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Development and reproduction of *Mallada basalis* (Neuroptera: Chrysopidae) on artificial diets

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Abstract

The green lacewing, *Mallada basalis* (Walker) (Neuroptera: Chrysopidae), has a broad prey range and effective searching abilities. Because rearing procedures based on natural or factitious foods for the larvae of this economically important predator are often time consuming and/or expensive, the main objective of our study was to develop an artificial diet suitable for mass rearing. We analyzed the development, survival, longevity, and reproduction of *M. basalis* (F1 generation) fed 3 artificial diets. These diets were formulated based on those of *Chrysoperla sinica* Tjeder and *Chrysopa pallens* (Rambur) (Neuroptera: Chrysopidae). All 3 of the diets contained chicken egg, beer yeast powder, sucrose, trehalose, vitamin C, and potassium sorbate. The first artificial diet (AD1) also contained honey and distilled water. AD2 also included pupal hemolymph of the Chinese oak silk moth (*Antheraea pernyi* Guérin-Méneville; Lepidoptera: Saturniidae), and AD3 also included whole *A. pernyi* pupae, which had been blended into all of the ingredients. The 2nd instars reared on AD3 required 2.91 d to develop to 3rd instars, which was significantly less time than the 3.69 d required on AD2. However, 3rd instars reared on AD1 required 4.48 d to develop to the pupal stage, which was significantly shorter than 3rd instars on AD2 and AD3 at 6.92 and 5.68 d, respectively. The development time of pupae in the AD3 treatment was 8.18 d, which was significantly shorter than that of pupae in the AD1 and AD2 treatments at 9.05 and 10.00 d, respectively. There were significant differences in adult longevity among the 3 diets, and these longevities in the AD1, AD2, and AD3 treatments were 39.40, 4.75, and 30.11 d, respectively. The oviposition period was significantly longer for females reared on AD1 (22.70 d) than for females reared on AD3 (5.25 d). The oviposition rate and total number of eggs laid from AD1 (16.41 eggs/day and 476.67 eggs, respectively) were significantly greater than those from AD3 (3.11 eggs/day and 19.75 eggs respectively). Females reared on AD2 laid no eggs. There were significant differences in egg hatch and pupation rates among the 3 diets. The egg hatch and pupation rate were largest on AD1, i.e., 100.0% and 63.3%, respectively, whereas they were the least on AD2, i.e., 53.33% and 16.19%, respectively. There were significant differences in hatch rates from newly laid eggs (F2 generation) between AD1 and AD3, i.e., 70.4% and 63.0%, respectively. We found that *M. basalis* was able to develop and reproduce when fed artificial diets AD1 and AD3. However, AD1 was much better than AD3. The development and reproduction of *M. basalis* fed AD2 were unacceptable. AD1 contained twice the amount of chicken egg compared with AD2 and AD3, and egg yolk is known to be a high-quality component of diets for entomophagous insects. The diet AD1 was also the only one that contained honey. All the 3 diets contained trehalose, which can be a partial substitute for insect components. Our findings may contribute to the mass production of this economically important predatory green lacewing.

Key Words: *Antheraea pernyi*; artificial diet; green lacewing; hemolymph; mass rearing, development; reproduction

Resumen

La crisopa verde, *Mallada basalis* (Walker) (Neuroptera: Chrysopidae), tiene una amplia gama de presas y una eficaz capacidad de búsqueda. Debido a que los procedimientos de cria basados en los alimentos naturales o ficticios para las larvas de este depredador de importancia económica son a menudo caros y/o requieren mucho tiempo, es que el objetivo principal de nuestro estudio fue desarrollar una dieta artificial adecuada para la cría masiva. Se analizó el desarrollo, la sobrevivencia, la longevidad y el rendimiento reproductivo de *M. basalis* (generación F1) alimentados con 3 dietas artificiales. Estas dietas fueron formuladas con base en las dietas de *Chrysoperla sinica* Tjeder y *Chrysopa pallens* (Rambur) (Neuroptera: Chrysopidae). Todas las 3 dietas tenían un huevo de pollo, levadura de cerveza en polvo, sacarosa, rehalose, la vitamina C y el sorbato de potasio. La primera dieta artificial (AD1) también tenía agua destilada. AD2 también incluyó hemolinfa de la pupa de la polilla de seda de roble chino (*Antheraea pernyi* (Gu'erin-M'eneville); Lepidoptera: Saturniidae), y AD3 también incluyó pupas completas de *A. pernyi*, que se había mezclado con todos los otros ingredientes. Los estadios segundos criados sobre AD3 requerieron 2.91 días para desarrollar los tercer estadios, lo cual fue mucho menos tiempo que los 3.69 días requeridos en AD2. Sin embargo, los estadios terceros criados en AD1 requerieron 4.48 días para desarrollarse el estadio de pupa, que fue significativamente más corto que los estadios terceros en AD2 y AD3 a los 6.92 y 5.68 días, respectivamente. El tiempo de desarrollo de pupas del tratamiento AD3 fue 8.18 días, que es significativamente más corto que el tiempo de desarrollo de las pupas en los tratamientos AD1 y AD2 a los 9.05 y 10.00 días, respectivamente. Hubo diferencias significativas en la longevidad de los adultos entre 3 dietas, y estas duración en los tratamientos AD1, AD2 y AD3 fue de 39.40, 4.75 y 30.11 días, respectivamente. El período de oviposición fue significativamente más largo en AD1 (22.70 días) que en AD3 (5.25 días). La tasa de oviposición y el número total de huevos puestos en AD1 (16.41 huevos/día y 476.67 huevos, respectivamente) fueron significativamente mayores que los de AD3 (3.11 huevos/día y 19.75 huevos respectivamente). Las hembras criadas en AD2 no pusieron huevos. Hubo diferencias significativas en la eclosión de los huevos y las tasas de pupación entre las 3 dietas. La eclosión de los huevos y la tasa de pupación fueron más altas en AD1, del 100.0% y 63.3%, respectivamente, mientras que fueran menos en AD2, del 53,33% y 16,19%, respectivamente. Hubo

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diferencias significativas en la tasa de eclosión de los huevos recién puestos (generación F2) entre AD1 y AD3, del 70,4% y 63,0%, respectivamente. Hemos encontrado que *M. basalis* fue capaz de desarrollar y reproducirse cuando son alimentados con dietas artificiales AD1 y AD3. Sin embargo, AD1 fue mucho mejor que AD3. El desarrollo y la reproducción de *M. basalis* alimentados con AD2 fueron inaceptables. Nuestros hallazgos pueden contribuir a la producción en masa de esta crisopa verde depredadora de importancia económica.

Palabras Clave: *Antheraea pernyi*; dieta artificial; crisopa verde; hemolinfa; cría en masa; desarrollo; reproducción

Green lacewings are among the most effective general entomophagous predators (Boo et al. 1998) because of their extensive range of prey species and wide distribution (Tauber et al. 2000). The green lacewing *Mallada basalis* (Walker) (Neuroptera: Chrysopidae) has a broad prey range and effective searching abilities (Li et al. 2011; Jiang et al. 2013; Ye et al. 2013). *Mallada basalis* larvae have been released to control *Icerya aegyptiaca* (Douglas) (Hemiptera: Margarodidae) in Bijia Mountain Forest Park, Shenzhen, China (Jiang et al. 2013). However, these *M. basalis* larvae were reared on factitious hosts, such as *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae), *Ferrisia virgata* (Cockerell) (Hemiptera: Pseudococcidae), and eggs of the rice grain moth *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) under laboratory conditions (Li et al. 2011; Ye et al. 2012, 2013; Jiang et al. 2013), which made this predator too expensive for use in large-scale open-field agricultural settings. Rearing procedures based on natural or factitious foods are often time consuming and/or expensive (Lee & Lee 2005).

Mass-rearing techniques, which are economical and possess high biological efficiency, need to be developed. Indeed, chrysopids reared on artificial diets have been used to control agricultural insect pests. For example, *Chrysoperla carnea* (Stephens) and *Chrysoperla rufilabris* (Burmeister) have been produced commercially for farmers (Cohen & Smith 1998). The following 3 standards are absolutely essential for successful commercial mass rearing: (i) production of continuous generations on an artificial diet and with no recourse to real prey, (ii) continuous increase in colony size, and (iii) ability of the mass-reared chrysopids to efficiently locate, kill, and feed on natural prey in the field (Cohen 1992).

The application of augmentative biological control measures is dependent upon the production of large numbers of high-quality predators (Smith & Nordlund 2000; Riddick 2009). The quality of the available food or diet is a key factor affecting the growth, development, and reproduction of predatory insects (Thompson 1999), and the biological characteristics of these species, e.g., their behavior, mortality, longevity, and reproduction, can be used to evaluate the quality of a diet (Grenier & De Clercq 2003; Sighinolfi et al. 2008). The main objective of our study was to develop a suitable artificial diet for mass rearing the economically important predator, *M. basalis*.

Materials and Methods

EXPERIMENTAL INSECTS

Mallada basalis larvae were collected in 2010 from guava (*Psidium guajava* L.; Myrtales: Myrtaceae) trees in Wenchang City, Hainan Province, China, and reared in the laboratory on a diet of eggs of the rice grain moth *C. cephalonica* (Ye et al. 2012). *Corcyra cephalonica* eggs, which had been laid by females reared on rice bran, were irradiated with ultraviolet light for 30 min to kill their embryos before being provided as the food of *M. basalis* (Ye & Cheng 1986). The *M. basalis* culture was reared at 26 ± 1 °C, 70 ± 5% RH, and a 16:8 h L:D photoperiod. The 19th generation of *M. basalis* was chosen for experiments, and for purposes of the present study, this 19th generation was designated as the parental generation, P. Their eggs and subsequent life stages were designated as the F1 generation. Likewise the eggs of F1 females and subsequent life stages belonged to the F2 generation.

PREPARATION OF ARTIFICIAL DIETS

The artificial diets were formulated based on the artificial diets of *Chrysoperla sinica* Tjeder (Ye et al. 1979) and *Chrysopa pallens* (Rambur) (Neuroptera: Chrysopidae) (Lee & Lee 2005). In the diets described below, the yolk and albumin (egg white) of a chicken egg were homogenized by blending in a food processor, and then the amount required in each diet was weighed on an electronic balance. Both the hemolymph in the 2nd artificial diet (AD2) and the body contents in the 3rd artificial diet (AD3) were obtained from newly eclosed pupae of the Chinese oak silk moth *Antheraea pernyi* Guérin-Méneville (Lepidoptera: Saturniidae). To obtain the body contents, the pupal integuments of several pupae were dissected with sharp scissors and discarded. Then, these integumentless pupae were immersed for 10 min in a 60 °C water bath to prevent melanization of the hemolymph. Next, the midgut of each pupa was extruded by lightly pressing the pupa with our fingers, and the extruded midgut was removed. Next, these pupal bodies were homogenized by blending in a food processor. Likewise, to obtain hemolymph, the pupal integuments of several pupae were dissected with sharp scissors and discarded. Then the hemolymph was collected by lightly pressing the pupae with the thumb and the fingers (Lü et al. 2013).

Three artificial diets were prepared for *M. basalis* larvae, and their compositions are shown in Table 1. The ingredients of the diets were blended in a food processor for 3 to 5 min until the entire mixture was homogenous. A 1 × 1 cm Parafilm membrane was stretched to about 3× *its original length and width (*Cohen & Smith 1998*).* Next, the diet was placed on the central part of the membrane. Then, the membrane was folded and stuck tightly together. The artificial diets were prepared every 2 wk and kept in a refrigerator at 5 °C. The weight of each diet packet was 0.05 g, and the weight of the diet itself was 0.03 g.

EXPERIMENTAL SETUP

Mallada basalis eggs (F1 generation) were individually placed in Petri dishes (9 cm diameter × 1.5 cm height). Fifty eggs in the Petri dishes were used for the AD1, AD2, and AD3 diet treatments. The eggs were placed on the bottom of the Petri dish between the diet and a moist wad of cotton. In each of the 3 treatments, beginning with the

Table 1. Compositions of 3 artificial diets for rearing larvae of the chrysopid *Mallada basalis.*

	Name of diet and amount of ingredient		
Ingredients	AD1	AD2	AD3
Chicken egg (homogenized)	40 g	20 g	20 g
Beer yeast powder	30 g	30 g	30 g
Honey	20 g	none	none
Sucrose	9 g	15 g	15 g
Trehalose	1 g	1 g	1 g
Vitamin C	0.1 g	0.1 g	0.1 g
Potassium sorbate	0.1 g	0.1 g	0.1 g
Distilled water	5 mL	none	none
Hemolymph of *Antheraea pernyi* pupae	none	30 g	none
Homogenized *A. pernyi* body contents	none	none	30 g

neonate larval stage, each larva was supplied with 5 diet packets and water in moist cotton every day. The durations of the development of each life stage from the neonate to the cocoon were determined by monitoring molting events every day. Mortality was recorded every day during development of the immatures. These experiments with the F1 generation were continued until the females died, and at that time, adult longevity was recorded. Dead males were replaced by males of similar age from the laboratory colony.

After the F1 generation adults had emerged, their sex was recorded, and then each adult female was paired with a male. The F1 adults were fed brewer's yeast powder, honey solution (honey to water = 1:2 by volume), and water in a moist cotton wad. The fecundity of each F1 female (eggs laid per day) was recorded. Ten Petri dishes each with the eggs of a single female were used for each treatment in order to determine the percentage of hatch from the eggs of the F2 generation, and this procedure was repeated 5 times. However, no observations were made on the development of F2 immatures on the 3 diets. The experiments were carried out in a growth chamber at 26 ± 1 °C, 70 ± 5% RH, and a 16:8 h L:D photoperiod.

STATISTICAL ANALYSES

Data were subjected to statistical analyses in SPSS (SPSS Inc., Chicago, Illinois, USA). Differences in larval and pupal development time, developmental parameters, and reproduction were analyzed by 1-way analysis of variance (ANOVA). These analyses were performed on data of 5 replicates per treatment, and the means were separated by Tukey's test at $P \leq 0.05$.

Results

There were significant differences in the development times among the 2nd instars, 3rd instars, and pupae in the 3 diet treatments (F = 3.91, 20.39, and 5.32, respectively; df = 2, 104, 2, 90, and 2, 52, respectively; $P < 0.05$) (Table 2). The 2nd instars reared on AD3 took significantly less time to develop to the 3rd instar than those fed AD2. The development time of the 3rd instars fed AD1 was significantly shorter than of those fed either AD2 or AD3. The development time of pupae derived from larvae fed AD3 was significantly shorter than that of pupae derived from larvae fed either AD1 or AD2.

There were significant differences in the longevities of adult F1 females in the 3 diet treatments, i.e. 37.27, 4.00, and 31.60 d in AD1, AD2, and AD3 treatments, respectively (F = 1.49; df = 2, 30; $P < 0.05$) (Table 3). Also, the average adult (female + male) F1 longevities among the 3 diet treatments, AD1, AD2, and AD3, differed significantly, i.e., 39.40, 4.75, and 30.11 d, respectively (F = 3.76; df = 2, 45; $P < 0.05$). The oviposition period of F1 females in the AD1 treatment (22.70 d) was significantly longer than in the AD3 treatment (5.25 d) (F = 5.51; df = 2, 22; $P < 0.05$), and F1 females in the AD2 treatment laid no eggs. The oviposition rate (eggs/female/day) and total number of eggs (eggs/female) in the AD1 treatment (16.41 and 473.67 eggs, respectively) were significantly greater than in the AD3 treatment (3.11 and 19.75 eggs, respectively) (F = 37.11 and 4.96, respectively; df = 2, 22; $P < 0.05$).

There were significant differences in egg hatch rate and pupation rate of the F1 generation among the 3 treatments (F = 14.80 and 11.73, respectively; df = 2, 14; $P < 0.05$) (Fig. 1), i.e., egg hatch and pupation rate were greatest in the AD1 treatment (100 and 63.33%, respectively) and smallest in AD2 treatment (53.33 and 16.19%, respectively). Moreover, there were significant differences in the hatch rate of F2 eggs between the AD1 and AD3 treatments, i.e., 70.40 and 63.00%, respectively (F = 57.04; df = 2, 14; $P < 0.05$) (Fig. 1).

Table 2. Development times (d) of the immature stages of the F1 generation of *Mallada basalis* fed 3 artificial diets at 26 ± 1 °C.

	Duration of development (d)			
Diet	1st instar	2nd instar	3rd instar	Pupa
AD1	3.53 ± 0.17a	3.07 ± 0.17ab	4.48 ± 0.20c	9.05 ± 0.21a
AD2	3.50 ± 0.13a	3.69 ± 0.31a	6.92 ± 0.45a	10.00 ± 0.32a
AD3	3.17 ± 0.09a	2.91 ± 0.09b	5.68 ± 0.20b	8.18 ± 0.26b

Notes: 116, 105, 91, and 53 individual 1st instars, 2nd instars, 3rd instars, and pupae, respectively, were tested in this experiment. Means (± SE) within a column followed by the same letter do not differ significantly (Tukey's test; $P > 0.05$). AD1, artificial diet 1; AD2, artificial diet 2; and AD3, artificial diet 3.

Discussion

The main objective of our study was to develop a suitable artificial diet for mass rearing the economically important chrysopid predator, *M. basalis*. Artificial diets must satisfy the nutritional requirements of predators and ensure the continuous production of progeny of high quality (Cohen 2004). In this study, we found that *M. basalis* was able to develop and reproduce when fed artificial diets AD1 and AD3. However, AD1 supported development and reproduction much better than AD3. The development and reproduction of *M. basalis* fed AD2 was not acceptable, because the females laid no eggs. Nettles (1990) and Grenier & De Clercq (2003) noted that adding insect components such as hemolymph to artificial diets enhanced their acceptability and improved their nutritional quality for a number of entomophagous insect species, especially parasitoids. However, in this study the development and reproduction of *M. basalis* that had been reared on AD1, which entirely lacked insect components, were much more successful than on AD2, which contained hemolymph of *A. pernyi* pupae, or on AD3, which contained the whole body contents of *A. pernyi* pupae. Indeed, the development and reproduction of *M. basalis* fed AD2 with pupal hemolymph of *A. pernyi* were much less successful compared with those fed AD3 with whole *A. pernyi* pupal body contents. Nevertheless, it is noteworthy that AD1 contained about 2-fold more homogenized chicken egg (i.e., 38.03% w/w) than either AD2 or AD3, each of which contained 20.79% of this component; and chicken egg yolk is known to be a high-quality dietary component for entomophagous insects. Only diet AD1 contained honey (20 g, i.e. 19.0%), which suggests that honey may be an important source of nutrients and possibly an important reason that AD1 was superior to AD2 and AD3. All 3 diets contained trehalose, which can be a partial substitute for hemolymph (Lü et al. 2013).

We evaluated suitability of the artificial diets with respect to development, survival, longevity, and reproductive performance of *M. basalis*, but it is also essential to compare the performance of these diets with other diets used to rear related species (Lee & Lee 2005). The development periods of the larval stages of *M. basalis* fed either AD1 or AD3 were longer than those previously found when they were fed eggs of *C. cephalonica* (Ye et al 2012), but were similar to those fed other natural prey species such as *I. aegyptiaca*, *F. virgata*, and *P. citri* (Ye et al 2012; Jiang et al 2013). The duration of the pupal stage of *M. basalis* fed AD1 was similar to that of *M. basalis* fed eggs of *C. cephalonica*, but was longer than of those fed *I. aegyptiaca*, *F. virgata*, and *P. citri*. The adult longevity of *M. basalis* fed AD1 was longer than of those fed eggs of *C. cephalonica*, *I. aegyptiaca*, *F. virgata*, or *P. citri*. The emergence rates of *M. basalis* fed AD1 and AD3 were greater than those fed *I. aegyptiaca*, *F. virgata*, or *P. citri*. The female proportions of *M. basalis* fed these 2 artificial diets were greater than those recorded

Table 3. Reproduction and oviposition parameters of the F1 adult progeny of *Mallada basalis* in the 3 artificial diet treatments at 26 ± 1 °C.

Parameter	AD1	AD2	AD3
Oviposition period (d)	22.70 ± 4.49a	0	5.25 ± 2.53b
Female longevity (d)	37.27 ± 8.32a	4.00 ± 3.00b	31.60 ± 9.49a
Average longevity (d) of females plus males	39.40 ± 6.78a	4.75 ± 1.44b	30.11 ± 6.46a
Female proportion	0.63 ± 0.03a	0.50 ± 0.00a	0.64 ± 0.07a
Oviposition rate (eggs/female/day)	16.41 ± 3.00a	0	3.11 ± 1.91b
Total number of eggs (eggs/female)	473.67 ± 132.54a	0	19.75 ± 8.86b

Notes: 46 adult males and 31 adult females were tested in this experiment. Means (± SE) followed by the same letter within a row do not differ significantly (Tukey's test; $P > 0.05$). AD1, artificial diet 1; AD2, artificial diet 2; and AD3, artificial diet 3.

on *I. aegyptiaca*, *F. virgata*, and *P. citri*. *Mallada basalis* fed AD1 and AD3 produced fewer eggs than when fed eggs of *C. cephalonica* (Ye et al 2012; Jiang et al 2013). This indicated that these artificial larval diets still have limitations with respect to assuring great fecundity.

The immature development time of *M. basalis* at 26 ± 1 °C on AD1 (20.13 d) was shorter than that of *C. pallens* (26.90 d) reared at 28 °C on an artificial diet consisting of infant formula, royal jelly, Neisenheimer's salt solution, beef liver and beef powder, homogenized *A. pernyi* pupal whole bodies, chicken egg yolk, and honey (Lee & Lee 2005). However, the average *M. basalis* female longevity and fecundity were 37 d and 474 eggs in the present study, compared with 89 d and 2,020 eggs for *C. pallens* adults fed an artificial diet containing chicken yolk, infant formula, beef liver, and beef powder (Lee & Lee 2005). This greatly enhanced reproductive performance of *C. pallens* adult females was obtained by feeding them an artificial diet (Lee & Lee 2005). Although the costs of the diets of Lee & Lee (2005) were much greater than those of our *M. basalis* diets, the nutritional quality of their immature and adult diets are superior to our *M. basalis* diets. Infant formula, royal jelly, beef liver, and beef powder are important nutrient sources. The liver-based diet showed high conversion efficiency when provided as a nutrient source (Lee & Lee 2005).

In conclusion, the artificial diet AD1 supported development and reproduction of *M. basalis* in the mass rearing of this economically important biological control agent. The balance of different nutrients is a critical and dominant factor that determines dietary acceptability and suitability (House 1974). However, nutritional imbalances within a diet may be expressed only in subsequent generations (De Clercq et al. 2005). Therefore, developmental and reproductive performance of *M. basalis* fed AD1 still need to be assessed over subsequent generations.

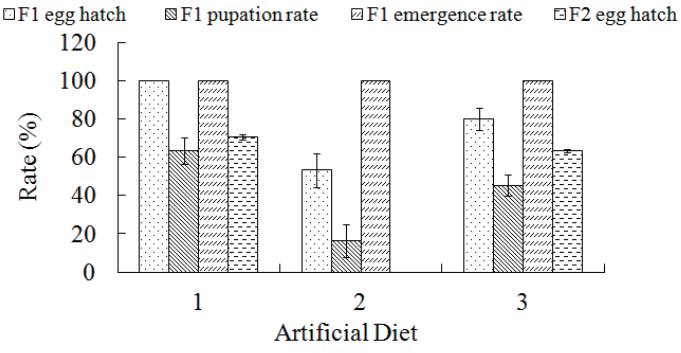

Fig. 1. Developmental parameters of *Mallada basalis* fed during the immature stages of the F1 generation on the 3 artificial diets at 26 ± 1 °C. F1 egg hatch and pupation rates differed significantly among the 3 diet treatments and both parameters were significantly the greatest in the AD1 treatment and unacceptably small in the AD2 treatment. F1 adult emergence rates among the 3 diets did not differ significantly ($P > 0.05$). The F1 generation produced no eggs in the AD2 treatment. The hatch rate from F2 eggs in the AD1 treatment was significantly greater than in the AD3 treatment.

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