 pupae (Table 2). Host mortality (the total percentage of adult emergence and host dead without producing parasitoid offspring) was more than 90% between 4th instar and day 1 pupal stages (Table 2).

Wäckers (2003) stated that sugar feeding could considerably increase a parasitoid's lifespan and also benefit a parasitoid's fecundity. Honey increased the longevity of females (Figure 2A), and the total number of hosts parasitised and

Table 4. Effect of superparasitism of *Tetrastichus brontispae*.

	No. of ovipositions/host			
	1	2	3	4
N	36	38	33	36
Mummification (%)[1]	94.4a	78.9ab	72.7ab	63.9b
Adult emergence (%)[1]	91.7a	71.1ab	66.7ab	55.6b
Host dead (%)[1]	5.6b	26.3ab	27.3ab	41.7a
Beetle emergence (%)[1]	2.8a	2.6a	6.1a	2.8a
Developmental time (days)[2]	19.4 ± 0.2a	18.9 ± 0.2a	19.1 ± 0.1a	19.1 ± 0.2a
No. of offspring emergence/host[2]	14.2 ± 0.4c	23.7 ± 1.1b	26.5 ± 1.6ab	31.3 ± 2.0a
No. of offspring dead/host (including 0 individual)[2]	0.3 ± 0.2b	3.2 ± 1.1ab	4.8 ± 1.6ab	7.2 ± 5.7a
Sex ratio (% female)[2]	85.5a	81.6ab	77.6b	80.3b
Forewing length (mm)[2,3]				
Female	1.03 ± 0.0aA	0.93 ± 0.0bA	0.85 ± 0.0cA	0.80 ± 0.0dA
Male	0.93 ± 0.0aB	0.83 ± 0.0bB	0.80 ± 0.0cB	0.77 ± 0.0dB

[1]Values followed by the same letter(s) within the same row do not differ significantly (Ryan's multiple-range test for proportions after χ^2 test, $P > 0.05$).
[2]Mean ± SE. Values followed by the same lower-case letter(s) within the same row are not significantly different (Tukey–Kramer HSD test following ANOVA, $P > 0.05$).
[3]Mean ± SE. Values followed by the same capital letter within the same column are not significantly different (*t*-test, $P > 0.05$).

offspring per female at one-host over those of water and no food provided (Figures 2B,D). However, oviposition frequency negatively affected longevity, and longevity at three-host was abruptly shortened even with honey provided (Figure 2A). In this case, sugar feeding did not present clear advantages, and only water was enough to increase the total number of host parasitised and offspring produced per female as much as those with honey (Figures 2B,D). Pro-ovigenic parasitoid species already have all or nearly all of their lifetime supply of eggs when they emerge as adults (Flanders 1950; Jervis, Heimpel, Ferns, Harvey and Kidd 2001). The number of mature eggs per female soon after eclosion (Figure 1) was similar to the total number of offspring produced per female at one-host treatments, when honey was provided (Figure 2D), which suggested that *T. brontispae* is pro-ovigenic. The reproduction abilities of the wasp with honey and water seemed to be equal at high host densities.

The female's age is also crucial to successful parasitism. The effect of the female's age on parasitisation has been reported in some other wasps (Hentz, Ellsworth, Naranjo and Watson 1998; Honda, Kainoh and Honda 1998; Aung, Takagi and Ueno 2010). They mentioned that the percentage of parasitism and number of offspring decreased in hosts parasitised by old females. Similar results were found in this study (Table 3). Although females of all ages examined in this study could parasitise hosts, females of days 1 and 3 had higher reproductive activity than old females. Females at days 15 and 21 had lower mummification and offspring emergence. There is the possibility that egg viability could be affected by time after emergence due to pro-ovigeny.

In the superparasitism experiment, the number of emerging wasps per host and percentage of host dead increased as the number of ovipositions per host increased. On the other hand, the mean number of eggs per attack for an unparasitised host was around 15 (Tables 1 and 4). This number was not directly counted by conducting host dissections, but was calculated from the total number of offspring emerging and the number of dead offspring per host. We estimated, in the same way, that the mean number of eggs laid by parasitoids ovipositing into hosts one, two, three and four times was 14.5, 12.4, 4.4 and 7.2, respectively, under superparasitism (Table 4). There are many reports that females discriminate against previously parasitised hosts and lay fewer eggs into them (Godfray 1994; Quicke 1997). Our results corroborate these studies. If all eggs oviposited were counted as emerging adults or as dead offspring, then *T. brontispae* actually decreased the number of eggs oviposited into previously parasitised hosts. This hypothesis is in need of further investigation.

When host density increases and oviposition frequency becomes high, *T. brontispae* females could perform well as a natural enemy of the beetle without honey but only a water supply (Figure 2). On the contrary, when host density becomes low and superparasitism occurs, a host could be parasitised and produce parasitoid offspring with up to four attacks per host. The wasp could also survive on average 17 days, and remain active until 10 days after emergence (Table 3) even when she seldom has the opportunity to encounter a host. *Tetrastichus brontispae* would serve as an efficient biological control agent of *B. longissima*, as they did in Celebes (Lever 1969), Tahiti and the Solomon Islands (Stapley 1973), and Taiwan (Chiu et al. 1985).

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