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Euborellia annulipes Mortality and Predation on *Diatraea saccharalis* Eggs after Application of Chemical and Biological Insecticides

Rodrigo Marin Arroyo[1], Joice Mendonça De Souza[1], Gilmar Da Silva Nunes[2], Dagmara Gomes Ramalho[1], Sergio Antonio De Bortoli[1]

[1]UNESP, São Paulo State University, Jaboticabal, SP, Brazil
[2]Manserv, Porto Nacional, Tocantins, Brazil
Email: dagmarabio@hotmail.com

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Abstract

Chemical and biological insecticides have been frequently used in sugarcane fields to control insects-pests, including the sugarcane borer, *Diatraea saccharalis*. Among the products used, those based on chlorantraniliprole and Metarhizium anisopliae entomopathogenic fungus, stand out. *Euborellia annulipes* is an insect of the order Dermaptera considered a potential predator of sugarcane borer eggs. This study aimed to evaluate the direct and indirect effects of the bioinsecticide based on *M. anisopliae* (Metarril®) and the chemical insecticide chlorantraniliprole (Altacor®) on the mortality of *E. annulipes* nymphs and adults, the predation and feeding preference of earwigs in eggs treated with the formulated products. Predator mortality was evaluated for seven days after treatment, while the effect on predation was analyzed by preference tests with and without choice, using prey eggs. The products tested were selective to the predator, causing ≤ 2% mortality and not affecting predation. The application of *M. anisopliae* on sugarcane borer eggs favored the food preference of fourth-instar nymphs, males, and females of the predator. Our results show that Metarril® and Altacor® can be used to control *D. saccharalis* when associated with the predator *E. annulipes*.

Keywords

Sugarcane Borer, Biological Control, Food Preference, Earwigs

1. Introduction

Biological pest control has become increasingly important in agriculture, mainly due to a growing demand for food free of pesticides and produced by more sustainable agricultural practices, increasing productivity, and reducing environmental impact [1] [2]. This method consists of using natural enemies (entomopathogens, parasitoids and predators) as an alternative to reduce pest population densities, keeping them below the level of economic damage [3].

In Brazil, biological control of sugarcane pests is one of the most important practices, with the larval endoparasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) being the most used against *D. saccharalis*; however, such method is followed by chemical control [4] [5]. Moreover, the use of entomopathogenic fungi to control pest insects has been highlighted as a key strategy for Integrated Pest Management (IPM). This method is usually selective to non-target insects and negatively affects the biological and reproductive characteristics of certain insect species. Furthermore, such a technique is often compatible with others and has low toxicity to humans and other animals [6].

The fungus *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) has been widely used in biological pest control, especially in areas that have adopted IPM [7] [8]. This fungus is characterized by attacking many insect pests and is widely distributed in nature and can be easily found in sugarcane growing areas, where it survives for long periods [9]. *Mahanarva fimbriolata* (Stal) (Hemiptera: Cercopidae) is one of the main targets of this entomopathogen, with studies demonstrating its pathogenicity to eggs and larvae of *Diatraea* spp. [10] [11], *i.e.*, its application can control both pests.

Among the natural enemies of agricultural importance, predators of the order Dermaptera, popularly known as earwigs, have shown great potential for use in IPM [12]. *Euborellia annulipes* (Lucas) (Dermaptera: Anisolabididae), a species with a wide geographic distribution [13], has drawn the attention of many researchers due to its feeding habits (omnivorous), as well as its predatory potential at different stages of life of agriculturally important insect pests, such as eggs and caterpillars of *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae), and caterpillars and pupae of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) [14] [15]. This predator has also been reported in sugarcane plantations and has been associated with predation by the sugarcane borer, *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae) [16] [17] [18].

Associating chemical and biological control tactics may bring positive results since insects can be found at different development stages in the field, increasing the mortality of insect pests. However, in this context, the use of selective insecticides is of great concern, as natural enemies are not targets [19]. Therefore, studies should be carried out to determine the effects of chemical and bioinsecticides on natural enemies, such as the predator *E. annulipes*, which occurs naturally in sugarcane fields and can be used in biological control programs.

Given the above, our objective was to evaluate the direct and indirect effects of the bioinsecticide based on *M. anisopliae* (Metarril®) and the chemical insecticide chlorantraniliprole (Altacor®) on *E. annulipes* mortality, as well as on its predation and food preference for *D. saccharalis* eggs treated with such products.

2. Material and Methods

The experiments were carried out at the Laboratory of Biology and Insect Rearing (LBCI) of the Department of Agricultural Production Sciences, College of Agricultural and Veterinary Sciences (FCAV), São Paulo State University "Júlio de Mesquita Filho" (UNESP), Campus in Jaboticabal, São Paulo State (Brazil). The study was conducted under controlled conditions of temperature (25˚C ± 2˚C), relative humidity (70% ± 10%), and photoperiod (12:12 LD).

2.1. Rearing of the Predator *Euborellia annulipes*

In the bioassays, we used fourth- and fifth-instar nymphs, males, and females of *E. annulipes* from rearing colonies at the LBCI, which were established using individuals from the Federal University of Paraíba, in Areia, Paraíba State (Brazil). Colonies were maintained in circular plastic containers for nymphs (9.0 cm high × 15.0 cm in diameter) and rectangular (13.0 cm × 20.0 cm × 7.0 cm) for adults. Each container held 40 nymphs and 36 adults (sexual ratio 3:1), toilet paper folded into a W shape (refuge substrate) and food substrate, which consisted of an artificial diet that had in its composition (amount for 1000 g): starter ration for chickens (350 g), brewer's yeast (220 g), wheat bran (260 g), powdered milk (130 g), and Methylparaben (40 g) [14]. Diet was supplied in Eppendorf tubes (2 mL).

2.2. Obtaining Prey and Insecticides

Eggs of D. saccharalis were acquired from a biofactory in Jaboticabal, São Paulo State (Brazil). The fungus *Metarhizium anisopliae* (CEPEA ESALQ E9) was obtained from the microbiological insecticide Metarril®, which was supplied by Koppert Biological Systems and kept in a freezer at a temperature of −1˚C ± 2˚C. The chemical insecticide Altacor® WG (chlorantraniliprole) was purchased from a commercial retailer and kept at room temperature.

The products were diluted in sterile deionized water containing 0.01% v/v of Tween® 80 (Polysorbate). The doses used were those recommended by the manufacturers for application in the field (**Table 1**). The fourth- and fifth-instar nymphs, males, and females of *E. annulipes* used in the bioassays were separated and placed in Petri dishes (16 cm in diameter).

2.3. Topical Effect of Insecticides on Earwigs

In this bioassay, 10-μL of each treatment were pipetted onto the back of the predator in the thoracic region. After application, the insects received a standard diet "*ad libitum*," and the Petri dishes (6 cm in diameter) were closed with PVC film and kept under the described environmental conditions. Each treatment consisted of 10 replicates containing five individuals per replicate, totaling 50 individuals per treatment.

Table 1. Treatments and doses of products used in bioassays.

Treatment	Composition	Dose
T1	Distilled water + Tween® 80 (Polysorbate) (0.01% v/v of Tween®)	0.01% of Tween® in 1 L
T2	Altacor® (chlorantraniliprole) – (0.01% v/v de Tween®)	0.3 g/L Altacor®
T3	Metarril® (M. anisopliae) – (0.01% v/v de Tween®)	2.5 g/L Metarril®

T1 = Distilled water + Tween® 80 (Polysorbate); T2 = Altacor® (chlorantraniliprole); T3 = Metarril® (*M. anisopliae*).

Predator mortality was observed daily for seven days, every 24 hours after treatment. Mortality by the fungal-based bioinsecticide was confirmed by verifying mycelial growth and sporulation in dead individuals externally disinfected and kept in a humid chamber.

2.4. Predation Rate under Different Choice Conditions

For the indirect effect, evaluations were performed to check whether applications affected predation. The postures of *D. saccharalis* (prey) were treated by immersion in the product solutions, postures aged up to 72 hours, and offered to predators in two situations: with and without choice. Untreated predators, unfed for 24 hours, were released onto the center of circular arenas (16-cm-diameter Petri dishes) divided into three equal areas, containing a central circle for the release of the predator, being a predator/arena and 300 treated eggs, with 100 eggs from each treatment in the free-choice condition. In no-choice tests, 150 eggs were randomly placed onto similar arenas for each treatment but without divisions. The average number of prey consumed was estimated as an average consumption percentage 12 h after treatment exposure. Ten replications were used for each treatment and condition, with and without choice.

2.5. Food Preference

Consumption of predator stages subjected to free-choice conditions was used to assess food preference. To this end, the preference index of [20] was estimated, using the equation:

$$\beta = \frac{\log\left(\frac{e_j}{A_j}\right)}{\sum_j^3 \log\left(\frac{e_j}{A_j}\right)}$$

wherein: β represents the preference index of the prey; j refers to the number of treatments submitted to the prey; e is the number of prey consumed during the exposure period (12 h), and A represents the number of prey provided the predator. This index produces values between 0 and 1, with values closer to 1 indicating a preference for the prey. This method considers the depletion of prey density due to predation during the experimental evaluation [21].

2.6. Statistical Analysis

The design used was a completely randomized design (CRD), with the results submitted to the Bartlett tests for homoscedasticity (PROC GLM) and Cramer von Mises tests for normality (PROC UNIVARIATE). The data met the ANOVA assumptions, and the means were compared using the Student-Newman-Keuls test ($p < 0.05$). All analyses were conducted using SAS software [22].

3. Results and Discussion

3.1. Topical Effect of Chemical Insecticide and Bioinsecticide on Earwigs

Regarding the mortality of *E. annulipes*, both for the predator phase ($F3.109 = 0.209$; $p = 0.8904$), and for the treatments ($F2.109 = 1.316$; $p = 0.2726$), and the interaction between them ($F5.109 = 1.562$; $p = 0.1769$), showed no significant differences. *E. annulipes* mortality was less than 2% for all treatments. The juvenile stages of earwigs may be more susceptible to chemical insecticides, but this depends on the active ingredient of the product to which they were subjected [23] [24]. The pesticide Chlorantraniliprole has been shown to be more toxic to caterpillars and sucking species [25] and more selective to beneficial insects such as bees and predators [26] [27], mainly to adult insects. As for the susceptibility of the nymphs, it can be explained by the structural modification mode of action caused by the presence of chlorine in the 5th position of the phenyl distribution in the cyano group, which increases susceptibility to several groups of insects [28] indicating that the young phase of the predator is more exposure to this action. According to [12], chlorantraniliprole proved to be one of the least lethal chemical insecticides to *Doru luteipes* (Scudder) (Dermaptera: Forficulidae), not affecting mortality and its behavior, the same occurring in this study with *E. annulipes*.

The earwig *E. annulipes* is reported to be tolerant to synthetic chemical insecticides (lambda-cyhalothrin, chlorfenapyr, and thiamethoxam) compared to other predators, with high survival (>90%), except for the organophosphorus methidathion insecticide, with a mortality of 100% [29] [30]. According to [31], the application of mycoinsecticides also does not cause significant mortality in *E. annulipes*, with a report of the earwig *Forficula auricularia* (L.) (Dermaptera: Forficulidae) presenting chemical defense mechanisms against fungal infections [32], condition this has not yet been studied in *E. annulipes*.

Although laboratory tests can determine insecticide lethal and sublethal effects on earwigs, field studies indicate results much closer to reality [33], which is still very little explored for dermapterans. Thus, the effects of chemicals used in sugarcane (e.g., herbicides, ant killers, among others) on earwigs must be studied to determine their tolerance level [34] [35]. In the case of earwigs, insecticides or bioinsecticides can affect maternal care, which is characteristic of this group of insects [31] [36] [37], which could negatively affect predator development.

3.2. Predation Rate under Different Choice Conditions

In the no-choice test, both treatments had no differences for the number of eggs consumed (F2.108 = 1.57; p = 0.2124), but predator stages did (F3.108 = 8.75; p < 0.0001) (**Table 2**), with females consuming more untreated eggs than the other stages. Borer eggs treated with the chemical insecticide were consumed in greater quantity by fourth- and fifth-instar nymphs and females. Conversely, the consumption of eggs treated with fungus-based biological insecticide showed no differences among predator stages (**Table 2**). In this regard, [15] reported that females are more aggressive and territorial, killing and consuming more prey.

In the free-choice test, significant differences were found between treatments (F2.108 = 4.87; p = 0.009) and among predator stages (F3.108 = 5.57; p = 0.001), but without any interaction between these factors (F6.108 = 1.71; p = 0.145). Fourth-instar nymphs consumed more eggs treated with Metarril®-based bioinsecticide than those treated with the other product, showing superior results to those of the other stages. Fifth-instar nymphs, males, and females consumed the same number of eggs, regardless of the treatment (**Table 3**).

3.3. Food Preference

Regarding food preference, no differences were found between nymphal instars or developmental stages (F3.108 = 0.00; p = 1.00), but with a difference between treatments (F2.108 = 6.19; p < 0.005). No interaction was observed between predator stages and treatments (F6.108 = 1.86; p = 0.095). According to the Manly index, fourth-instar nymphs, males, and females preferred to consume eggs treated with the bioinsecticide (**Figure 1**).

Table 2. Average number (±SE) of *Diatraea saccharalis* eggs contaminated with biological and chemical insecticides consumed by *Euborellia annulipes* within 12 h in a free-choice laboratory test.

Predator	Treatment		
	T1	T2	T3
4th instar	46.90 ± 9.48 aA	64.90 ± 8.83 bA	68.30 ± 6.89 aA
5th instar	93.40 ± 16.88 abA	71.10 ± 16.98 bA	62.10 ± 19.66 aA
Males	44.60 ± 13.42 aA	13.70 ± 9.53 aA	26.30 ± 8.84 aA
Females	116.20 ± 16.32 bA	89.50 ± 11.40 bA	70.00 ± 9.64 aA

Means followed by the same lowercase letter in the column and uppercase on the line does not differ significantly by the Student-Newman-Keuls test (p < 0.001). T1 = Distilled water + Tween® 80 (Polysorbate); T2 = Altacor® (chlorantraniliprole); T3 = Metarril® (*M. anisopliae*).

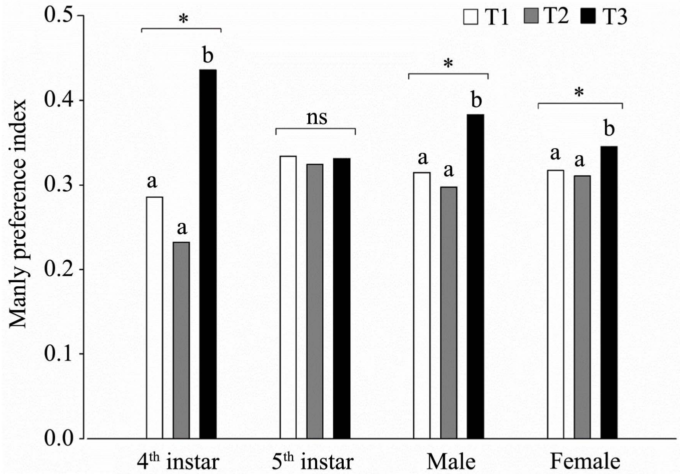

Figure 1. Manly preference index (*β*) ± EP for the developmental stages of *Euborellia annulipes* (4th instar, 5th instar, male, and female) consuming eggs treated with T1 = Distilled water + Tween® 80 (Polysorbate); T2 = Altacor® (chlorantraniliprole) and T3 = Metarril® (*M. anisopliae*), under laboratory conditions over a period of 12 h. Different lowercase letters indicate statistical differences by the Student-Newman-Keuls test ($p > 0.005$).

Table 3. Average number (±SE) of *Diatraea saccharalis* eggs contaminated with biological and chemical insecticides and consumed by *Euborellia annulipes* within 12 h in a free-choice laboratory test.

Predator	Treatment		
	T1	T2	T3
4th instar	26.2 ± 9.01 aA	6.60 ± 6.6 aA	56.40 ± 6.18 bB
5th instar	19.4 ± 9.88 aA	19.30 ± 8.05 bA	18.20 ± 8.53 bA
Males	40.60 ± 12.74 aA	46.70 ± 9.53 bA	48.30 ± 11.84 abA
Females	9.10 ± 16.32 aA	6.10 ± 4.5 aA	35.40 ± 10.40 abA

Means followed by the same lowercase letter in the column and uppercase in the row do not differ significantly by the Student-Newman-Keuls test ($p < 0.001$). T1 = Distilled water + Tween® 80 (Polysorbate); T2 = Altacor® (chlorantraniliprole); T3 = Metarril® (*M. anisopliae*).

Other research has also shown that some synthetic chemical insecticides, such as spinosad, acetamiprid, and chlorpyrifos-ethyl, have no effects on the predation activity of earwigs [38], when the insects are exposed to a dose or concentration that isn't lethal [39]. On the other hand, infection by pathogens is a factor that affects predation in some insects [40] [41], however, according to [42], *E. annulipes* consumes *P. xylostella* caterpillars infected or not with bioinsecticide based on *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae). According to [43], females of *E. annulipes* are less selective than the other stages of their development. The application of the fungus on the eggs can break the physical barrier that is the egg's chorion, which, due to the mode of action of the fungus that initially releases appressoria and initiates an invagination in the egg, may make it more susceptible to the enzymatic action of the fungus [28] [44] and more easily the predator could feed on it, explaining that consumption is higher in eggs contaminated by the fungus and not by chemical insecticide, which has a more effective mode of action against other stages of the insects through sodium channels.

Overall, earwigs are naturally omnivorous and some species mycophagous [45] [46], which may have favored attraction and consumption of prey treated with the *M. anisopliae* based bioinsecticide. In this sense, fungi represent a nutrient-rich diet with several good nutritional attributes for insects, contributing to their biological development [47] [48].

In this study, both chlorantraniliprole (Altacor®) and *M. anisopliae* (Metarril®)-based products were not harmful to *E. annulipes*, with the predation of *D. saccharalis* eggs treated with these products not being affected, and even being stimulated by the bioinsecticide. Our results indicate that Metarril® and Altacor® can be used to reduce *D. saccharalis* populations or to maintain pest populations to a level below economic damage when associated with the predator *E. annulipes*. And the results bring us one main alternative to control the pest that is the biological control, in general, offering fewer risks to the environmental and human health.

4. Conclusions

1) Earwig predation on *Diatraea saccharalis* eggs has no effect from chemical and biological insecticide treatments.

2) The control of *Diatraea saccharalis* eggs can be done with agents and products simultaneously.

3) *Euborellia annulipes* prefers eggs treated with Metarril®.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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