



Alternative food sources for the ladybird *Brumoides foudrasii* (Mulsant) (Coleoptera: Coccinellidae)

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Abstract

The predator *Brumoides foudrasii* (Mulsant) (Coleoptera, Coccinellidae) has been naturally found in plants infested by mealybugs. In this study, the striped mealybug *Ferrisia dasyliirii* (Cockerell) (Hemiptera, Pseudococcidae) and *Anagasta kuehniella* Zeller (Lepidoptera, Pyralidae) eggs were evaluated as diets for the development and reproduction of *B. foudrasii*. *Brumoides foudrasii* immatures developed faster when fed with *A. kuehniella* eggs than when fed with *F. dasyliirii*. However, the survival and longevity of *B. foudrasii* adults were not affected by the diets. Oviposition was more frequent when *B. foudrasii* females were fed with *F. dasyliirii* (95%) than those fed with *A. kuehniella* eggs (65%). *Brumoides foudrasii* females fed with *F. dasyliirii* were 2.5 times more fecund than those fed with *A. kuehniella* eggs. Although both diets may be considered proper for *B. foudrasii*, *A. kuehniella* eggs were more suitable for immature development, while *F. dasyliirii* provided more nutritious resources for adult biological parameters as oviposition period, egg viability and fecundity. These results suggests that this predator can play an important role in regulating populations of the striped mealybug *F. dasyliirii* in the field.

Keywords: coccinellids, biological control, essential food, diet, laboratorial rearing.

Fontes de alimento alternativo para a joaninha *Brumoides foudrasii* (Mulsant) (Coleoptera: Coccinellidae)

Resumo

O predador *Brumoides foudrasii* (Mulsant) (Coleoptera, Coccinellidae) tem sido encontrado naturalmente sobre plantas infestadas por cochonilhas. Neste estudo, a cochonilha listrada *Ferrisia dasyliirii* (Cockerell) (Hemiptera, Pseudococcidae) e ovos de *Anagasta kuehniella* Zeller (Lepidoptera, Pyralidae) foram avaliados como dietas para o desenvolvimento e reprodução de *B. foudrasii*. Formas amatuross de *B. foudrasii* desenvolveram-se mais rapidamente quando alimentados com ovos de *A. kuehniella* do que quando alimentados com *F. dasyliirii*. No entanto, a sobrevivência e longevidade de adultos de *B. foudrasii* não foram afetadas pelas dietas. A oviposição foi mais freqüente em *B. foudrasii* alimentadas com *F. dasyliirii* (95%) do que aquelas alimentadas com ovos de *A. kuehniella* (65%). Fêmeas de *B. foudrasii* alimentadas com *F. dasyliirii* foram 2,5 vezes mais fecundas do que aquelas alimentadas com ovos de *A. kuehniella*. Embora ambas as dietas possam ser consideradas apropriadas para *B. foudrasii*, ovos de *A. kuehniella* foram mais adequadas para o desenvolvimento de imaturos, enquanto *F. dasyliirii* proporcionou mais recursos nutritivos para os parâmetros biológicos do adulto, tais como período de oviposição, viabilidade de ovos e fecundidade. Estes resultados sugerem que este predador pode ser potencialmente eficiente na regulação populacional da cochonilha *F. dasyliirii* em campo.

Palavras-chave: coccinélidos, controle biológico, alimento essencial, dieta, criação de laboratório.

1. Introduction

The genus *Brumoides* (Coleoptera, Coccinellidae, Chilocorini) consists of about ten recognized species. *Brumoides*, like other Chilocorini genera, features a wide range of prey including aphids, whiteflies, psyllids, mealybugs,

and mites (Chapin, 1965). They are predominantly found in the old world (Kovář, 1995); however, four species have been reported in the New World: *Brumoides blumi* (Nunenmacher), *Brumoides histrio* (Fall), and *Brumoides*

septentrionis (Weise) are found in North America (Gordon, 1985) while *Brumoides foudrasii* (Mulsant) is found in South America (Giorgi et al., 2014).

The first observations of *B. foudrasii* in Brazil indicate an association of this predator with some species of mealybugs such as *Dactylopius opuntiae* (Cockerell) (Hemiptera, Dactylopiidae) in forage cactus (Lima et al., 2011) and *Ferrisia dasyliirii* (Cockerell) (Hemiptera: Pseudococcidae) in cotton (Giorgi et al., 2014). Recent studies suggest that the latter species can pose a threat to cotton crops in the semiarid region of Brazil (Oliveira et al., 2013).

Giorgi et al. (2014) report *F. dasyliirii* predation by *B. foudrasii*. However in the fields, *Brumoides* species can behave as opportunistic predators feeding on a wide range of prey (Arif et al., 2012), suggesting that this behavior may not clearly indicate the suitability of prey (Evans et al., 1999). According to Dixon (2000), prey used by coccinellids depend on the abundance of prey available in the environment where they live. Since coccinellids feed on a wide range of prey, it is difficult to determine for some species a correct relationship between predator and prey (Hodek, 1973). This behavior is often seen as an emergency strategy when the preferred food is scarce (Castro-Guedes et al., 2016). The amount and quality of prey is very important because influences directly on the biological aspects of Coccinellidae (Dixon, 2000). Various parameters, including developmental time, survival, and female fecundity, have been used to determine the suitability of prey in coccinellids (Matos and Obrycki, 2006). Hagen (1987) and Hodek and Honěk (1996) report that some prey consumed in the field may not be nutritionally appropriate for the development and reproduction of coccinellids. When the ingested food is few or of poor quality, the development time usually increases and the oviposition, fecundity and fertility decrease. Prey species used by Coccinellidae are commonly classified as essential or alternatives (Hodek and Honek, 1996). Food that provides complete development and reproduction of coccinellids is considered essential while that which serves only as an energy source and is able to prolong survival is characterized as alternative (Hodek, 1996).

Coccinellids have been the group of predators most often associated with biological control (Hodek and Honěk, 1996; Jervis and Kidd, 1996). According to Dixon (2000), coccidophagous coccinellids have been the most effective as biological control agents, especially in classical biological control programs. Studies with *Brumoides* species suggest that these coccinellids have potential as biological control agents for mealybug species (Wheeler, 2003; Han et al., 2007; Arif et al., 2012; Chakraborty and Korat, 2013).

The natural occurrence of *B. foudrasii* on cotton plants infested with *F. dasyliirii*, as well as its predatory behavior on this species in the field, suggests that this coccinellid can act as a natural enemy of mealybugs (Giorgi et al., 2014). However, evidence to support this hypothesis requires the confirmation that *B. foudrasii* can use *F. dasyliirii* as the only source of food that allows its development and reproduction.

Lepidopteran eggs, particularly those of the Mediterranean flour moth *Anagasta kuehniella* (Lepidoptera, Pyralidae) have been extensively used as factitious food for rearing Coccinellidae in place of natural prey (Kato et al., 1999; Santos et al., 2009; Silva et al., 2013; Zazycki et al., 2015; Castro-Guedes et al., 2016). Moreover, *A. kuehniella* eggs can be produced easily and at low cost, making this prey propitious for rearing Coccinellidae (Specky et al., 2003; De Clercq et al., 2005; Silva et al., 2009). This study evaluated and compared the food suitability of *F. dasyliirii* and *Anagasta kuehniella* eggs to *B. foudrasii*, which may help to implement rearing methods of this species.

2. Material and Methods

The study was conducted at the Insect Biology and Insect Plant Resistance Laboratory (LBIRPI) at the Universidade Federal Rural de Pernambuco (UFRPE). Insect rearing techniques were adapted from Sanches et al. (2002). Stocks of the mealybug *F. dasyliirii* in LBIRPI were used to prepare test cultures of mealybug; test cultures of the predator *B. foudrasii* were generated from insects collected on cotton crops in the municipality of Surubim in Pernambuco State. Insect rearing and experiments were carried out in a room with the following controlled conditions: 25 ± 1 °C, $70 \pm 10\%$ RH, and 12 hours photoperiod.

Pumpkins of the jacarezinho variety [*Cucurbita moschata* (Duch. ex Poir.)] were acquired from the local food supply center (CEASA) in Recife at the early stage of maturation. Pumpkins were washed, dried at room temperature, and placed in plastic trays ($30 \times 45 \times 4$ cm) lined with paper towel. Actively reproductive mealybug females were released in the stalk region of non-infested pumpkins.

The period from infestation to complete pumpkin colonization with adult mealybugs is approximately 30 days. Subsequent infestations were performed by overlapping non-infested pumpkins on infested pumpkins allowing the passage of nymphs and adults; this condition was maintained for two days. Infested pumpkins were used to feed *B. foudrasii* in mass-rearing conditions.

Brumoides foudrasii adults collected in the field were transferred to the laboratory and confined in plastic cages of $50 \times 30 \times 25$ cm (length \times width \times height) containing rectangular openings (35×20 cm) on the sides, which were sealed with voile to allow air circulation. Pumpkins infested with the mealybug *F. dasyliirii* at different stages of development were placed inside the boxes to feed the coccinellids. Water was offered through moistened cotton balls inside 80 mL plastic containers. Infested pumpkins were continuously provided to maintain food abundance.

Plastic Petri dishes (5.5 cm diameter) lined with filter paper to serve as a substrate for oviposition were used to house adult predator couples. Couples were approximately 48 h old. Twenty couples were housed in twenty Petri dishes which were sealed with plastic film. *Ferrisia dasyliirii* nymphs and adult females were provided in abundance to feed these couples. The filter paper and entire petri

dish were surveyed once a day in a stereomicroscope to count the number of laid eggs. After hatching, a total of 100 larvae were manually transferred to individual Petri dishes lined with filter paper where *F. dasyliirii* nymphs were offered as feed.

Larvae instar change was monitored daily in a stereomicroscope by observing exuviae left after molting. Prey from the previous day was replaced daily with fresh and abundant prey. *Brumoides foudrasii* larvae were fed with *F. dasyliirii* neonate nymphs up until the second instar. After that, they received mealybug adult females as food up until the pre-pupal stage, which was characterized by cessation of movement. The daily observations of immature stages enabled the determination of the duration and viability of these stages.

The experiment of *B. foudrasii* individuals fed with *A. kuehniella* eggs was carried out according to the methodology described above. Petri dishes were lined with filter paper, and *A. kuehniella* eggs were provided in abundance daily. Immature forms were monitored as described before to determine the development and viability from immature stages to adulthood.

Newly emerged *B. foudrasii* adults were sexed using morphological characteristics. Males have six visible sternites while females have only five (Corrêa and Almeida, 2010). Couples were formed and individually placed in Petri dishes (5.5 cm diameter) sealed with plastic film. These couples were divided into two groups of 20 each; one was fed with *F. dasyliirii* while the other was fed with *A. kuehniella* eggs, with both foods were provided in abundance. These dishes were lined with filter paper as the substrate for oviposition.

The filter paper and entire petri dish were surveyed once a day in a stereomicroscope to count the number of laid eggs. These eggs were separated into Petri dishes and identified to evaluate the duration and viability of this phase. The following parameters were determined through these daily observations: pre-oviposition, oviposition, and post-oviposition period, the number of eggs/female, egg viability, and female longevity. These parameters were compared between the groups fed with *F. dasyliirii* and *A. kuehniella* eggs.

The T-test [Proc TEST, Method: pooled (for equality of variances)] (SAS, 2002) was used to analyze the effect of each diet on the developmental time of each *B. foudrasii* immature stage. Viability in the immature

period was evaluated by survival curves estimated using the Kaplan-Meier method and compared using the Log-Rank test (Proc LIFETEST) (SAS, 2002). The diet effect on the biological parameters evaluated in *B. foudrasii* females (longevity, pre-oviposition, oviposition, and post-oviposition period, the number of eggs/female, and egg viability) were analyzed by the T-test [Proc TEST, Method: pooled (for equality of variances)] (SAS, 2002).

3. Results

3.1. Development of *B. foudrasii* immature

The development of *B. foudrasii* from the larval to adult stage was faster when they were fed with *A. kuehniella* eggs compared to those fed with *F. dasyliirii* nymphs and adults ($T_{152} = -17.72$; $P < 0.0001$) (Table 1). With the exception of the third larval instar that did not differ between diets ($T_{152} = -0.99$; $P = 0.32$), all the other immature stages showed longer duration when fed with *F. dasyliirii* ($T_{152} > 3.43$; $P < 0.0008$) (Table 1).

Diet did not influence the survival of *B. foudrasii* immature stages (larvae to adults) ($\chi^2 = 0.65$; $P = 0.4175$), which corresponded to 85% in the group fed with *A. kuehniella* eggs and 81% in the group fed with *F. dasyliirii* (Figure 1); the mortality of immatures was concentrated in the first two larval instars (up to the 10th day after hatching) in both groups.

3.2. Longevity and reproduction of *B. foudrasii* adults

Brumoides foudrasii females showed similar longevity regardless of diet ($t_{38} = -1.47$; $P = 0.15$) (Table 2). The pre-oviposition period was longer in the group fed with *A. kuehniella* eggs compared to that fed with *F. dasyliirii*, while oviposition and post-oviposition periods were longer in the group fed with *F. dasyliirii* compared to that fed with *A. kuehniella* eggs ($t_{30} > 6.33$; $P < 0.0001$) (Table 2).

It is worth noting that 7 out of the 20 *B. foudrasii* females fed with *A. kuehniellai* eggs did not lay eggs (65% of females) compared to only 1 out of 20 females fed with *F. dasyliirii* (95% of females). The first eggs were laid after 16 days of feeding when females were fed with *A. kuehniella* eggs and after 4.5 days of feeding when females were fed with *F. dasyliirii*. The number of eggs per females was approximately 2.5 times higher when the predator was fed with *F. dasyliirii* than in the group fed with *A. kuehniella* eggs ($t_{30} = -2.29$; $P = 0.029$) (Table 2).

Table 1. Developmental periods (days) of *B. foudrasii* when reared on different diets.

	Prey	<i>A. kuehniella</i> eggs (n= 73)	<i>F. dasyliirii</i> (n= 81)
Larva	1 st Instar	4.1 ± 0.08 B	5.5 ± 0.17 A
	2 nd Instar	4.3 ± 0.07 B	5.3 ± 0.14 A
	3 rd Instar	4.2 ± 0.09 A	4.0 ± 0.10 A
	4 th Instar	5.1 ± 0.09 B	5.9 ± 0.12 A
	1 st -4 th Instar	18.0 ± 1.40 A	20.5 ± 0.40 A
Pupa		7.9 ± 0.17 B	11.1 ± 0.152 A
Immature stages		32.3 ± 0.26 B	37.5 ± 0.15 A

Averages followed by same letters in the rows do not differ significantly (*t*-test; $p > 0.05$).

In addition, *B. foudrasii* fed with *F. dasyliirii* provided higher egg viability ($t_{30} = -5.78$; $P < 0.0001$) and shorter embryo development time ($T_{152} = 3.43$; $P = 0.0008$) compared with the group fed with *A. kuehniella* eggs. The sex ratio was 0.52 in individuals fed with *F. dasyliirii* and 0.46 in individuals fed with *A. kuehniella* eggs (Table 2).

4. Discussion

Both tested prey (*A. kuehniella* eggs or *F. dasyliirii*) enabled the development and reproduction of *B. foudrasii*. According to the results obtained in this study, *A. kuehniella* eggs and *F. dasyliirii* are considered essential prey to their predator *B. foudrasii*, following the classification of Hodek and Honek 1996. Similar results were obtained by Lima et al. (2016), using *Zagreus bimaculosus* Mulsant (Coleoptera: Coccinellidae) as predator.

Although considered essential prey, *A. kuehniella* eggs and *F. dasyliirii* mealybugs influenced *B. foudrasii* immatures and adults differently. *Brumoides foudrasii*

immature forms fed with *A. kuehniella* eggs developed faster than those fed with *F. dasyliirii*, however, the diet did not affect the viability of immature forms of *B. foudrasii*. According to Panizzi and Parra (2009), differences in the developmental time of predators may be associated with nutritional differences between preys. Thus, our results suggest that there are nutritional differences between *F. dasyliirii* and *A. kuehniella* eggs, being the latter nutritionally more advantageous to the development of *B. foudrasii*. In addition, several studies have indicated that the initial instars of coccinellids have difficulty feeding on active prey (Majerus, 1994; Phoofolo and Obrycki, 1995; Dixon, 2000; Jalali et al., 2009). This may explain the longer duration of the 1st and 2nd instars of *B. foudrasii* fed with *F. dasyliirii* nymphs (crawlers). It is worth noting that when in contact with new prey, some predators, especially those with pre-oral digestion such as coccinellids, need some time to adapt to their predation behavior (handling the prey) and ingest its content (Cohen, 1998; Grenier and De Clercq, 2003). Thus, despite the fact that the predator *B. foudrasii* was fed with *F. dasyliirii* for a few generations, its performance might improve through the passing of generations.

The viability of immature stages seems to be associated with water content in the food. According to Michaud and Grant (2005), the amount of available water in the food can affect the survival of ladybird larvae. Since it is difficult to evaluate water content in both prey (eggs and mealybug nymphs) they were offered ad libitum, hence, it is possible that predators did not experience water limitation, which would explain the observed similarity in viability rates.

Brumoides foudrasii females showed similar longevity regardless of diet. However, only 65% of females fed with *A. kuehniella* eggs initiated reproduction by laying eggs after 16 days of feeding. A total of 95% of females fed with *F. dasyliirii* laid eggs; the first eggs were laid after 4.5 days of feeding. According to Seagraves (2009), coccinellids need to consume a certain amount of nutrients above the level needed for maintenance, in order to start reproduction.

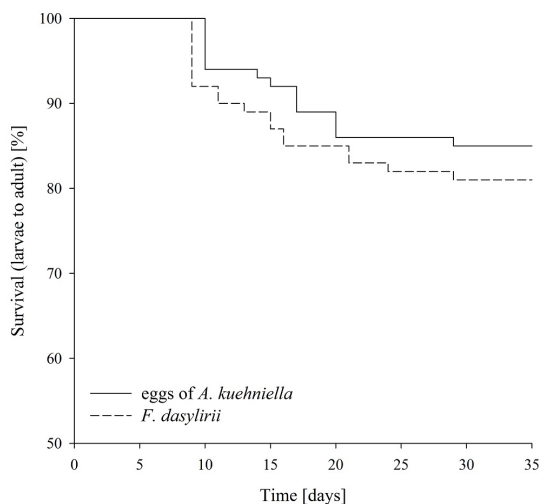


Figure 1. The survival rate of *B. foudrasii* immature stages reared on different diets. Survivorship curves are not significantly different by the Log-Rank test.

Table 2. Reproductive parameters of *B. foudrasii* when reared on different diets.

Prey		<i>A. kuehniella</i>	<i>F. dasyliirii</i>
N ¹		20	20
Longevity		67.2 ± 4.98 A	77.4 ± 3.71 A
n*		13	19
Fertility	Pre-oviposition	16.1 ± 2.32 A	4.5 ± 0.44 B
	Oviposition	31.4 ± 3.23 B	45.6 ± 0.64 A
	Post-oviposition	19.7 ± 4.12 B	27.3 ± 0.22 A
	Eggs/female	26.5 ± 8.07 B	68.0 ± 15.26 A
	Viability of eggs	46.6 ± 3.88 B	83.1 ± 4.28 A
Duration of the egg stage		6.3 ± 0.10 A	5.9 ± 0.06 B
Sex ratio		0.46	0.52

Averages followed by same letters in the rows do not differ significantly (*t*-test; $p > 0.05$); ¹Number of repetitions; *Number of females that had started oviposition.

Our results show that *F. dasyliirii* or *A. kuehniella* eggs were nutritionally adequate for the reproduction of *B. foudrasii*. However, *B. foudrasii* females fed with *F. dasyliirii* were about 2.5 times more fecund than those fed with *A. kuehniella* eggs. Although both diets allowed *B. foudrasii* reproduction, differences in the nutritional content of each prey may explain the higher oviposition rate of females fed with *F. dasyliirii* compared to those fed with *A. kuehniella* eggs. Egg viability of *B. foudrasii* was greater when fed with *F. dasyliirii* (83.1%) than when fed with *A. kuehniella* eggs (46.6%). In natural conditions, *B. foudrasii* can feed on other prey such as other species of mealybugs and aphids, which can maximize the fitness and performance of adults in the field.

Our study provides evidence of divergent nutritional requirements between larvae and adults of *B. foudrasii*. Although *A. kuehniella* eggs were more suitable for immature development, while *F. darsyrii* provided more nutritious resources for adult biological parameters, as oviposition period, egg viability and fecundity (Table 1 and 2). These results are in agree with Michaud (2005), who has reported that prey suitability may differ for larval and adult coccinellids. Still according to him, adult coccinellids have stronger mandibles and more highly developed digestive systems than do larvae and likely process some types of food more efficiently. Moreover, larvae utilize resources for growth and development, whereas adults use them for dispersal and reproduction, and the nutritional demands of these various functions may differ.

The results obtained in this study show that *F. dasyliirii* enabled the development and reproduction of *B. foudrasii*, and thus this mealybug may be included as an essential food for *B. foudrasii*. This suggest that the predator may play a potentially important role regulating mealybug's populations in agroecosystems. Moreover, our study demonstrates the possibility of *B. foudrasii* mass rearing fed with *A. kuehniella* eggs and the use of this alternative prey as a diet for *B. foudrasii* can help reducing the cost of mass production, which represents a major limitation in biological control programs.

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