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Source: Florida Entomologist, 97(3) : 1075-1080

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.097.0311

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EFFECT OF CANNIBALISM ON THE GROWTH AND DEVELOPMENT OF *MALLADA BASALIS* (NEUROPTERA: CHRYSOPIDAE)

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ABSTRACT

The green lacewing, *Mallada basalis* (Walker) (Neuroptera: Chrysopidae), is one of the most important natural enemies used in biological control programs for forestry and agricultural pests. However, it is difficult to mass rear *M. basalis* instars because they are voracious cannibals. This study was conducted to determine the effect of cannibalism on the development and fecundity of *M. basalis*. We analyzed the developmental parameters of 3rd instars that cannibalized *M. basalis* eggs and 1st instars, and the relationship between the proportion of each of the 3rd instars cannibalized and the number of folded paper barriers provided in an arena to minimize the frequencies of encounters. In all treatments the green lacewing instars were provided eggs of the pyralid, *Corcyra cephalonica* (Stainton). In Treatment I the pyralid eggs were supplemented with *M. basalis* eggs and in Treatment II, the pyralid eggs were supplemented with *M. basalis* 1st instars. The developmental times of the 3rd instars and pupae of Treatment I and Treatment II were significantly shorter than in the control. The pupal weights, fecundities and egg hatch rates of Treatments I and II were significantly larger than in the control. When 3rd instars cannibalized *M. basalis* eggs or 1st instars, the pupal weights significantly increased from 10.3 (control) to 11.0 and 10.9 mg, respectively, fecundities significantly increased from 91 (control) to 141 and 121 eggs, respectively, and the egg hatch rates significantly increased from 62% (control) to 70% and 68%, respectively. Moreover, as the number of folded paper barriers per arena increased, the proportions of 3rd instars cannibalized at first tended to decrease but then increased. When the different instars were studied separately, the proportion of 3rd instars cannibalized was larger than the corresponding proportions of 1st and 2nd instars. The research indicated that cannibalism enhances both development and fecundity of *M. basalis*. The use of paper barriers to frequent encounters reduced cannibalism somewhat, but this method was not adequately effective for the 3rd instars. Our findings may contribute to the mass production of this economically important predatory green lacewing.

Key Words: *Mallada basalis* (Walker); cannibalism; intra-specific feeding; barrier

RESUMEN

La crisopa verde, *Mallada basalis* (Walker) (Neuroptera: Chrysopidae), es uno de los enemigos naturales más importantes utilizados en programas de control biológico de plagas agrícolas y forestales. Sin embargo, es difícil criar en masa los estadios de *M. basalis* porque son caníbales voraces. Se realizó este estudio para determinar el efecto de canibalismo en el desarrollo y la fecundidad de *M. basalis*. Se analizaron los parámetros de desarrollo de los terceros estadios que canibalizan los huevos y primeros estadios de *M. basalis*, y la relación entre la proporción de cada uno de los terceros estadios cannibalizados y el número de barreras hechas de papel doblado puestas en una arena para minimizar la frecuencia de los encuentros. En todos los tratamientos, se proporcionó los estadios de crisopa verde con huevos del pirálido, *Corcira cephalonica* (Stainton). En el Tratamiento I, los huevos de los pirálidos fueron complementados con huevos de *M. basalis,* y en el tratamiento II, de los huevos pirálidos se complementaron con el primer estadio de *M. basalis*. El tiempo de desarrollo del tercer estadio y las pupas en el Tratamiento I y Tratamiento II fue significativamente más corto que en el control. El peso de la pupa, la fecundidad y tasa de eclosión de huevos de los Tratamientos I y II fueron significativamente mayores que en el control. Cuando los terceros estadios canibalizaron los huevos o primeros estadios de *M. basalis*, el peso de las pupas aumentó significativamente de 10.3 (de control) a 11.0 y 10.9 mg, respectivamente, la fecundidad aumentó significativamente de 91 (de control) a 141 y 121 huevos, respectivamente, y la tasa de eclosión de los huevos aumentó significativamente de 62 % (de control) a 70 % y 68 %, respectivamente. Por otra parte, con el aumentó en el número de barreras de papel plegado en la arena, la proporción de los terceros estadios cannibalizados al principio tendieron a disminuir, pero luego se incrementó. Cuando se estudiaron los diferentes estadios por separado, la proporción de los terceros estadios cannibalizados fue más grande que la proporción correspondientes de los primeros y segundos estadios. La

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investigación indicó que el canibalismo mejora tanto el desarrollo como la fecundidad de *M. basalis*. El uso de barreras de papel para frecuentar los encuentros redujó el canibalismo un poco, pero este método no fue adecuadamente eficaz para los terceros estadios. Nuestros hallazgos pueden contribuir a la producción en masa de esta económicamente importante crisopa verde depredadora.

Palabras Clave: *Mallada basalis* (Walker); canibalismo; alimentación intraespecífica; barrera

The green lacewing, *Mallada basalis* (Walker) (Neuroptera: Chrysopidae), distributed in South China, is one of the most important natural enemies used in the biological control of insect pests of forestry and agriculture (Ye et al. 2013). Its larvae prey on several kinds of pests, such as mealy bugs, whiteflies, the eggs and larvae of Lepidoptera, and other orders (Li et al. 2011). However, *M. basalis* displays substantial cannibalism which may impede mass rearing. Also, larval cannibalism in apredatory insect species is a manifestation of significant intra-specific competition.

Cannibalism, defined as killing and eating an individual of the same species, is a behavioral trait seen in a number of species. Cannibalism occurs in many different ecological and social contexts, and is present during any or all life stages from just after hatching and throughout development or mating (Fox 1975; Elgar & Crespi 1992). Cannibalism has been observed in a number of animals and is seen as a force that structures predatory insect communities (Polis et al. 1989; Dong & Polis 1992). Newly eclosed coccinellid larvae may improve their survival rate by feeding on conspecific eggs (Gagné et al. 2002), and larval cannibalism of coccinellids increases their rates of success of pupation and emergence (Wang 2010). Thus, cannibalism is adaptively significant for ladybirds to raise their survival rate in adversity (Agarwala & Dixon 1992). Female mantis (Mantodea: Mantidae) and spiders (Arachnida: Araneae) may gain nutritional benefits from male consumption that consequently translates into increased fecundity (Birkhead et al. 1988; Newman & Elgar 1991; Barry et al. 2008).

The biology and ecology of *Chrysopa sinica* Tiedet, *Chrysopa carnea* Stephens, *Chrysopa formosa* Brauer, *Chrysopa phyllochrom* Brauer have been studied in China and other countries. Techniques for improving the mass rearing of these green lacewings have been studied, including the use of folded paper, straws and copper wire as partitions to reduce cannibalism during the mass rearing (Cai et al. 1983; Xu et al. 1997; Su et al. 1999; Zhang et al. 2004; Muhammad et al. 2006; Li et al. 2010). However, there has been little research on the mass rearing of *M. basalis*, and especially on the biological significance and the mechanism of larval cannibalism. Thus, the effect of cannibalism, in which the 3rd instars cannibalize eggs and 1st instars, on the development and fecundity of *M. basalis*, and the relationship between the proportion of larvae cannibalized and different amounts of folded paper were studied, to provide a theoretical foundation and technical support for mass rearing.

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, and reared in the laboratory with eggs of the rice moth [*Corcyra cephalonica* Stainton) (Pyralidae)] (Ye et al. 2012). The 13th generation of *M. basalis* was chosen for experiments. *Corcyra cephalonica*, whose eggs were irradiated with ultraviolet light for 30 min to kill their embryos before being used, was reared with rice bran. Rearing conditions were 26 ± 1 °C, 70 ± 5% RH and 16:8 h L:D.

Developmental Parameters of *Mallada basalis*' under Various Intra-Specific Feeding Conditions

About 100 newly hatched *M. basalis* larvae were individually reared until the 3rd instar with eggs of *C. cephalonica* and with moistened cotton enclosed in a little glass tube. Then, two treatments were carried out. In Treatment I each *M. basalis* 3rd instar was put in a Petri dish (9 cm diam × 1.5 cm) and reared with 50 *C. cephalonica* eggs (Li et al. 2012) and with cotton moistened in water every day. Five *M. basalis* eggs were added to feed each *M. basalis* 3rd instar every day until the 3rd instars pupated. Next, the pupa was weighed and its weight was recorded. After the adults emerged, their sex was recorded, and then each adult female was paired with a male and put into a Petri dish, and fed brewer's yeast powder, honey and water moistened in cotton. The female's fecundity (eggs laid per day) and longevity were recorded. After the adults had laid eggs, one hundred eggs were randomly chosen, and their hatching rate was recorded. Ten Petri dishes were used for each treatment, which was repeated 5 times.

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In Treatment II five *M. basalis* 1st instars were added everyday to feed each *M. basalis* 3rd instar until the 3rd instars pupated; other steps were the same as Treatment I. In the control neither *M. basalis* eggs nor 1st instars were added to each Petri dish, and other steps were the same as Treatment I. The experiments were carried out in a growth chamber at 26 ± 1 °C, 70 ± 5% RH and 16:8 h L:D.

Relationship between the Proportion of Larvae Cannibalized and Number of Paper Barriers

Five *M. basalis* larvae were reared in a 9 cm diam × 1.5 cm Petri dish with 50 *C. cephalonica* eggs and moistened cotton. A paper strip (7 cm × 2.5 cm), folded twice along the long axis, was placed into the Petri dish as a barrier to frequent encounters of larvae. Then, no piece, or 1, 2, 3, and 4 pieces of similarly folded paper, respectively, were put into various Petri dishes. The following 3 treatments were carried out separately with newly hatched larvae, 2nd instars and 3rd instars. The number of larvae that were killed was recorded each day. Such records were taken until the next molt, i.e., in Treatment #1 until all of the 1st instars had transformed into 2nd instars, in Treatment #2 until all of the 2nd instars had transformed into 3rd instars, and in Treatment #3 until all of the 3rd instars had pupated. The proportion of cannibalized larvae was calculated by dividing the number killed by the initial number of *M. basalis* larvae (5).

Statistical Analyses

Data were subjected to statistical analysis (SPSS Inc., Chicago, Illinois, USA). Differential effects of cannibalism on development parameters and the developmental times of third instars, pupae and adults, and the relationship between the proportion of cannibalized larvae and the number of paper barriers to encounters were analyzed by one-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

Effect of Cannibalism on Developmental Times of Various Life Stages

The developmental times of adults (Table 1) did not differ significantly among the control, Treatment I (cannibalizing eggs) and Treatment II (cannibalizing 1st instars) ($F = 0.254$; df = 113; $P = 0.776$). However, there were significant differences in the developmental times of the 3rd instars and pupae between the control and Treatments I and II ($F = 16.53, 10.75$; df = 149, 143; $P < 0.05$). The developmental times of the 3rd instars and the pupae of the control were 5.06 days and 9.13 days respectively; those of Treatment I were significantly shorter at 4.32 days and 8.61 days, respectively, and those of Treatment II also were significantly shorter than the control, i.e., 4.14 days and 8.69 days, respectively (Table 1).

Effect of Cannibalism on Developmental Parameters

Different treatments had no significant influence on the sex ratio (Table 2) with the proportion of females ranging insignificantly from 0.51 to 0.60 ($F = 3.24$, df = 14, $P = 0.075$). The pupation rate and emergence rate did not differ significantly among the control, Treatment I and Treatment II ($F = 0.46, 3.81$; df = 14; $P = 0.64, 0.06$). However, there were significant differences in the pupal weights, fecundity and egg hatching rate among the control and other treatments ($F = 41.02, 16.37$ and 16.46, respectively; df = 143, 63 and 14, respectively; $P < 0.05$). In individuals that had cannibalized eggs or 1st instars, the pupal weight was significantly increased to 10.96 mg or 10.88 mg, as compared to 10.33 mg in the control. Fecundity in Treatment I was 140.50 eggs, and in Treatment II it was 120.77 eggs, and these levels were significantly larger than the control level of 90.56 eggs. Also the egg hatch rates were 69.60% in Treatment I and 67.80% in Treatment II, both of which were significantly larger than the control, 61.80% (Table 2).

TABLE 1. DEVELOPMENTAL TIMES (DAYS) OF *MALLADA BASALIS* THIRD INSTARS, PUPAE AND ADULTS WHEN 3RD INSTARS CANNIBALIZED EITHER *M. BASALIS* EGGS OR 1ST INSTARS.

Treatments	Duration of Developmental Stage (Days)		
	3rd Instar	Pupa	Adult
Control	5.06 ± 0.12 a	9.13 ± 0.12 a	40.57 ± 4.10 a
Treatment I – cannibalized eggs	4.32 ± 0.13 b	8.61 ± 0.12 b	41.10 ± 4.06 a
Treatment II – cannibalized 1st instars	4.14 ± 0.11 b	8.69 ± 0.13 b	44.31 ± 3.87 a

Notes: 150, 144 and 114 larvae, pupae and adults, respectively, were tested in this experiment. Means (± SE) followed by the same letter within columns do not differ (Tukey's test; $P > 0.05$).

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TABLE 2. DEVELOPMENTAL PARAMETERS OF *MALLADA BASALIS* WHEN 3RD INSTARS CANNIBALIZED EITHER *M. BASALIS* EGGS OR 1ST INSTARS.

Treatments	Pupal weight (mg)	Female proportion	Pupation rate (%)	Emergence rate (%)	Fecundity (eggs per female)	Egg hatch (%)
Control	10.29 ± 0.08 b	0.51 ± 0.03 a	94.00 ± 4.00 a	74.60 ± 2.04 a	90.56 ± 3.84 b	61.80 ± 1.20 b
Treatment I	10.96 ± 0.05 a	0.60 ± 0.02 a	98.00 ± 2.00 a	81.60 ± 2.14 a	140.50 ± 6.27 a	69.60 ± 0.93 a
Treatment II	10.88 ± 0.04 a	0.56 ± 0.03 a	96.00 ± 2.45 a	81.40 ± 1.94 a	120.77 ± 6.84 a	67.80 ± 0.86 a

Notes: 144, 114, 64 and 100 pupae, adults, adult females and eggs respectively were tested in this experiment. Means (± SE) followed by the same letter within columns do not differ (Tukey's test; $P > 0.05$). In Treatment I and Treatment II the *M. basalis* 3rd instars cannibalized the eggs and 1st instars, respectively.

Relationship between the Proportion of Larvae Cannibalized and Number of Paper Barriers

Generally the larvae did not hide in the folded paper barriers; however the 3rd instars tended to hide in the paper crevices just prior to pupation. Fig. 1, shows that as the number of folded paper barriers increased, the proportions of all 3 instars that were cannibalized tended to decrease at first and then to increase. There were significant differences among the proportions of cannibalized instars and the number of folded paper barriers ($F = 3.07$, 5.63 and 7.89, respectively; df = 24; $P < 0.05$). The proportion of cannibalized 3rd instars was the largest regardless of the number of barriers to encounters. The proportion of cannibalized 3rd instars was the largest with no paper barrier, i.e., 0.50, whereas with 2 paper barriers it was the smallest, i.e., 0.24. The proportion of cannibalized 2nd instars was smaller than that of 1st and 3rd instars. The proportion of cannibalized 2nd instars was the largest with no paper barrier, i.e., 0.14, and it was the smallest with 2 paper barriers, i.e., 0.05. The proportion of cannibalized 1st instars was the largest with no paper barrier, i.e., 0.33, while with 3 barriers it was the smallest, i.e., 0.10.

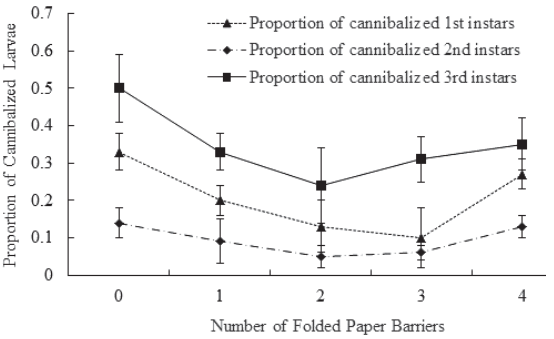


Fig. 1. Relationship between the Proportion of *Mallada basalis* Larvae Cannibalized and the Number of Paper Barriers to Encounters. Each curve shows the proportion of larvae in a Petri dish that were cannibalized by the time all had molted, i.e., in Treatment #1 until all of the 1st instars had transformed into 2nd instars, in Treatment #2 until all of the 2nd instars had transformed into 3rd instars, and in Treatment #3 until all of the 3rd instars had pupated. The proportion of cannibalized larvae was calculated by dividing the number killed by 5, the initial number in each Petri dish. There were significant differences among the number of folded paper barriers and the proportions of cannibalized larvae ($P < 0.05$).

DISCUSSION

Studies on cannibalism in arthropods have revealed several important factors affecting the occurrence of cannibalism: size, developmental stage, hunger, density, and food availability. The first 3 factors largely determine the vulnerability of the victim, whereas the latter 2 factors largely affect the environmental potential for cannibalism (Dong & Polis 1992). Studies of the proximate factors of cannibalism have found that a relative size difference between the cannibal and its victim is the most frequently cited factor determining the vulnerability of the victim, with developmental stage and hunger level are considered to be secondary determinants (Semlitsch & West 1988; Dong & Polis 1992; Lounibos et al. 1996; Sowig 1997; Samu et al. 1999).

The larvae of green lacewings have the habit of killing each other (Ridgeway et al. 1970). Field experiments have established that predatory terrestrial arthropods are frequently food- and nutrient-limited in nature (Wise 1993; Fagan et al. 2002; Denno & Fagan 2003; Fagan & Denno 2004; Wise 2006), and these limitations may be the reason that cannibalism occurs. In this paper the eggs and 1st instars were used to feed the 3rd instars. Even though sufficient *C. cephalonica* eggs were provided as food, cannibalism still happened. Qiu (1997) found that in mass rearing, if food was insufficient, cannibalism intensified, and if food was sufficient, cannibalism still could not be completely avoided. In this paper, the developmental times of the 3rd instars and pupae were shorter when they cannibalized eggs and 1st instars than when no cannibalism occurred. and when 3rd instars were allowed to cannibalize eggs and 1st instars, their pupal weights were significantly larger than those of

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3rd instars that had no opportunity to cannibalize. These results indicated that cannibalism favored the growth and development of larvae and pupae. Also cannibalism significantly increased fecundity and percent egg hatch. Thus our findings indicated that cannibalism was positive for the experimental population.

Cannibalism has effects at both the individual and the population level. At the individual level, a cannibal gains food, whereas from the point of view of the victim, cannibalism increases the death rate (Frank & Wilfried 1997). In the study on impeding encounters between and among *M. basalis* instars by folded paper barriers, we found that as the number of barriers increased, the proportion of cannibalized all three instars tended to decrease at first and then to increase, and there were significant differences among the number of folded paper barriers and the proportion of cannibalized instars. This suggested that use of paper barriers can indeed reduce cannibalism to a limited extent. The more barriers provided, the less the amount of space was available per larva, and thus as crowding increased, it became easier for the larvae to meet and cannibalize. Therefore, the increased use of paper barriers caused the rate of cannibalism to increase. We found that the cannibalism proportion of 3rd instars was the largest. It could be that the tendency toward cannibalism of 3rd instars was much greater than in the earlier instars. Thus it may be necessary to mass rear 3rd instars by keeping them individually in separate cells. Perhaps surprisingly the proportion of dead 1st instars was larger than of 2nd instars, which might be due to a lower survival rate of neonates. In an experiment using paper barriers, cannibalism was greater in the first instars of *Chrysopa septempunctata* Wesmael larvae and the survival rate was the lowest for the 1st instars, which indicated that more barriers to encounters were needed for rearing first instars (Zhang et al 2004). This was different from our results.

Our findings may contribute to the mass production of this economically important predatory green lacewing. However, several issues require further study, such as the correlation between larval density and cannibalism as well as between food availability and cannibalism.

Acknowledgments

This study was supported by the Youth Foundation of Guangdong Academy of Sciences (qnjjsq201113), the Special Fund for Agro-scientific Research in the Public Interest (201103026-1) and the Science and Technology Plan Project of Guangdong Province (2011B031500026).

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