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Pollen did not provide suitable nutrients for ovary development in a ladybird *Brumoides foudrasii* (Coleoptera: Coccinellidae)

El polen no proporcionó nutrientes adecuados para el desarrollo del ovario en una mariquita *Brumoides foudrasii* (Coleoptera: Coccinellidae)

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ABSTRACT: Coccinellids can exploit a wide variety of foods and other sources of nutrients to supplement their diet. The use of these secondary sources of food, is called an alternative diet. Some species are able to maintain their development and reproduction by feeding exclusively on an alternative diet, while the fecundity of others are compromised. One of the major sources of alternative food exploited by predator coccinellids is pollen. The aim of this research is test the hypothesis that pollen affects the fecundity of the *B. foudrasii*. A group of females were fed with nymphs and adults of *F. dasylirii* mealybug. A second groupe were feed with *F. darsylirii* along with pollen, and a third group was fed only pollen. All female beetles were observed for 10 days. The eggs were counted and the females were dissected, to access oocyte maturation. The results showed that only females fed with mealybugs oviposited. These females had an average of 18.9 mature oocytes. Females fed with the mix of mealybugs and pollen had an average of 17.4 mature oocytes. Females fed exclusively pollen had no mature oocytes. Our results suggest that pollen appeared to exert an inhibitory effect and oviposition behavior.
KEYWORDS: oocyte maturation, supplementary food, oviposition, predatory ladybird, coccidophagous ladybird.

RESUMEN: Los coccinélideos puenden explorar una amplia variedad de alimentos y otras fuentes de nutrientes para complementar su dieta. El uso de estas fuentes secundarias de alimentos, se llama una dieta alternativa. Algunas especies pueden mantener su desarollo y reproducción alimentándose exclusivamente con una dieta alternativa, mientras que la fecundidad de otras se ve comprometida. Una de las principales fuentes de alimentos alternativos explorados por los coccinelídeos depredadores es el polen. El objetivo de esta investigación es probar la hipótesis de que el polen afecta la fecundidade de *Brumoides foudrasii*. Un grupo de hembras fueron alimentadas con ninfas y adultos de la cochinilla *Ferrisia dasylirii*. Un segundo grupo se alimento con *F. dasylirii* junto con polen y un tercer grupo se alimento solo con polen. Se observaron todas las mariquitas hembras por 10 días. Se contaron los huevos y se diseccionaron las hembras para aceder a la maduración de los ovócitos. Los resultados mostraron que solo las hembras alimentadas con cochinillas ovipositaron. Estas hembras tenian un promedio de 18.9 ovocitos maduros. Las hembras alimentadas con la mezcla de cochinillas y polen tuvieron un promedio de 17.4 ovocitos maduros. Nuestros resultados sugieren que el polen ejerce un efecto inhibitorio en el comportamiento de oviposición.
PALABRAS-CLAVE: Alimento suplementar, mariquita depredadora, mariquita coccidofaga, maturación de los ovócitos, oviposición.

INTRODUÇÃO

Coccinellidae are the predators most frequently associated with biological control. Coccinellidae coccidophagous have been the most effective in this group, especially in classical biological control programs (HODEK; HONĚK,1996; JERVIS; KIDD, 1996; DIXON, 2000).

Although coccinellids are predators of phytophagous insects, they can exploit a wide variety of foods (SLOGGETI; MAJERUS. 2000). Coccinellidae may resort to other sources of nutrients to supplement their diet or as the only food source if their prey is absent in the environment (HODEK; HONEK, 1996; GIORGI et al., 2009; ALMEIDA et al., 2011). The use of these secondary sources of food, most often non-essential, is called an alternative diet (SEAGRAVES, 2009).

Some species of Coccinellidae predators are able to maintain their development and reproduction by feeding exclusively on an alternative diet (BEKVENS et al., 2010), while the fecundity of others are compromised with a diet exclusively of plant origin (LUNDGREN; WIEDENMANN, 2004).

One of the major sources of alternative food exploited by predator coccinellids is pollen. Although it is widely used as an alternative or complementary food by a large number of coccinellidae species (GIORGI et al., 2009), only consuming pollen seems to be a limiting factor for their reproductive performance (BEKVENS et al., 2007). Even as an alternative diet, the presence of pollen can increase the reproduction of some species and limit the reproduction of others (MICHAUD, 2000).

Even though pollen plays a key role in the maturation of eggs in some species of bees (CANE, 2016), in Coccinellidae the information about its importance in the diet is based on studies with aphidophagous species (GIORGI et al., 2009). For at least one coccidophagous species, *Cryptolaemus montrouzieri*, the consumption of pollen in addition to the consumption of *Ferrisia virgata* mealybugs compromises the fecundity of these ladybugs (MARQUES et al., 2015).

The maturation of the ovaries occurs through vitellogenesis (CHAPMAN, 1998). The maturation process may occur before the emergence of adults, using nutritional resources obtained in the larval stage, a reproductive strategy called proovigeny (JERVIS et al., 2005). When maturation occurs after emergence, adults still need to obtain the necessary resources for ovarian development, which commonly occurs via feeding (DAVEY, 1997) or via transfer of nutritionally rich seminal fluid during intercourse (POIANI, 2006). In species where the maturation of the ovaries depends on resources acquired in adulthood, the type of diet should influence fertility through ovarian maturation.

Thus, this work aims to assess the hypothesis that pollen affects the fecundity of coccidophagous coccinellids acting on their ovarian development. Thus, we observed the effects of a diet composed of prey and/or pollen on the reproduction and ovarian development of *Brumoides foudrasii* (Mulsant), which has great potential as of biological control agent for several species of scale insects (ARIF et al., 2012; CHAKRABORTY; KORAT, 2013; LIMA et al., 2018).

METHODOLOGICAL PROCEDURE

Specimens of *B. foudrasii* were collected from cotton plants in the municipality of Surubim in Pernambuco and taken to the Laboratory of Insect Biology and Plant Resistance to Insects at the Universidade Federal Rural de Pernambuco. There, they remained under constant conditions of 25 ± 1 °C, 70 ± 10% RH, and 12 hours of photoperiod, which were also the conditions during the experiment. The colony of *B. foudrasii* was maintained using as main food the mealybug *Ferrisia dasylirii*, raised in laboratory kept on Pumpkins of the jacarezinho variety [*Cucurbita moschata* (Duch)]. Duch. ex Poir.], which were acquired from the local food supply center (CEASA) in Recife at the early stage of maturation. Larvae from the last instar of the colony were separated and individualized in plastic petri dishes (8cm × 1.5 cm) covered with leaked plastic film to allow exchange of air. Soon after the emergence (< 24h), individuals were sexed and used in the experiments.

To test the effects of pollen on ovarian reproduction and development, females *B. foudrasii* were individualized on petri dishes and fed *ad libitum* with nymphs and adults of *F. dasylirii* mealybug offered along with a commercial pollen (Dehydrated Organic Bee Pollen Breyer - content: energy - 10 kcal, carbohydrates - 2.5 g, proteins - 1.2 g, and dietary fiber - 0.7 g). A second group of females were fed only pollen, and a third group was fed only nymphs and adults of the mealybug *F. dasylirii*, as a control. Each petri dish containing a *B. foudrasii* female was considered a repetition. Each treatment had 10 repetitions.

All female beetles were observed for 10 days after the pre-oviposition period, which is approximately 4.5 days (LIMA et al., 2018). The eggs were counted daily and separated into a new petri dish and monitored for up to five days after oviposition to observe larvae hatching. After this period, the eggs were considered infertile. Soon after, the females were dissected, and the ovaries were examined under stereomicroscope. Oocytes were considered mature when fully developed and yellowish in color, following the description proposed by OBATA (1988).

RESULTS AND DISCUSSION

Only females fed exclusively with mealybugs oviposited (Table 1), and an average of 13.6 eggs were laid per female. These females had an average of 18.9 mature oocytes (Fig. 1A). The females fed with the mixture of mealybugs and pollen had an average of 17.4 mature oocytes (Fig. 1B). Females fed exclusively pollen had no mature oocytes (Fig. 1C).

In all treatments where pollen was included, no oviposition occurred, suggesting that pollen may inhibit oviposition, however it did not affect ovary development.

The pollen as an alternative food even with the availability of mealybug inhibited oviposition in *B. foudrasii.* The presence of mature oocytes indicates that the nutritive resources required for maturation came exclusively from the consumption of the mealybug The number of mature oocytes was equal to those found in females fed exclusively with *F. dasylirii.* Unlike with *C. montrouzieri* where pollen consumption did not affect the fecundity (MARQUES et al., 2015), pollen as exclusive food resource affected the maturation of oocytes in *B. foudrasii*, and also inhibited the oviposition behavior (Table 1).

Table 1. Number of mature oocytes and eggs laid by the ladybird *B. foudrasii* feeding 10 days on three different diets

Diet	oocytes/female	Eggs/female	mean of oocytes/female	χ^2	*P*
F. dasylirii	189	136	18.9 ± 1,61		
F. dasylirii + pollen	122	-	17.4 ± 0,97	0.4897	0.48
pollen	-	-	-		

Chemical composition of pollen varies among plant species, it can contain different levels of proteins, amino acids, and starch, and to a lesser extent other components, such as lipids, sugars, flavonoids, carotenoids, and minerals (ATROUSE et al., 2004; LUNDGREN, 2009). Nevertheless, pollen is not sufficient to allow multiple species of insect to reproduce itself but only as a source of energy for somatic maintenance (HODEK; HONEK, 1996).

However, polyphagous coccinellidae species can take a favorable balance of important nutrients from various foods including pollen that may contain phytostimulant compounds that could influence food absorption (HODEK; HONEK, 1996). This fact was confirmed by DE CLERCQ et al., (2005), who obtained a higher rate of oviposition and viability of eggs for *Adalia bipunctata* when fed with pollen as a dietary supplement. Other species, such as *Coleomegilla maculata*, complete their entire cycle and reproduce themselves feeding exclusively on pollen (LUNDGREN; WIEDENMANN, 2004).

Pollen from some species of plants may have secondary metabolites (BOPPRÉ et al., 2005) that are toxic to insects, especially for general pollinators (MESQUITA et al., 2010; ASSIS JUNIOR et al., 2011). Intoxication by consuming pollen metabolites can lead to adverse effects on insect, which include the compromising of larval development, as recorded in bee larvae (PRAZ et al., 2008). On the other hand, the deleterious effects caused by pollen can be attributed to the intrinsic indigestibility of pollen. Variations in the ability of insects to digest pollen have been reported among bees (PENG; DOBSON, 1997) and coleopterans (JOHNSON; NICHOLSON, 2001), which may compromise the level of nutrient uptake and consequently affect the development of important tissues, including those of the reproductive system (HUMAN et al., 2007).

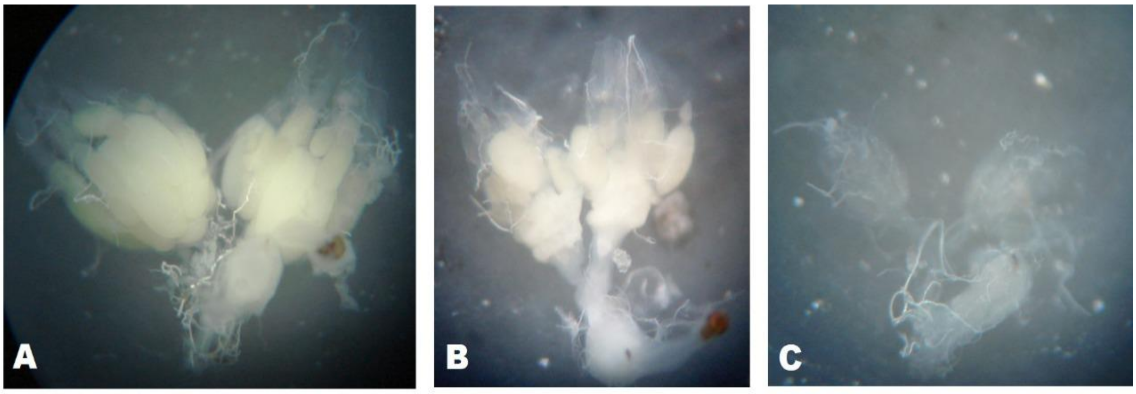

Figure 1. Ovaries of *B. foudrasii* feed on three different diets: A) the mealybug *F. dasylirii* as the only food source; B) mealybug and pollen simultaneously available, and C) only with pollen.

The dynamics of ovarian maturation among coccinellids seems to be quite variable and influenced by physiological and environmental conditions. The phytophagous ladybird beetle *Epilachna niponica* can reabsorb its mature oocytes when, due to population fluctuations of its host plant, food becomes scarce (OHGUSHI, 1996). The predatory ladybird beetle *Harmonia axyridis* synchronizes its ovarian development only with the acceptability of copulation (OBATA, 1988). The phytophagous ladybird beetles, *Coccinella septempunctata* and *C. transversoguttatarichardsoni* Brown also present variations of ovarian maturation according to the availability of food (KAJITA; EVANS, 2009). As the absence of adequate food in sufficient quantities can induce oocyte reabsorption, the nutrients in the pollen offered to *B. foudrasii* may have been nutritionally insufficient to induce ovarian maturation.

In summary, females of *B. foudrasii* that eat exclusively pollen did not present ovarian maturation, and when pollen is given as an alternative food, oocyte maturation is allowed. The number of mature oocytes was inferior to that of females that ate exclusively mealybugs. In addition, pollen appeared to exert an inhibitory effect on oviposition behavior Thus, the use of pollen as an alternative food for *B. foudrasii* was detrimental to its reproduction, either as a single source of food or as a supplement to its natural food, the mealybugs.

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