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RESEARCH ARTICLE

Artificial diets for rearing the coconut hispine beetle, *Brontispa longissima* (Coleoptera: Chrysomelidae), and their suitability to two specialist parasitoids

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The coconut hispine beetle, *Brontispa longissima* (Gestro), is a serious invasive pest that infests young unopened fronds of coconut palms (*Cocos nucifera* L.) in Southeast Asia. We previously developed the first artificial diet for rearing *B. longissima* larvae, which contained a leaf powder of young coconut fronds. Because the fronds are required for healthy growth of coconut palms, it is necessary to reduce their use for rearing the beetles. In this study, we tested two new artificial diets for the beetle larvae, which contained the leaf powders of mature coconut leaves or orchard grass (*Dactylis glomerata* L.). *Brontispa longissima* successfully developed from hatching to adulthood on both the mature coconut leaf diet and orchard grass diet. The beetles reared on the mature coconut leaf diet and orchard grass diet developed faster than those reared on the young coconut leaf diet. Fecundity and egg hatchability of beetles did not differ among the three diet treatments. We then examined the suitability of beetle larvae or pupae reared on each diet as hosts for two specialist endoparasitoids, *Asecodes hispinarum* Boucek and *Tetrastichus brontispae* Ferriere. The survival rate from oviposition to adult emergence for *A. hispinarum* was 43.8% in hosts reared on a young coconut leaf diet, 77.1% on a mature coconut leaf diet, and 85.7% on an orchard grass diet. For *T. brontispae*, the survival rate was 70.0% in hosts reared on the young coconut leaf diet, 38.1% on the mature coconut leaf diet, and 66.7% on the orchard grass diet. Our results indicate these artificial diets can be useful for rearing *B. longissima* and its two parasitoids, helping to reduce the costs of mass rearing these insects.

Keywords: *Cocos nucifera*; biological control; invasive insect pest; larval parasitoid; pupal parasitoid; artificial diet

Introduction

Because many insect studies rely on laboratory-reared individuals, providing information on how to successfully rear them in the laboratory has long been a fundamental research goal (Cohen 2003). Pest control studies often require artificial rearing systems to obtain enough healthy individuals of the target pest to allow

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studies of the pest itself. Artificial rearing systems are also necessary for rearing biological control agents including parasitoids and predators (van Lenteren and Tommasini 2003), as well as sterile insects (Knipling 1966).

Coconut palm, *Cocos nucifera* L. (Arecales: Arecaceae), is an economically and industrially important crop for millions of people in Southeast Asia who depend on income from coconut products, such as copra, coconut oil, and coconut shell charcoal (Liebregts and Chapman 2004; Guarino 2006). Recently, coconut trees have suffered heavy damage from the invasive coconut hispine beetle, *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae) (Liebregts and Chapman 2004; Nakamura, Konishi, and Takasu 2006). The beetle is believed to be native to Indonesia and Papua New Guinea, but was accidentally introduced into Vietnam, Thailand, the Philippines, and China's Hainan Province, where *C. nucifera* is widespread (Liebregts and Chapman 2004; Nakamura et al. 2006; Lu, Tang, Peng, Salle, and Wan 2008; Nakamura, Konishi, and Takasu 2008). Larvae and adults of *B. longissima* damage leaflets of the unopened fronds of coconut palms by eating away the surface tissues (Brown and Green 1958). Such infestations turn the coconut leaves brown and decrease fruit production.

Invasion by *B. longissima* is of great concern internationally, and the beetle is listed in the Global Invasive Species Database (2010). To control populations of *B. longissima*, importation biological control has been conducted mainly using two specialist parasitoids, *Asecodes hispinarum* Boucek (Hymenoptera: Eulophidae) and *Tetrastichus brontispa* Ferriere (Hymenoptera: Eulophidae). *Asecodes hispinarum* was introduced into Western Samoa from Papua New Guinea in 1982, and populations of *B. longissima* subsequently decreased (Voegelé 1989). *Tetrastichus brontispa* is native to Java and has been successfully introduced to several countries, including Sulawesi, Tahiti, Solomon Islands, New Guinea, and Western Samoa (Gutiérrez 1978). Recent studies on the basic biology of *A. hispinarum* and *T. brontispa* demonstrated that these two parasitoids can parasitize the larvae and pupae of *B. longissima* with a high rate of parasitism under laboratory conditions, suggesting these parasitoids have the potential to reduce coconut hispine beetle populations (Lu et al. 2008; Chen et al. 2010).

Artificial rearing systems can be useful for obtaining enough healthy *B. longissima* individuals for mass rearing of *A. hispinarum* and *T. brontispa*. We previously developed the first artificial diet for rearing *B. longissima* (Ichiki, Dung, Takano, and Nakamura 2009) with leaf powder of young unopened fronds of coconut palms as a main ingredient. However, it is difficult to obtain a sufficient number of unopened fronds because cutting them damages the coconut trees and the fronds are relatively expensive, even in Southeast Asia. To reduce the costs of artificial rearing systems we need to find alternatives for unopened fronds of coconut trees. The mature leaves of coconut trees are a more suitable food for immature development and egg production of *B. longissima* compared with the young leaves (Yamashita, Winotai, and Takasu 2008). In preliminary laboratory tests we also found that *B. longissima* larvae ate orchard grass (*Dactylis glomerata* L.). We therefore assumed that we could rear the beetle larvae on artificial diets containing leaf powders of mature *C. nucifera* leaves or orchard grass instead of unopened *C. nucifera* fronds.

In this study we tested two new artificial diets for immature *B. longissima*. We also examined the suitability of beetles reared on the diets as hosts for *A. hispinarum* and *T. brontispa*. The results reveal a convenient and inexpensive rearing technique that can reduce the costs of parasitoid production.

Materials and methods

Insects

The colony of *B. longissima* used in this study originated from beetles collected on Ishigaki Island, Okinawa, Japan in August and September 2008. Because fresh coconut leaves are difficult to obtain in Japan, we reared the beetle colony on fresh leaves of the narrow-leaf cattail, *Typha angustifolia* L. (Poales: Typhaceae), which is an alternative host plant of this beetle (Winotai, Sindhusake, and Morakote 2007; Yamashita et al. 2008). Larvae and adults were maintained separately on fresh leaves in plastic containers (15.5 × 11.5 × 5.0 cm, long × wide × high) with a screened window (60 mesh, 7.0 × 3.0 cm, long × wide) in the lid. Approximately 100 larvae were kept in the plastic container. In addition, approximately 30 female and 30 male adults were kept in the plastic container and were allowed to mate frequently. Females laid eggs on the leaves, and the eggs were carefully removed from leaves with forceps and kept in plastic Petri dishes (6.0 × 2.5 cm, diameter × high; ca. 50 eggs per dish) with moistened cotton until hatching.

The *A. hispinarum* colony was obtained from parasitized *B. longissima* larvae that had been collected at Rabaul, New Britain, Papua New Guinea in February 2009, and was maintained using the final instar larvae of *B. longissima* as hosts. The *T. brontispae* colony was obtained from Taiwan in September 2009 and was maintained using *B. longissima* prepupae and young pupae (within 48 h after pupation) as hosts. Adult wasps of each parasitoid species were maintained in groups ($n = 15\text{--}30$) and fed a 30% honey solution in plastic Petri dishes (6.0 × 2.5 cm, diameter × high). All experimental procedures were conducted at $25 \pm 1^\circ\text{C}$ under a 12 h L:12 h D photoperiod and $65 \pm 5\%$ relative humidity.

Artificial diet for *B. longissima*

We have previously developed an artificial diet for *B. longissima* by modifying the diets used for the West Indian sweetpotato weevil, *Euscepes postfasciatus* (Fairmaire) (Coleoptera: Curculionidae), by Shimoji and Kohama (1996) and Sakakibara (2003). In that diet, we used dried leaf powder of unopened fronds of *C. nucifera*, which is the main food for larvae of *B. longissima* (Ichiki et al. 2009). In this study we prepared two new artificial diets in which we used leaf powders of mature coconut leaves or orchard grass.

Table 1 lists the ingredients of the artificial diets. The young coconut leaf diet (control), mature coconut leaf diet, and orchard grass diet were identical except for the type of dried leaf powder they contained. We made the three diets following the method described by Ichiki et al. (2009). To make a dried leaf powder, plant leaves were dried at 80°C for 12 h and then ground for 20 min in a flour mill (T-429, Rong Tsong Iron Co., Taiwan). To prepare the diet we stirred all ingredients using an electric blender and then autoclaved the mixture at 121°C for 16 min. The mixture was then stirred until the temperature dropped to about 60°C . We dipped a tightly woven polyethylene sheet (60 mesh, 3.0 × 3.0 cm) into the mixture once with forceps to daub the artificial diet (ca. 0.5 g) onto the mesh sheet to a thickness of about 1 mm. The mesh sheets plus diet were stored in a refrigerator at 4°C until use.

Table 1. Ingredients of the artificial diet developed for *Brontispa longissima*.

Ingredients	Amount
Agar ¹	2 or 4 g
Dried leaf powder	10 g
Sucrose	4 g
Casein	2.4 g
Powdered cellulose	2 g
Yeast	1 g
Vitamin mixture ²	0.5 g
Salt mixture ³	0.3 g
Sorbic acid	0.2 g
Methyl p-hydroxybenzoate	0.2 g
Cholesterol	0.08 g
Distilled water	100 ml

¹For first-instar larvae 4 g of agar was used in the hard-type diet, and 2 g was used in the soft-type diet for later instars.

²Vanderzant vitamin mixture for insects (Sigma-Aldrich Japan, Tokyo, Japan) was used.

³Wesson's salt mixture (Sigma-Aldrich Japan, Tokyo, Japan) was used.

Rearing B. longissima larvae on the artificial diets

Larvae of *B. longissima* were reared on a young coconut leaf diet, a mature coconut leaf diet, or on an orchard grass diet. For each of the three diets, 10 newly hatched larvae (within 24 h after hatching) were inoculated onto a mesh sheet and kept together in a Petri dish (6.0 × 2.5 cm, diameter × high). Each treatment was replicated 10 times (i.e., 100 larvae per diet). The diet was exchanged for a fresh one every other day. Our previous study showed that agar content in the artificial diet was important for immature development of this beetle (Ichiki et al. 2009). Therefore, the hard-type diet containing 40 g/L agar was provided to first instar larvae; this was changed to the soft-type diet containing 20 g/L agar after 50% of the surviving larvae had molted into the second instar. The soft-type diet was then used until pupation.

We recorded the numbers of individuals surviving to each larval instar stage every other day. After the larvae reached the prepupal stage, prepupae were transferred to new Petri dishes and maintained individually until adult emergence. From the prepupal stage onward, we recorded the numbers of individuals surviving to the pupal and adult stages every day. The rates of both pupation and adult emergence were calculated based on the number of hatched larvae. We measured total body length (head tip to abdominal tip) of adults and determined their sex under a stereomicroscope.

Reproductive ability of B. longissima reared on artificial diets during the larval stage

Newly emerging adults obtained from the rearing experiment were used to examine the reproductive ability of *B. longissima* reared on the artificial diets. We created 11,

6, and 7 pairs of adult beetles from the young coconut leaf diet, mature coconut leaf diet, and orchard grass diet rearing treatments, respectively. Each pair was provided with fresh leaves of *T. angustifolia* in Petri dishes (6.0 × 2.5 cm, diameter × high; one pair per dish). Every other day we replaced the food and counted the eggs that had been laid by the beetles. For each pair, the eggs ($n = 1-14$) laid over 2 days were kept in separate Petri dishes. The eggs were monitored to determine the hatching rate on days 3, 5, 7, and 9 after they were collected. These experiments were continued for 60 days following female emergence.

Suitability of B. longissima larvae reared on artificial diets as hosts for A. hispinarum and T. brontispae

We used Petri dishes (6.0 × 2.5 cm, diameter × high) to rear the hatched larvae of *B. longissima* on the three diets, using the same method as in the rearing experiments. The final instar larvae were used as hosts for *A. hispinarum*, and prepupae or young pupae (within 48 h after pupation) were provided to *T. brontispae*. Each adult of the two wasp species was allowed to mate for more than 12 h after adult emergence. One to three hosts were placed in a Petri dish containing 15–30 wasps (more than 80% females). The behavior of the wasps and hosts was observed. When a female wasp inserted its ovipositor into a host, the wasp and host were carefully transferred to a Petri dish and kept there until the wasp finished ovipositing. The parasitized host was then retained in the Petri dish until it became mummified. A host parasitized by *A. hispinarum* or *T. brontispae* was provided with the same artificial diet that it was reared on. After mummification, the host was maintained without food until adult wasps emerged. The parasitized hosts were monitored daily to determine the percentages of mummification and adult wasp emergence. We determined the sex of the newly emerging adult wasps and measured the length of forewing (one to five individuals for each sex per host) under a stereomicroscope.

Statistical analyses

Differences in the survival rates of beetles and parasitoids among the three diet treatments were analyzed using a chi-squared test and then compared using Ryan's multiple-range test for proportions (Ryan 1960). The same method was used to compare the sex ratio of emerging beetles among the three diet treatments. The chi-squared test was conducted to determine whether the sex ratio of emerging beetles differed from 1:1. The chi-squared test was used to examine differences in the composition of instar of the beetle larvae when they pupated among the three diets. Analyses were conducted using version 2.12.1 of R software (R Development Core Team 2010). Differences in developmental times, body lengths, pre-oviposition period, fecundity, and egg hatchability of beetles among the three diet treatments were subjected to one-way ANOVA and then compared using a Tukey–Kramer test. The same method was used to compare the number of wasps per host, sex ratio, developmental times, and body size of parasitoids. Student's *t*-tests were used to compare the body sizes of the beetles or parasitoids between sexes. These analyses were conducted using version 5.1.1 of JMP software (SAS Institute 2004).

Results

Rearing of B. longissima larvae on the artificial diets

Brontispa longissima successfully developed from hatching to adulthood in all the artificial diet treatments, but a significantly higher proportion of adult beetles emerged when reared on the young coconut leaf diet than on the other two diets (Ryan's multiple-range test for proportions following a chi-squared test, $P < 0.05$; Table 2). The sex ratio of emerging beetles had a bias toward males in the young coconut leaf diet ($\chi^2 = 4.923$, $df = 1$, $P = 0.023$), whereas it did not differ significantly from 1:1 in mature coconut leaf diet ($\chi^2 = 0.533$, $df = 1$, $P = 0.465$) and orchard grass diet ($\chi^2 = 0.444$, $df = 1$, $P = 0.505$). A significant difference was found in female body size between the young coconut leaf diet or the mature coconut leaf diet and the orchard grass diet and in male body size between the young coconut leaf and orchard grass diets (Tukey–Kramer test following ANOVA, $P < 0.05$). In all three diet treatments, females were significantly larger than males (t -test, young coconut leaf diet: $t = 5.922$, $df = 50$, $P = 0.0001$; mature coconut leaf diet: $t = 8.127$, $df = 28$, $P < 0.0001$; orchard grass diet: $t = 5.854$, $df = 34$, $P < 0.0001$).

The number of larval instars varied among the three diets (Table 3). The composition of instar of the larvae when they pupated differed significantly among the three diets ($\chi^2 = 106.557$, $df = 6$, $P < 0.0001$). In each artificial diet treatment, 59–70% of the surviving larvae pupated after the fifth instar. The majority of the other surviving larvae pupated after the fourth or sixth instars, although in the young coconut leaf diet treatment three larvae molted to the seventh instar. Within each final instar, larval developmental time did not differ significantly among the three diets (ANOVA, $P > 0.05$), except for the larvae pupated after the fourth instar (Tukey–Kramer test following ANOVA, $P < 0.05$). Pupal developmental time did not differ significantly among the three diets (ANOVA, $P > 0.05$). When total developmental time from hatching to adulthood was compared among the three diet treatments, the young coconut leaf diet was significantly longer than the orchard grass diet (Tukey–Kramer test following ANOVA, $P < 0.05$).

Reproductive ability of B. longissima reared on the artificial diets

Brontispa longissima females of each diet treatment laid 79–89 eggs, and the egg hatchability was over 90% (Table 4). The fecundity and egg hatchability were not significantly different among the three diet treatments (ANOVA, $P > 0.05$). The pre-oviposition period was significantly shorter in the young coconut leaf diet than in the mature coconut leaf diet treatment (Tukey–Kramer test following ANOVA, $P < 0.05$).

Suitability of B. longissima larvae reared on artificial diets as hosts for A. hispinarum and T. brontispae

In *A. hispinarum*, the percent wasp emergence and the number of wasps emerged per host were significantly lower in the young coconut leaf diet treatment than in the mature coconut leaf diet or orchard grass diet treatments (Ryan's multiple-range test for proportions following a chi-squared test, $P < 0.05$; Table 5). The sex ratio was female-biased in all three diets. Developmental time of *A. hispinarum* from

Table 2. Survival rates, sex ratios, and body sizes of *Brontispa longissima* reared on the artificial diets.

Artificial diet	No. of hatched larvae examined	Pupation (%) ¹	Adult emergence (%) ¹	Sex ratio (to female) ¹	Body lengths (mm) ²			
					<i>n</i>	Female	<i>n</i>	Male
Young coconut leaf	100	55.0 a	52.0 a	0.346 a	18	8.9±0.1 a	34	8.2±0.1 a
Mature coconut leaf	100	37.0 b	30.0 b	0.567 a	17	9.1±0.1 a	13	8.0±0.1 ab
Orchard grass	100	38.0 b	36.0 b	0.556 a	20	8.7±0.1 b	16	7.9±0.1 b

¹Values followed by the same letters within the same column do not differ significantly (Ryan's multiple-range test for proportions following a chi-square test, $P > 0.05$).

²Mean ± SE. Values followed by the same letters within a column do not differ significantly (Tukey-Kramer test following ANOVA, $P > 0.05$).

Table 3. Developmental times of *Brontispa longissima* reared on the artificial diets.

Artificial diet	Larval developmental times (days) for various larval instars ^{1, 2}								Pupal developmental times (days) ²	Total developmental times from hatching to adult (days) ²	
	<i>n</i>	4	<i>n</i>	5	<i>n</i>	6	<i>n</i>	7			
Young coconut leaf	2	28.0 ± 1.0 ab	38	39.7 ± 1.0 a	12	53.8 ± 2.1 a	3	69.3 ± 7.5	52	6.5 ± 0.1 a	49.3 ± 1.3 a
Mature coconut leaf	7	35.1 ± 1.8 a	22	38.7 ± 0.6 a	8	53.8 ± 2.8 a	0	—	30	6.5 ± 0.1 a	47.6 ± 1.3 ab
Orchard grass	8	29.9 ± 0.9 b	26	37.2 ± 1.0 a	4	53.0 ± 4.1 a	0	—	36	6.3 ± 0.1 a	42.8 ± 1.1 b

¹Larvae pupated after the fourth, fifth, sixth, or seventh instars. Larval development times were compared within each larval instar.

²Mean ± SE. Values followed by the same letters within a column do not differ significantly (Tukey-Kramer test following ANOVA, $P > 0.05$).

Table 4. Reproductive ability of *Brontispa longissima* adults reared on the artificial diets during the larval stage.

Artificial diet	No. of examined females	Pre-oviposition period (days) ¹	No. of eggs per female ¹	Egg hatchability (%) ^{1,2}
Young coconut leaf	11	14.5 ± 0.6 a	82.9 ± 3.0 a	91.6 ± 1.2 a
Mature coconut leaf	6	18.8 ± 1.38 b	79.2 ± 5.1 a	92.0 ± 2.9 a
Orchard grass	7	15.9 ± 0.9 ab	89.3 ± 4.7 a	90.4 ± 1.5 a

¹Mean ± SE. Values followed by the same letters within a column do not differ significantly (Tukey–Kramer test following ANOVA, $P > 0.05$).

²Egg hatchability per female.

oviposition to adult emergence was significantly longer in the young coconut leaf diet treatment than in the mature coconut leaf diet treatment (Tukey–Kramer test following ANOVA, $P < 0.05$). A significant difference was found in female body size between the mature coconut leaf diet and orchard grass diet treatments and in male body size between the young coconut leaf diet and orchard grass diet treatments ($P < 0.05$). Female wasps were significantly larger than male wasps in all three treatments (t -test, young coconut leaf diet: $t = 4.224$, $df = 37$, $P < 0.001$; mature coconut leaf diet: $t = 10.819$, $df = 245$, $P < 0.0001$; orchard grass diet: $t = 2.134$, $df = 93$, $P = 0.036$).

For *T. brontispae*, percent wasp emergence in the young coconut leaf diet treatment was significantly higher than that in the mature coconut leaf diet treatment (Tukey–Kramer test following ANOVA, $P < 0.05$). However, female body size of *T. brontispae* in the young coconut leaf diet treatment was significantly smaller than that in the orchard grass diet treatment, and male body size in the young coconut leaf diet treatment was significantly smaller than that in the other two treatments. No significant difference was found in the sex ratio, the number of wasps emerged per host, or developmental time among the three diet treatments (ANOVA, $P > 0.05$). Female *T. brontispae* were significantly larger than male wasps in all three treatments (t -test, young coconut leaf diet: $t = 9.790$, $df = 118$, $P < 0.0001$; mature coconut leaf diet: $t = 3.720$, $df = 51$, $P < 0.001$; orchard grass diet: $t = 6.790$, $df = 67$, $P < 0.0001$).

Discussion

We developed two new artificial diets for rearing larvae of *B. longissima* containing the leaf powders of mature coconut leaves or orchard grass instead of young unopened coconut fronds. *Brontispa longissima* successfully developed from hatching to adulthood on both the mature coconut leaf diet and the orchard grass diet (Table 2). Although the survival rate of *B. longissima* reared on a young coconut leaf diet was significantly higher than that on a mature coconut leaf diet or orchard grass diet (Table 2), the beetles reared on a young coconut leaf diet needed a longer time for larval development, especially when compared to the orchard grass diet (Table 3), and the sex ratio of emerging beetles was biased toward males in the young coconut leaf diet treatment (Table 2). In addition, fecundity did not differ among the three diets (Table 4). These results suggest that the mature coconut leaf diet and orchard grass diets are useful for rearing larvae of *B. longissima*.

Table 5. Host suitability of *Brontispa longissima* reared on the artificial diets for *Asecodes hispinarum* and *Tetrastichus brontispae*.

Artificial diet	No. hosts examined	Mummification (%) ¹	Wasp emergence (%) ¹	No. wasps per host ²	Sex ratio (to female) ²	Developmental time from oviposition to adult emergence (days) ²	Forewing lengths (mm) ²			
							<i>n</i>	Female	<i>n</i>	Male
<i>Asecodes hispinarum</i>										
Young coconut leaf	23	91.3 a	30.4 a	11.3±4.41 a	0.668±0.098 a	23.4±0.67 a	22	0.764±0.020 ab	17	0.651±0.016 a
Mature coconut leaf	35	82.9 a	80.0 b	33.0±3.29 b	0.774±0.035 a	21.5±0.24 b	140	0.751±0.005 a	107	0.673±0.005 ab
Orchard grass	22	86.4 a	54.5 b	35.3±5.68 b	0.778±0.080 a	22.0±0.30 ab	44	0.722±0.012 b	51	0.691±0.008 b
<i>Tetrastichus brontispae</i>										
Young coconut leaf	30	76.7 A	70.0 A	12.71±0.68 A	0.859±0.009 A	20.00±0.44 A	84	0.982±0.007 A	36	0.858±0.010 A
Mature coconut leaf	21	47.6 B	38.1 B	13.38±0.75 A	0.871±0.025 A	19.75±0.49 A	40	1.006±0.007 AB	13	0.950±0.014 B
Orchard grass	15	84.7 A	66.7 AB	13.54±1.23 A	0.887±0.021 A	19.73±0.45 A	44	1.030±0.008 B	25	0.937±0.010 B

¹Values followed by the same letters within the same column do not differ significantly (Ryan's multiple-range test for proportions following a chi-square test, $P > 0.05$).

²Mean±SE. Values followed by the same letters within a column do not differ significantly (Tukey-Kramer test following ANOVA, $P > 0.05$).

It is preferable and easier to obtain mature leaves of *C. nucifera* than young unopened fronds because cutting a small amount of mature leaves is unlikely to damage the coconut trees. Orchard grass is a versatile bunchgrass that grows rapidly and is used worldwide for pasture, hay, green chop, and silage (Hall 2008). Orchard grass is easy to obtain and costs markedly less than unopened fronds of *C. nucifera*. Therefore, using artificial diets with mature coconut leaves or orchard grass would help to reduce the costs of mass rearing of *B. longissima*.

Both *A. hispinarum* and *T. brontispae* have high potential for use as biological control agents against populations of *B. longissima* (Lu et al. 2008; Chen et al. 2010). We demonstrated that these parasitoids were able to develop on larvae or pupae reared on artificial diets. *Brontispa longissima* larvae reared on the mature coconut leaf diet and the orchard grass diet were more suitable hosts for *A. hispinarum* than those reared on the young coconut leaf diet. *Asecodes hispinarum* showed a higher rate of parasitism (80%; Table 5) on larvae reared on the mature coconut leaf diet, and the parasitism rate was similar to that of larvae reared on fresh coconut leaves (approximately 75%; Lu et al. 2008). In the case of *T. brontispae*, the mature coconut leaf diet did not produce suitable host prepupae and pupae, and the survival rate of wasps was lower in the mature coconut leaf diet-reared hosts than for the other two diets. However, prepupae and pupae reared on the orchard grass diet or the young coconut leaf diet appear to be suitable hosts for *T. brontispae*. The successful rearing of these two parasitoids using beetle larvae and pupae reared on the artificial diets suggests that these diets can significantly improve the efficiency of mass rearing of these natural enemies.

The survival rates of immature *A. hispinarum* and *T. brontispae* were affected by the ingredients of the artificial diets on which *B. longissima* larvae were reared. In *A. hispinarum* the survival rate was significantly higher in the mature coconut leaf diet-reared larvae than in the young coconut leaf diet-reared larvae, whereas *T. brontispae* had the exact opposite results (Table 4). Because the artificial diets used in this study were identical except for the dried leaf powders, our findings indicate that the leaf powder affects the development of immature parasitoids. Others have reported the effect of host diets on the performance of immature parasitoids (Godfray 1994). For example, larger adults of *Brachymeria intermedia* (Nees) (Hymenoptera: Chalcididae) emerged from gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), fed on the leaves of red oak, *Quercus rubra* L. (Fagales: Fagaceae), in comparison with alternative food plants (Greenblatt and Barbosa 1981). When nicotine was added to the artificial diet of host caterpillars, parasitoids developing within the hosts showed significant differences in their development and survival (Barbosa et al. 1986). The effects of nicotine were more severe on *Hyposoter annulipes* (Cresson) (Hymenoptera: Ichneumonidae) parasitizing fall armyworm, *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae), than on *Cotesia congregata* (Say) (Hymenoptera: Braconidae) parasitizing tobacco hornworm, *Manduca sexta* (L.) (Lepidoptera: Sphingidae).

This study demonstrated that two newly developed artificial diets are useful for rearing larvae of *B. longissima* and its two parasitoids. These diets, which contain mature coconut leaf and orchard grass powders, will aid researchers in obtaining healthy *B. longissima* individuals and in rearing their natural enemies, *A. hispinarum* and *T. brontispae*.

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