

# Predation of diamondback moth larvae and pupae by Euborellia annulipes

Gilmar da Silva Nunes<sup>1</sup>, Thais Aparecida Vitoriano Dantas<sup>2</sup>, Wennia Rafaely Souza Figueiredo<sup>2</sup>, Mileny dos Santos de Souza<sup>2</sup>, Izabela Nunes do Nascimento<sup>2</sup>, Jacinto de Luna Batista<sup>2</sup>

- <sup>1</sup> Universidade Estadual Paulista Júlio de Mesquita Filho, Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal, Departamento de Fitossanidade, Jaboticabal, SP, Brasil. E-mail: gilmarsilvanunes@gmail.com (ORCID: 0000-0002-8310-1987)
- <sup>2</sup> Universidade Federal da Paraíba, Campus II, Departamento de Fitotecnia, Areia, PB, Brasil. E-mail: thaisvitorianodantas@gmail.com (ORCID: 0000-0002-6409-1365); wennia\_figueiredo@hotmail.com (ORCID: 0000-0003-0992-6849); mileny.lopes67@gmail.com (ORCID: 0000-0001-6321-6835); izabelaufpb@gmail.com (ORCID: 0000-0003-0425-7236); jacinto@cca.ufpb.br (ORCID: 0000-0001-8860-8085)

**ABSTRACT:** Diamondback moth, *Plutella xylostella*, is considered the most important pest species of brassica crops and *Euborellia annulipes* can exhibit potential for its control. This work recorded the consumption of 4<sup>th</sup>-instar larvae and pupae of *P. xylostella* by nymphs and adult females of the ring-legged earwig *E. annulipes* at different ages: 2, 4, 6, 8 and 10 days old. Nymphs in the 3<sup>rd</sup>-instar and females of *E. annulipes* consumed daily more *P. xylostella* larvae than pupae. The consumption of diamondback moth pupae became constant as the 5<sup>th</sup>-instar nymphs of the predator develop. In the other hand, the consumption of larvae decreases when nymphs are closer to molt. Female, regardless of its age, consumed more larvae than pupae of *P. xylostella*.

**Key words:** brassica; earwigs; integrated management; *Plutella xylostella* 

# Predação de lagartas e pupas da traça-das-crucíferas por Euborellia annulipes

**RESUMO:** A traça-das-crucíferas, *Plutella xylostella*, é considerada a principal praga das brássicas e *Euborellia annulipes* pode ter importante contribuição no seu controle. Este trabalho avaliou o consumo de lagartas de quarto instar e pupas de *P. xylostella* em função de cinco idades de ninfas e fêmeas adultas (2, 4, 6, 8 e 10 dias) da tesourinha *E. annulipes*. Ninfas no terceiro instar e fêmeas da tesourinha consumiram maior quantidade de lagartas que pupas de *P. xylostella*. Na medida em que as ninfas de quinto instar do predador se desenvolveram, o consumo de pupas da traça-das-crucíferas tornou-se constante. Em contrapartida, o consumo de lagartas decresce quando as ninfas do predador se aproximam da muda. As fêmeas, independente da idade, consomem mais lagartas que pupas de *P. xylostella*.

Palavras-chave: brássicas; dermápteros; manejo integrado; Plutella xylostella

# Introduction

The production of brassica (Brassicaceae) has fundamental social, medicinal and economic importance worldwide. Most of these plants have nutraceutical properties such as kale (Brassica oleracea var. acephala), cabbage (Brassica oleracea var. capitata), arugula (Eruca sativa), broccoli (Brassica oleracea var. italica) and cauliflower (Brassica oleracea var. botrytis), used in human nutrition (Carvalho et al., 2013; Gonçalves et al., 2013). They can be also used as raw material in the oil and biodiesel industry (Schmidt & Bancroft, 2010), as it is the case of canola (Brassica napus).

The diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae) is considered the main pest affecting brassica in all producing countries. This moth has high reproductive capacity and rapid selection for resistance to insecticides, resulting in dramatic crop productivity losses and significantly increased control costs (De Bortoli et al., 2011; Zalucki et al., 2012). Biological control is indicated as an emergent and efficient alternative for reducing insect pest infestations. It also works in integrated production and sustainable agriculture, helping to reduce the use of insecticides composed of products that incur toxic effects to non-target organisms such as natural enemies, domestic animals, man and the environment. Thus, predatory insects have been the target of increasing research aimed at the control of pests (Almeida et al., 2009; Brito et al., 2009; Vacari et al. 2012).

The ring-legged earwig Euborellia annulipes (Lucas) (Dermaptera: Anisolabididae) has been reported as predator of larvae and pupae of Anthonomus grandis Boheman (Coleoptera: Curculionidae) (Lemos et al., 1998) and eggs and larvae of Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae) (Silva et al., 2009a). The natural occurrence of ring-legged earwigs in kale plantations intercropped or not with pigeon pea, Cajanus cajan (Fabaceae), and sweet alyssum, Lobularia maritima (Brassicaceae), was cited by Ramos (2015) and Ribeiro & Gontijo (2017). The ring-legged earwig has negative phototropic behavior and therefore a nocturnal habit, being able to find protection in dark places, between leaves and alike, under natural conditions, besides exhibiting high mobility and ability to climb surfaces (Klostermeyer, 1942; Bharadwaj, 1966). Ring-legged earwig can explore plants in search of food resources, as described by Abramson et al. (2007). In this context, E. annulipes may have contact with the different stages of development of the diamondback moth while exploring cabbage, broccoli, chard, cauliflower, and other plants in brassica agroecosystems.

Based on the above, the investigation of the predatory potential of the ring-legged earwig *E. annulipes* on the diamondback moth is relevant for future studies about its use in the strategies to control this pest. The age of the predator is a feature that can provide information about predatory behavior and dynamics as it develops. In addition,

the development phase of the prey may interfere with the consumption rate of the predator. Thus, the predation of *P. xylostella* larvae and pupae by nymphs of different ages and females of *E. annulipes* was determined in laboratory conditions in the present study.

# **Material and Methods**

## Location and conditions of the experiment

The experiment was conducted at the Laboratory of Entomology of Campus II, Federal University of Paraíba (UFPB), Areia - PB, under temperature of  $25 \pm 2$  °C, relative humidity of  $70 \pm 10\%$  and 12-h photophase. Nymphs and females of *E. annnulipes* and fourth instar larvae and pupae of *P. xylostella* were used in the research, both obtained from breeding in laboratory conditions.

#### **Predator production**

The ring-legged earwig *E. annulipes* was produced in laboratory with feed based on the use of artificial diet described by Silva et al. (2009b). Twenty-four hours after hatching, the nymphs were separated into clear plastic containers (13 cm wide x 20 cm long x 7 cm high) containing filter paper moistened with distilled water for shelter and diet. Each vessel had an average density of 40 nymphs on the moistened substrate. Periodic cleanings in the containers and replacement of diet were performed every three days or when needed.

Instar transitions of *E. annulipes* nymphs was observed daily until adults were obtained. The sex was differentiated based on forceps (males have curved left forceps, females have same-size forceps) (Klostermeyer, 1942). Females and males were pooled into a total of 32 insects/container at a ratio of one male to three females. The eggs obtained were separated together with the female on moistened filter paper in Petri dishes (8 cm diameter x 11 cm width).

### **Prey production**

The diamondback moth was obtained in a commercial production of cabbage in the city of Areia - PB and taken to the laboratory. The larvae were grown in transparent plastic containers (25 cm wide x 36 cm long x 12 cm high) closed with voile tissue (50 cm long x 35 cm wide) and fed with kale leaves (*B. oleracea* var . *acephala*) of the Georgia cultivar. The pupae were removed from the leaves with the aid of a brush and packed until emergence in flat bottom glass tubes (8.5 cm height x 2 cm diameter) sealed with hydrophilic cotton.

After emergence, males and females were packed in plastic cages (6.5 cm diameter x 15 cm height) covered in the top with voile tissue and containing kale leaves for oviposition. Leaves with eggs were removed and packed into another type of container (13 cm wide x 20 cm long x 7 cm high) until hatching; fresh leaves were added in the cages

every two days. Adults were fed with a solution of honey (5%) + water (95%) disposed on hydrophilic cotton in the upper part of the cage.

#### **Predation rate**

Predation of *P. xylostella* larvae and pupae by *E. annulipes* was evaluated according to the development of nymphs and females (2, 4, 6, 8 and 10 days of age) as a function of the development stages of the pest, i.e. fourth instar larvae and pupae. Prey consumption until the day of ecdysis was analyzed as a function of instar transition of the predator. Each treatment consisted of 10 nymphs of each instar of the predator (first to fifth instar) and 10 adult females of *E. annulipes*, respectively. The number of preys consumed per day and per age group of the predator and observations of predatory behavior were recorded during the experiment.

#### Data analysis

The statistical design adopted was completely randomized with plots subdivided in time. Data on number of prey consumed by ring-legged earwig nymphs and females per day were submitted to analysis of variance (Proc GLM; SAS Institute, 2015), using a  $(5+1) \times 2$  scheme (five instars + adult females of the predator x two phases of prey), and compared by Tukey and F tests (p  $\leq$  0.05). Data on prey consumption as a function of predator age were submitted to polynomial regression analysis (Proc REG, SAS Institute, 2015). The comparison of the consumption of larvae and pupae by the predator at each independent interval was compared by F test (p  $\leq$  0.05).

# **Results**

# **Daily predation**

The daily consumption of *P. xylostella* by nymphs and females of *E. annulipes* varied according to development stages of the predator (F = 603.5, p < 0.001) and the prey (F = 1103.0, p < 0.001), with significant interaction between the sources of variation (F = 603.5, p < 0.001). *P. xylostella* larvae were consumed in greater number by *E. annulipes* nymphs of third instar and upwards; females consumed the largest number of prey daily (Table 1).

The daily consumption of diamondback moth larvae and pupae by first- and second-instar nymphs did not present statistical difference. Nymphs from the third instar onwards and females predated a larger number of larvae than pupae (Table 1).

### Predation according to age of predator

The interaction of prey and predator development stages with the predator development days was statistically significant (F = 39.7, p < 0.001). No regression model fitted the consumption of *P. xylostella* larvae and pupae and there was no statistical difference between prey stages consumed by *E. annulipes* nymphs during the first instar (Figure 1A).

The consumption of prey pupae increased linearly and the consumption of prey larvae increased as the nymphs developed, resulting in a stabilization of consumption

**Table 1.** Mean number (± SE) of *Plutella xylostella* fourth instar larvae and pupae consumed per day by *Euborellia annulipes* nymphs and females in laboratory.

Phases	Phases of prey <sup>2</sup>	
of predator <sup>1</sup>	Larvae	Pupae
1 <sup>st</sup> instar <sup>ns</sup>	0.6 ± 0.06 e	0.3 ± 0.04 d
2 <sup>nd</sup> instar <sup>ns</sup>	2.5 ± 0.07 e	1.2 ± 0.05 cd
3 <sup>rd</sup> instar**	7.5 ± 0.11 d	1.9 ± 0.07 cd
4 <sup>th</sup> instar**	14.5 ± 021 c	3.2 ± 0.07 c
5 <sup>th</sup> instar**	25.6 ± 0.18 b	7.7 ± 0.14 b
Females**	45.0 ± 0.17 a	11.3 ± 0.11 a
CV (%)	19.08	

<sup>1</sup>Difference of consumption between prey stages analyzed by F test (\*\* p < 0.001; <sup>ns</sup>not significant). <sup>2</sup>Means of prey consumption by predator stages followed by the same lowercase letters in the column do not differ statistically by the Tukey test at 5% probability.

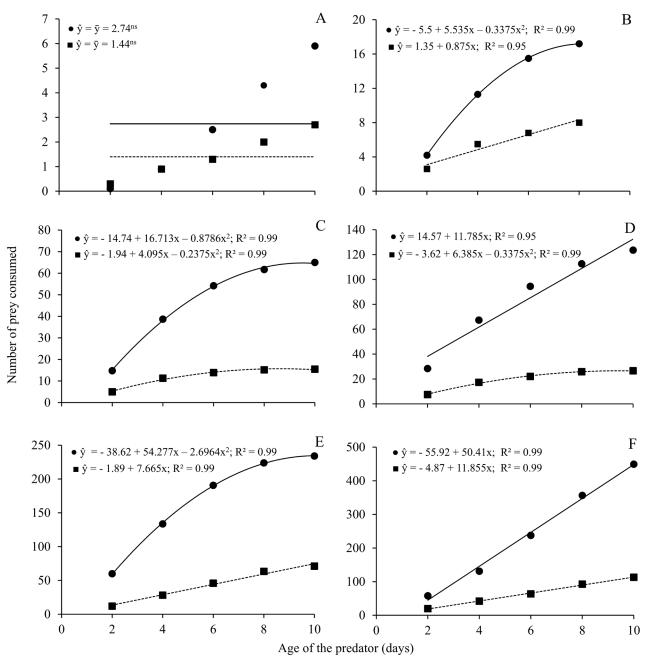
represented by a quadratic response (Figure 1B). The maximum point estimated by derivation of the equation was obtained at eight days of development, corresponding to 17 larvae consumed. Second instar nymphs showed no difference in intake of the different prey stages and the transition to the third instar occurred, on average, at 7.8 days; thus, the curve was not evaluated at 10 days.

During the first two days of age, third instar *E. annulipes* nymphs consumed the same amount of *P. xylostella larvae* and pupae. From this age, i.e. third instar onwards, they began to consume more larvae at all age ranges. The prey consumption response followed a quadratic function from 2 to 10 days of age of the predator at this instar (Figure 1C). The maximum estimated consumption was 64.7 larvae at 9.5 days and 15.7 pupae at 8.6 days. The consumption of larvae by fourth instar nymphs presented a linear response, while the consumption of pupae had a quadratic response (Figure 1D), where the estimated maximum point was obtained at 9.5 days, corresponding to 26.6 pupae consumed. Pupae consumption was statistically lower than that of larvae of *P. xylostella* on any day of development of fourth instar predators.

As for fifth instar nymphs, it was observed that according to their development, the consumption of larvae fitted a quadratic regression model and the consumption of pupae, a linear model. These *E. annulipes* nymphs consumed approximately 234 larvae within 10 days (Figure 1E). Females, too, consumed more larvae than pupae, exhibiting a linear consumption of both prey stages (Figure 1F). Ring-legged earwigs consumed 20 pupae and 58 larvae at the beginning of the adult phase (female), predating 113 pupae and 449 larvae of *P. xylostella* in 10 days of observations.

## **Predatory behavior**

First instar ring-legged earwig nymphs are smaller than *P. xylostella* larvae and pupae, and this influences the attack and consumption. The pupae have a cocoon of silk threads that surrounds them and are more chitinized. Therefore, nymphs at this stage needed to break the cocoon to consume them and at times, they would shelter among the pupae not yet consumed (Figure 2A). On the other hand, the movement of



<sup>ns</sup>Non-significant differences.

Figure 1. Regression curves adjusted for the average number of *Plutella xylostella* fourth instar larvae (→→) and pupae (···■···) consumed by nymphs of first (A), second (B), third (C), fourth (D) and fifth (E) instars and females (F) of *Euborellia annulipes*, according the age of the predator.

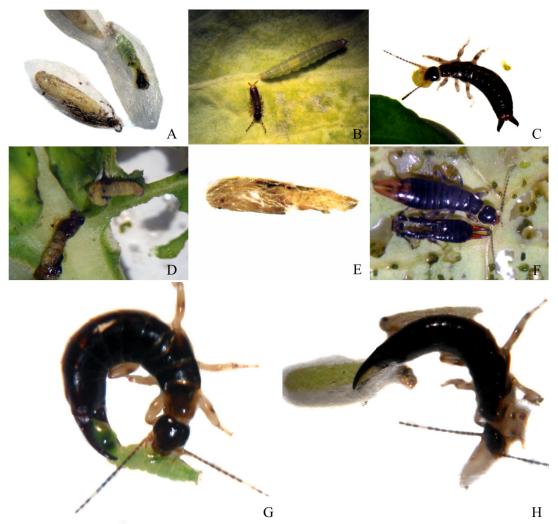
larvae stimulated the removal of the first instar nymphs (Figure 2B). However, even dodging its prey, the aggressiveness of the predators was stimulated and they attacked again, for there was no other type of prey to be consumed.

Fourth instar *E. annulipes* nymphs were more voracious and larger in size than *P. xylostella* larvae; they consumed the prey completely (Figure 2C). During the attack, predator nymphs, especially younger ones, only killed prey or partially consumed their contents, discarding part of the integument (Figure 2D, Figure 2E). The nymphs ceased their predation when they approached instar transition, indicated by the presence of liberated exuviae (Figure 2F).

Females immobilized the larvae with the forceps due to the evasive movement of the prey (Figure 2G). Then they consumed the entire larva or they only killed it when another larva was detected. The use of forceps by the predator has also been evidenced in pupae, when prey move the abdomen (Figure 2H). However, this behavior was practically inexpressive in the observations made.

## **Discussion**

Predation of *E. annulipes* was observed to be more intense on *P. xylostella* larvae than pupae. Increased predation



**Figure 2.** Predation behavior of *Plutella xylostella* fourth instar pupae and larvae by *Euborellia annulipes* nymphs of first (A, B) and fifth (C) instar, prey consumed by third instar nymphs (D, E), exuviae and post-ecdysis nymph-adult (F) and behavior of attack of females on caterpillars (G) and pupae (H) under laboratory conditions.

rate was also observed with the development of instars of the predator (Table 1). The nymphs were more agile and consumed more prey from the third instar onwards. Adult ring-legged earwig individuals showed greater predation than nymphs, of both prey larvae and pupae.

Many authors have verified that predation of first and second instar *E. annulipes* on *S. frugiperda* is higher as ringlegged earwigs develop (Silva et al., 2009a), corroborating the results obtained in the present study with *P. xylostella* larvae and pupae. The stage of *P. xylostella* (egg to pupa) defined the increase in the number of individuals predated by *Nabis kinbergii* Reuter (Hemiptera: Nabidae) according to the predatory stage, i.e. nymph to adult (Ma et al., 2005). Therefore, higher larvae consumption by predators as nymphal development advances is a common result (Vacari et al., 2013; De Jesus et al., 2014).

Nabis kinbergii nymphs only consume fourth instar larvae, and third to fifth instar pupae of the diamondback moth *P. xylostella* (Ma et al., 2005), while *P. nigrispinus* nymphs consume only from the second instar onwards, since in the first instar the nymphs consume only water

(Vacari et al., 2012; Botteon et al., 2016). Thus, the action of *E. annulipes* on diamondback moth is already evident in the first days of life of the nymphs, which probably makes possible a more successful control of the pest by this predator.

The lower predation of diamondback moth pupae by the ring-legged earwig may be related to the protection of the prey or its nutritional aspect, capable of satisfying the predator. It was reported by Eubanks & Denno (2000) that prey movement attracts the predator *Geocoris punctipes* (Say) (Hemiptera: Pentatomidae), influencing its preferential consumption; however, this prey is nutritionally less suitable for the predator. Furthermore, prey age can cause a decrease of consumption by predators, as reported by Silva et al. (2009b) who fed this same species of ring-legged earwig with eggs of *S. frugiperda* of different ages.

Diamondback moth pupae are chitinous and surrounded by silk threads formed during the pupation process (Philips et al., 2014). This is the primary defense mechanisms that hinder access and serve as a form of protection against predator attacks (Sarfraz et al., 2011). Different forms of rupture of the chitinous protection of *P. xylostella* pupae by predators may occur. Prey age may ensure greater or lower flexibility of the integument within the first 24 hours after its formation. The predator's oral apparatus may have greater action on the prey and may vary according to the natural enemy (Morrison et al., 2016). Predator stage is also important, where older nymphs and adult insects may have larger size and predation ability.

First instar *E. annulipes* nymphs, although smaller than the *P. xylostella* pupae, broke the silk threads that enclosed them and sometimes used it as a shelter (Figure 2a). With this they had an easier access to food. Older ring-legged earwig nymphs easily accessed the pupae because of their size. Some prey defense mechanisms may interfere with predation behavior and influence the success of biological development and number of prey consumed by the predator (Tapajós et al., 2016).

Some larvae respond to predator attacks through natural, chemical, behavioral or physiological stimuli, and also by induction, with evasive or defense movements (Grosman et al., 2008; Greeney et al., 2009). Prey movements may also stimulate the attack and influence the predation activity and success (Zanuncio et al., 2008; Silva et al., 2012; Vacari et al., 2012; Santos et al., 2016), as observed in *E. annulipes*. However, other food resources may be present in the field and the predator may consequently show greater selection of food due to its omnivorous nature (Kocarek et al., 2015).

There was a trend of increasing consumption of *P. xylostella* by *E. annulipes* females throughout their days of age. It is worth mentioning that after oviposition, there may be a decrease in predation activity because the energetic cost of females starts to be destined for maternal care, which is the natural behavior of *E. annulipes* females; from this point onwards, they devote much of their attention to eggs and hatched nymphs (Butnariu et al., 2013; Wong et al., 2013).

Due to the lack of information in the literature on the action of predators of *P. xylostella* in Brazil, knowledge about aspects related to the predatory action of *E. annulipes* on this pest is essential for the development of research aimed at biological control strategies associated with the integrated management of *P. xylostella*.

## Conclusion

Euborellia annulipes nymphs consume more P. xylostella larvae than pupae from the third instar onwards, and consumption varies according to their stages and age. Likewise, E. annulipes females consume more P. xylostella larvae than pupae.

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