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Exposure to chlorantraniliprole reduces locomotion, respiration, and causes histological changes in the midgut of velvetbean caterpillar *Anticarsia gemmatalis* (Lepidoptera: Noctuidae)

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HIGHLIGHTS

- Effective dominance of resistance of *Anticarsia gemmatalis* to insecticides and bioinsecticides have been reported in Brazil.
- The insecticidal effects of the anthranilic diamide chlorantraniliprole were tested on *A. gemmatalis.*
- Chlorantraniliprole was toxic to *S. frugiperda* at different lethal and sublethal concentrations.
- Chlorantraniliprole exhibited significant activity to altering the locomotor activity and reducing the respiration rate.
- Lethal and sublethal effects of chlorantraniliprole in *A. gemmatalis* can be an alternative to control for this pest.

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ABSTRACT

The anthranilic diamide, chlorantraniliprole is a systemic insecticide affecting ryanodine receptors. This insecticide is used to control caterpillars in soybean crops because it has low toxicity to non-target organisms. The objective was to identify side-effects of chlorantraniliprole on midgut histopathology, respiration and behavior of the velvetbean caterpillar *Anticarsia gemmatalis* in laboratoty. Chlorantraniliprole has $LC_{50} = 0.61$ (0.58–0.64) mg mL^{-1} for *A. gemmatalis* fourth instar caterpillars after 96 h. The insecticide causes severe histopathological effects in the midgut with epithelial disorganization, microvilli degeneration, cytoplasm vacuolization, cell fragmentation, and peritrophic matrix disorganization. The respiratory rate and the walking speed decrease, whereas the resting period increase for caterpillars exposed to this insecticide. Chlorantraniliprole is toxic to *A. gemmatalis* at median lethal concentrations causing severe histological and ultrastructural changes with degeneration of the midgut epithelium, reduction of respiratory rates and inducing an arresting behavioral response of this insect.

1. Introduction

Anticarsia gemmatalis Hübner (Lepidoptera: Noctuidae), is an important soybean pest in the New World (Sosa-Gomes, 2004; Panizzi, 2013). Its caterpillars cause severe defoliation and reduce soybean production (Da Silva-Júnior et al., 2020). In Brazil, successive soybean crops result in vulnerable agricultural systems for the development of *A. gemmatalis* (Sosa-Gomes, 2004) and it is controlled mainly with neurotoxic (as cyclodienes, organophosphates or pyrethroids), inhibitors of chitin biosynthesis insecticides (as benzoylureas), and bio-insecticides based on *Bacillus*

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thuringiensis and *Baculovirus anticarsia* (Haase et al., 2015; Castro et al., 2019). However, the occurrence of resistant populations of *A. gemmatalis* by these pesticides has been reported (Abot et al., 1996; Sosa-Gómez and Miranda, 2012).

New synthetic molecules with different modes of action are necessary to prevent or delay widespread insecticide resistance. Diamides are efficient to pest control, with a new target site ryanodine receptors (Sparks and Nauen, 2015), promoting excessive Ca^{2+} release, muscle paralysis, feeding inhibition, and insect death (Qi et al., 2014; Lahm et al., 2005; Sparks and Nauen, 2015). Thus, the mode of action of chlorantraniliprole [3-bromo-4′-chloro-1-(3-chloro-2-pyridyl-2′-methyl-6′-methylcarbamoyl) pyrazole-5-carboxanilide] (Lahm et al., 2005) differs of others neurotoxic insecticides (Lahm et al., 2009), without risks to human health, since the structure of the ryanodine receptors differs between insects and mammals (Malhat et al., 2012). This insecticide is used in different crops with high efficiency against insect pests (Liu et al., 2017; Plata-Rueda et al., 2019a; Agrofit, 2018). Chlorantraniliprole has low toxicity to natural enemies of pests and soil invertebrates (Lavtizar et al., 2016; Whalen et al., 2016). Also, this insecticide is used in Brazilian soybean crops to control *Chrysodeixis includens* Walker, *Helicoverpa armigera* Hübner, *Heliothis virescens* Fabricius, *Pseudoplusia includens* Walker, *Spodoptera eridania* Cramer, *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae), *Elasmopalpus lignosellus* Zeller, *Hedylepta indicata* Fabricius (Lepidoptera: Pyralidae), and *Phyllophaga cuyabana* Moser (Coleoptera: Scarabaeidae) (Agrofit, 2018).

Although chlorantraniliprole acts on the central nervous system and muscles, some side effects have been reported in other insect organs such as digestive tract (Hu et al., 2019), ovary (Liu et al., 2012), and fat body (Huang et al., 2016). In this sense, the digestive tract is one of the first contact organs when the food contaminated by insecticides is ingested (Fiaz et al., 2018a; Martínez et al., 2018; Arthidoro de Castro et al., 2020). In insects, the digestive tract is anatomically differentiated into three main regions: the foregut and hindgut (from the ectodermal origin) and midgut (from the endodermal origin) (Chapman, 2013).

Chlorantraniliprole affects the development and reproduction of insects and biochemical mechanism processes have been studied (Cao et al., 2017; Liu et al., 2018; Wu et al., 2018), but its side-effects on the midgut, behavior, and respiration are poorly studied.

In this study, we evaluated the toxicity and sublethal effects of chlorantraniliprole on the locomotion, respiration, and midgut histopathology of *A. gemmatalis* caterpillars.

2. Materials and methods

2.1. Insects

Anticarsia gemmatalis caterpillars in the fourth instar were obtained from the mass rearing of the Insect Biological Control Laboratory of the 'Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO)' of the 'Universidade Federal de Viçosa' in Viçosa, Minas Gerais state, Brazil. These caterpillars were fed on an artificial diet (Greene et al., 1976) and kept at 25 ± 2 °C, 75 ± 5% relative humidity and 12:12 h (light:dark) photoperiod. *A. gemmatalis* caterpillars without amputations or apparent malformations were caged in polystyrene pot (1590 cm^3) for bioassays.

2.2. Toxicity test

The insecticide chlorantraniliprole (Premio® OD, Dupont, Alphaville, Brazil) 200 g L^{-1} was used from an aqueous stock solution with 100 mg mL^{-1} to obtain the tested concentrations. Six concentrations of chlorantraniliprole (0.04, 0.09, 0.19, 0.30, 0.78, and 1.56 mg mL^{-1}) were used to determine the lethal concentrations in laboratory. The deionized ultrapure water was used as control. Chlorantraniliprole (0.5 μL) each concentration was mixed on 1 g of an artificial diet. *Anticarsia gemmatalis* caterpillars were individualized in Petri dishes (90 mm dia × 1.5 mm hight) and fed on artificial diet with different insecticide concentrations *ad libitum*. Three replicates of 50 (n = 150) caterpillars were used for each concentration tested following a completely randomized design and the number of dead caterpillars was counted for 96 h.

2.3. Locomotion test

Anticarsia gemmatalis caterpillars were individualized in open Petri dishes (90 × 1.5 mm) with a filter paper Whatman N° 1 covering the bottom. The inner walls of the plates were covered with Teflon® polytetrafluoroethylene (PTFE) (Dupont, Wilmington, DE, USA) to prevent insect escape. Behavioral bioassays were performed using the paper filter as arenas with half of their surface treated with chlorantraniliprole and distilled water as a control. A solution of 250 μL of estimated lethal concentrations LC_{50} and LC_{90} of chlorantraniliprole were sprayed on the half-arena and one caterpillar released in the center of the arena. Twenty caterpillars were used per lethal concentration and control group in a completely randomized design. The movement of each caterpillar in the arena was recorded for 10 min with a Canon® NTSC (XL1 3CCD, Canon USA, Lake Success, NY, USA) video camera, equipped with a 16 × video lens (5.5–88 mm XL zoom lens) and the images were digitally submitted to computerized analysis using a video tracking system (ViewPoint LifeSciences, Montreal, Quebec, Canada). The measurements evaluated were walked distance and the resting time in each half-arena following a method adapted from previous studies (Plata-Rueda et al., 2020a, 2020b).

2.4. Respiration rate

Respiration rate of *A. gemmatalis* caterpillars was conducted by 3 h after ingestion of LC_{50} and LC_{90} of chlorantraniliprole and water as a control. The carbon dioxide (CO_2) production was measured using a TR3C CO_2 analyzer (Sable System International, Las Vegas, NE, USA) with a methodology adapted from previous studies (Fiaz et al., 2019; Plata-Rueda et al., 2019b). One *A. gemmatalis* caterpillar was placed in a respirometer tube (25 mL). CO_2 produced by caterpillar in each respirometer tube was quantified with compressed oxygen gas (99.99% pure) passing through the respirometer at a flow of 100 mL min^{-1} for 2 min. Ten replicates (chambers with

Table 1
Lethal concentrations of chlorantraniliprole against *Anticarsia gemmatalis* caterpillars after 96 h exposure obtained from probit analysis (df = 5, Slope ± SE = 2.246 ± 0.43, intercept = 3.672).

Number Insects	Lethal concentrations	Estimated concentration (mg mL^{-1})	95% Confidence Interval (mg mL^{-1})	χ^2 (*P*–value)
150	LC_{25}	0.526	0.484–0.558	5.69 (0.33)
150	LC_{50}	0.616	0.588–0.643	
150	LC_{75}	0.707	0.679–0.740	
150	LC_{90}	0.797	0.760–0.848	

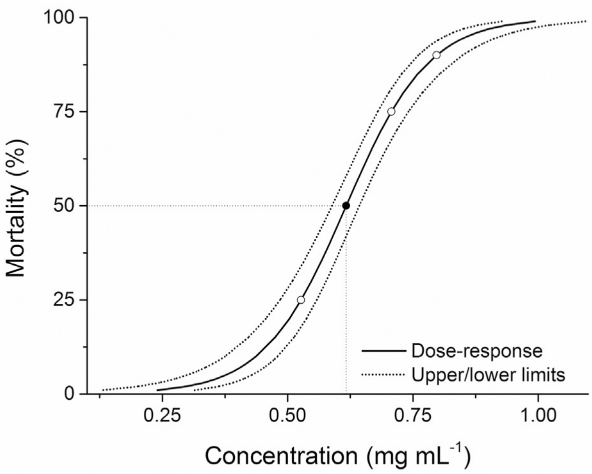

Fig. 1. Toxic effects of chlorantraniliprole on *Anticarsia gemmatalis* caterpillars. Mortality caused by chlorantraniliprole at lethal concentrations (LC_{25}, LC_{50}, LC_{75}, and LC_{90}) (χ^2. = 5.69; df = 5; $P > 0.05$). Dotted lines represent 95% confidence intervals. The black dot represents the concentration (LC_{50}) selected to evaluate histopathological and cytotoxic effects.

one caterpillar) were used per treatment.

2.5. Histopathology

Twenty *A. gemmatalis* caterpillars were fed on LC_{50} chlorantraniliprole and evaluated after 3, 4, 8, 16, and 32 h. Caterpillars were cryoanesthetized at −4 °C, the midgut was dissected in insect saline solution (0.1 M NaCl + 0.2 M KH_2PO_4 + 0.2 M Na_2HPO_4) and transferred to Zamboni's fixative solution (Stefanini et al., 1967) for 12 h at 5 °C. Samples were dehydrated in ethanol series (70, 80, 90, and 95%), embedded in Leica historesin (Leica Biosystem Nussloch GmbH, Wetzlar, Germany), and sectioned at 3 μm thickness with a Leica RM2255 microtome. Sections were stained with hematoxylin and eosin and analyzed under Olympus BX-60 light microscope (Olympus Corporation, Tokyo, Japan).

2.6. Cytotoxicity

Ten *A. gemmatalis* caterpillars were exposed to LC_{50} of chlorantraniliprole via ingestion for 32 h. Caterpillars were cryoanesthetized at −4 °C, the midguts were dissected in insect saline solution, and transferred to 2.5% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) with 0.2 M sucrose for 4 h at room temperature. Samples were post-fixed in 1% osmium tetroxide in the same buffer for 2 h, washed in buffer, dehydrated in a graded ethanol series (70, 80, 90, and 99%), and embedded in LR White resin (London Resin Company Ltd.). Ultrathin sections (80–90 nm thick) were obtained with glass knives a Power Tome-X ultramicrotome (Boeckeler Instruments, Tucson, AZ, USA), stained with 1% aqueous uranyl acetate and lead citrate (Reynolds, 1963), and examined under Zeiss Libra 120 transmission electron microscope (Carl Zeiss, Jena, Germany).

2.7. Statistical analysis

Lethal concentrations (LC_{25}, LC_{50}, LC_{75}, and LC_{90}) and confidence limits were determined by Probit analysis based on dose-response relationship (Finney, 1964) with the PROC PROBIT procedure of SAS v. 9.0 for Windows (SAS Institute, Campus Drive Cary, NC, USA). For locomotion test, the walked distance (cm) and resting time (s) data were subjected to one-way ANOVA with treatment as a fixed effect and Tukey's honest significance difference HSD test ($P < 0.05$) was used a mean separation test. Data on locomotion test were arcsine–transformed to satisfy the premises of normality and

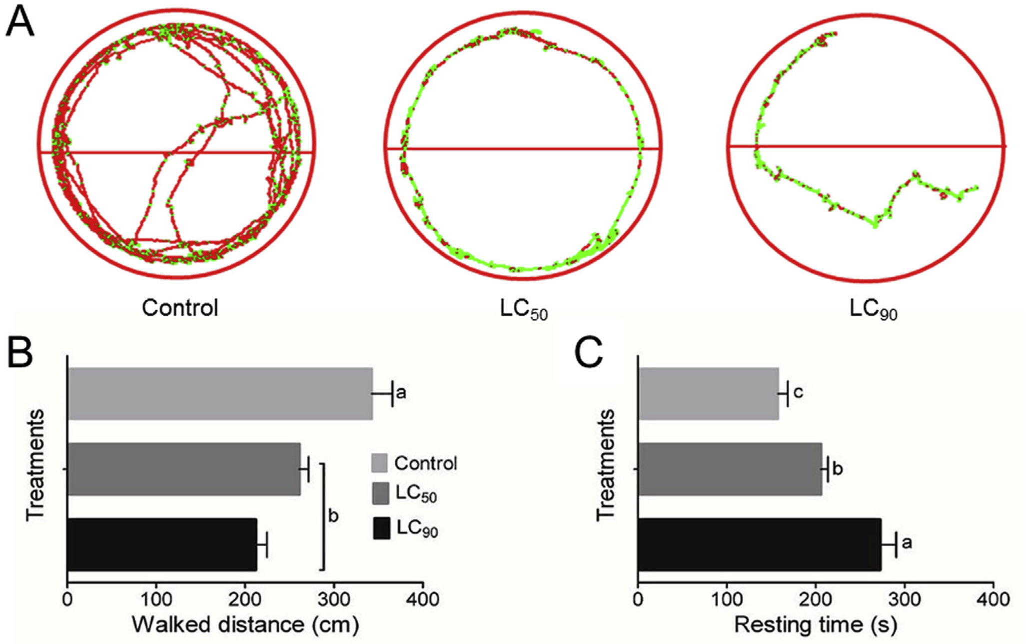

Fig. 2. Locomotor activity of *Anticarsia gemmatalis* caterpillars exposure to chlorantraniliprole. (A) Representative tracks showing the walking activity of caterpillars over a 10-min period on paper-filter arenas half impregnated with chlorantraniliprole (upper half of each arena). Red tracks indicate high walking velocity; green tracks indicate low (initial) velocity. (B) Distance walked and (C) resting time (mean ± SEM) for insects exposed to treatments (control, LC_{50} and LC_{90} estimated values) for 10 min. Different letters show differences among treatments at $P < 0.05$ (Tukey's mean separation test). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

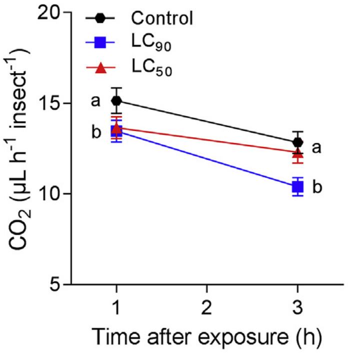

Fig. 3. Respiration rate (mean ± SEM) of *Antacarsia gemmatalis* caterpillars exposed to chlorantraniliprole (control, LC_{50} and LC_{90} estimated values) for 3 h. Different letters show differences among treatments at $P < 0.05$ (Tukey's mean separation test).

homoscedasticity. Respiration rate (volume measured in μL CO_2 h^{-1} $insect^{-1}$) data were subjected to two-way ANOVA with time, treatment, and the interaction as fixed effects and Tukey's HSD test ($P < 0.05$) was used a mean separation test. For one and two-way ANOVAs, statistical analyses were performed using PROC GLM with SAS for Windows v. 9.0.

3. Results

3.1. Toxicity test

The dose-response model used had good fit ($P > 0.05$) and four lethal concentrations of chlorantraniliprole were estimated by Probit analysis with $LC_{50} = 0.61$ (0.58–0.64) mg mL^{-1} confirming the toxicity of this insecticide to *A. gemmatalis* (Table 1; Fig. 1). The mortality in the control was <0.93%.

3.2. Locomotion test

Representative tracks of *A. gemmatalis* caterpillar movement in half-arenas treated showed lower walking speed for those exposed to the insecticide (Fig. 2A). The walked distance by the caterpillars was higher in the control than in half-arenas treated with chlorantraniliprole LC_{50} and LC_{90} ($F_{2,19} = 16.76$; $P < 0.05$) (Fig. 2B). Caterpillars had longer resting periods when exposed to the LC_{90} than to the LC_{50} and in the control ($F_{2,19} = 33.57$; $P < 0.05$; Fig. 2C).

3.3. Respiration rate

The respiration rate of *A. gemmatalis* was influenced by exposure to chlorantraniliprole at the LC_{50} and LC_{90}. Respiration rate of *A. gemmatalis* differed between control group (15.14 μL CO_2 h^{-1}), LC_{50} (13.65 μL CO_2 h^{-1}), and LC_{90} (13.47 μL CO_2 h^{-1}) 1 h after exposure, but after 3 h, respiration rate decreased to 12.84 μL CO_2 h^{-1} in the control group, followed by LC_{50} with 12.31 μL CO_2 h^{-1}, and LC_{90} with 10.41 μL CO_2 h^{-1} (Fig. 3). There was a significant effect of treatments ($F_{2,54} = 8.27$; $P < 0.001$), time ($F_{1,54} = 29.3$; $P < 0.001$), and not differed between interaction treatments × time ($F_{2,54} = 1.43$; $P = 0.246$) (two-way ANOVA, $P < 0.05$, Table S1).

3.4. Histopathology

The midgut epithelium of *A. gemmatalis* caterpillars not exposed to the insecticide had columnar and goblet cells with cytoplasm with few vacuoles, well-developed brush border, and evident peritrophic matrix in the lumen. The nucleus was elongated, occupying the medial-basal portion of the cells, with the predominance of decondensed chromatin (Fig. 4A). Histological changes after insecticide ingestion in the midgut of *A. gemmatalis* caterpillar included apocrine secretion 2 h after exposure to chlorantraniliprole (Fig. 4B). The epithelium was irregular with the onset of cell degeneration and release of cell fragments into the lumen after 8 h of the insecticide ingestion (Fig. 4D). Vacuolization in the cytoplasm (Fig. 4B–F) and peritrophic matrix disorganization (Fig. 4E and F) increased according to the time of feeding on chlorantraniliprole. Fragments of cell some with pyknotic nucleus were released into the midgut lumen with a progressive increase during 4–32 h of exposure (Fig. 4D–F).

3.5. Cytotoxicity

The ultrastructure of the *A. gemmatalis* caterpillar midgut cells in the control was characterized by digestive cells with well-developed apical microvilli cytoplasm with some vacuoles, mitochondria and rough endoplasmic reticulum (Fig. 5A), the nucleus with decondensed chromatin and well-developed basal labyrinth (Fig. 5B). The digestive cells in the caterpillars exposed to the insecticide showed apical protrusions to the midgut lumen with disorganized microvilli (Fig. 5C) and cytoplasm with autolysosomes and damaged mitochondria (Fig. 5C and D). Some cells showed a rupture of the plasma membrane in a necrotic pathway (Fig. 5E).

The Goblet cells of the control insects showed the cavity rich in well-organized microvilli with mitochondria inside them and basal nucleus with some clumps of condensed chromatin (Fig. 6A and B).

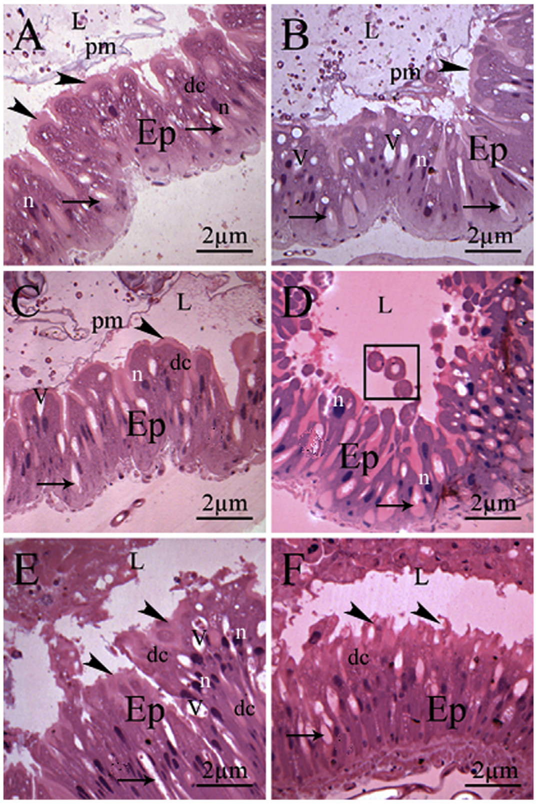

Fig. 4. Light micrographs of the midgut of *Anticarsia gemmatalis* caterpillars. (A) Epithelium (ep) with digestive cells (dc), nuclei with decondensed chromatin (n), brush border (arrowheads), goblet cells (arrows) and preserved peritrophic matrix (pm) of caterpillars non-exposed to chlorantraniliprole. (B–F) Insects exposed to chlorantraniliprole after 2 h (B), 4 h (C), 8 h (D), 16 h (E), and 32 h (F) showing epithelium (Ep) with digestive (dc) and goblet cells (arrows) with vacuoles (V) nuclei with condensed chromatin (n), disorganized brush border (arrowheads), and cell fragments (square) released to the lumen (L).

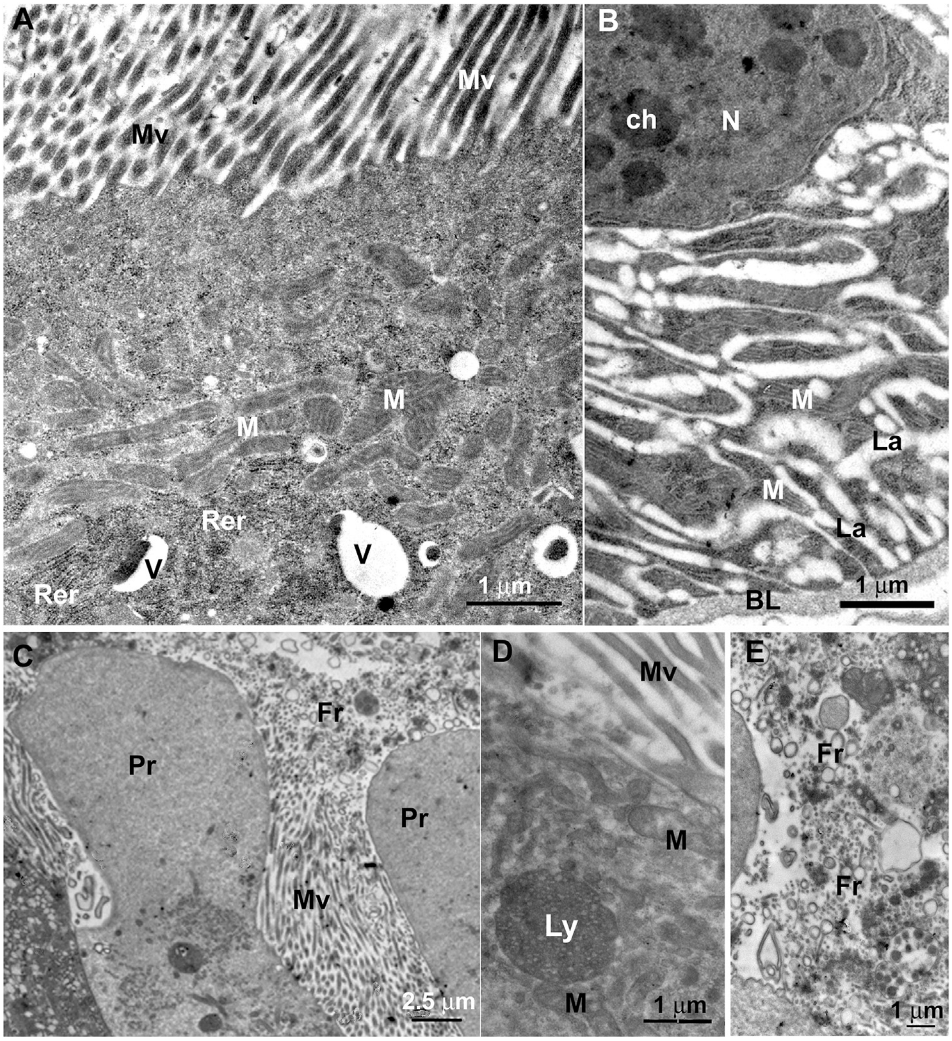

Fig. 5. Transmission electron micrographs of the digestive cells in the midgut of *Anticarsia gemmatalis* caterpillars. (A) Apical region showing well-developed microvilli (Mv), mitochondria (M), rough endoplasmic reticulum (Rer) and vacuoles (V) of caterpillars non-exposed to chlorantraniliprole. (B) Basal region showing enlarged labyrinth (La) associated with mitochondria (M), and nucleus (N) with few clumps of condendesed chromatin (Ch) of caterpillars non-exposed to chlorantraniliprole. (C) Apical region showing protrusions (Pr) with disorganized microvilli (Mv) and cell fragments (Fr) in the lumen of caterpillars exposed to chlorantraniliprole. (D) Detail of apical protrusion with autolysosome (Ly) and damaged mitochondria (M). (E) Detail of cell fragments (Fr) of a necrotic cell released in the lumen (L).

Also, the goblet cells showed disorganization in the microvilli, cytoplasm rich in large vacuoles with membranous content (Fig. 6C) and autolysosomes (Fig. 6D) in the inscts exposed to the insecticide.

4. Discussion

The chlorantraniliprole is toxic to *A. gemmatalis* affecting survivor, locomotion, respiration, and the midgut epithelium. Chlorantraniliprole is toxic to caterpillars of this insect and had a strong effect by ingestion ($LC_{50} = 0.61$ mg mL^{-1}) and cause mortality in a dose-dependent manner, as reported for other Lepidoptera pest (Sial and Brunner, 2012). The high toxicity and rapid action on *A. gemmatalis* caterpillars caused by chlorantraniliprole are similar to those reported in *Agrotis ipsilon* Hufnagel, *Helicoverpa armigera* Hübner and *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) (Liu et al., 2017). On the other hand, the parasitoid wasps and predators that are natural enemies of Lepidoptera caterpillars have low susceptibility to chlorantraniliprole (Brugger et al., 2010; Liu et al., 2016). This reinforces the importance of this insecticide to Lepidoptera pest control with a low impact on non-target insects (Sparks and Nauen, 2015; Lahm et al., 2009; Qi et al., 2014). Overall, the results showed that small volumes of chlorantraniliprole were sufficient to cause toxicity in *A. gemmatalis*.

The behavioral response assay indicates that chlorantraniliprole promotes an arresting effect on *A. gemmatalis*. Changes in the behavioral pattern are due to the action of toxic compounds that reduce or stimulate insect mobility (Plata-Rueda et al., 2020a, 2020b). Arrested effect of chlorantraniliprole on *A. gemmatalis* behavior is a common feature of the insect locomotion reduction after exposure with the insecticide (Hannig et al., 2009). Antranilic diamides, the insecticidal class of chlorantraniliprole, have the insect muscle as target organ, stimulating release of intracellular calcium resulting in depletion of calcium stored in the sarcoplasmic reticulum, promoting muscle paralysis, cessation of feeding, lethargy, and death (Hannig et al., 2009; Plata-Rueda et al., 2019a). In *A. gemmatalis*, caterpillars exposed to chlorantraniliprole gradually reduce the distance walked and resting time on the arenas sprayed with the insecticide, since this insecticide also has contact action (Thorne et al., 2015; Van Herk et al., 2015).

Chlorantraniliprole negatively affects the respiration rate of *A. gemmatalis* 3 h after exposure, indicating physiological stress.

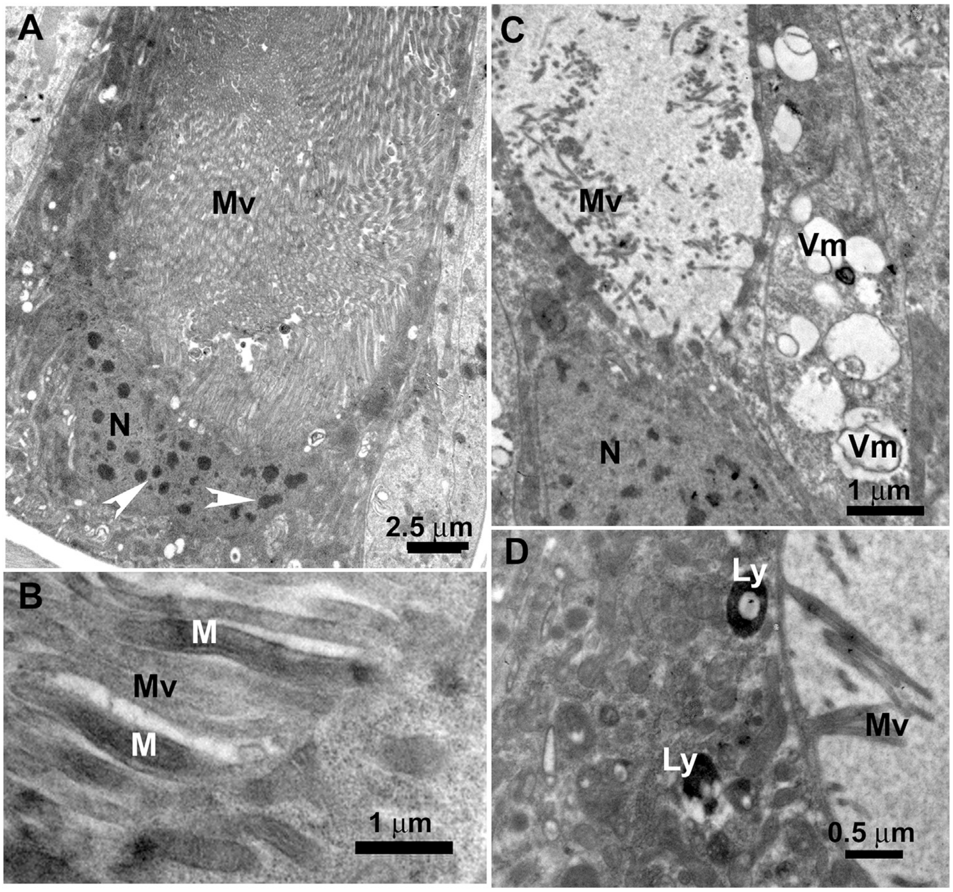

Fig. 6. Transmission electron micrographs of the goblet cells in the midgut of *Anticarsia gemmatalis* caterpillars. (A) Cavity with well-developed microvilli (Mv) and nucleus (N) with some clumps of condensed chromatin (white arrowheads) of caterpillars non-exposed to chlorantraniliprole. (B) Detail of microvilli (Mv) with mitochondria (M) of caterpillars non-exposed to chlorantraniliprole. C) Disorganization of microvilli (Mv) in the cell cavity and cytoplasm rich in vacuoles with membranous content (Vm) of caterpillars exposed to chlorantraniliprole. D) Cytoplasm with autolysosomes (Ly) of caterpillars exposed to chlorantraniliprole.

Similar results have been reported in *A. gemmatalis* exposed to tebufenozide (Fiaz et al., 2018b). The respiration of insects is influenced by the energy demands of the metabolic functions that are necessary to produce defense mechanisms against toxic compounds (Fiaz et al., 2018a; Plata-Rueda et al., 2018; Brügger et al., 2019). Low respiration rates result in a high fitness cost, as resources and energy must be reallocated at the expense of physiological processes (Fiaz et al., 2018b; Plata-Rueda et al., 2019a) and can also impair muscle activity, leading to permanent paralysis (Brügger et al., 2019; Plata-Rueda et al., 2019b). This study reveals that *A. gemmatalis* caterpillars exposed to chlorantraniliprole have decrease in the respiration rates, suggesting a possible fitness costs and energy reallocation.

Cell degeneration of the midgut epithelium with cell fragments released to the lumen, apical protrusions with damaged organelles, increased cytoplasmic vacuolization and peritrophic matrix and microvilli disorganization in the digestive cells of *A. gemmatalis*, reveal side-effect of chlorantraniliprole in this non-target organ. The apical protrusions followed by elimination of cell fragments into the midgut lumen have claimed to be a physiological response in insects (Carneiro et al., 2020; Plata-Rueda et al., 2020b) which may be involved in the apocrine secretion of digestive enzymes (Cristofoletti et al., 2001; Martínez et al., 2019; Santos-Junior et al., 2020), renewal of cytoplasm content (Cruz-Landim et al., 1996) or cell death (Tettamanti et al., 2007; Azevedo et al., 2009; Santos et al., 2015). Our study suggests that those features are cell responses to the toxic effect since they are found only in caterpillars exposed to chlorantraniliprole, which may reduce the digestion efficiency in this pest. A decrease in the digestive capacity caused by insecticides has been reported for *H. armigera* (Barbeta et al., 2008). Vacuoles in the digestive cells are commonly found in insects (Carneiro et al., 2020; Santos-Junior et al., 2020), but the increase in their number and size in the cytoplasm has been associated with response to the elimination of waste, toxins, and recycling of cellular components (Levy et al., 2008; Gomes et al., 2013; Fiaz et al., 2018a; Cossolin et al., 2019; Plata-Rueda et al., 2020b).

Damages in the goblet cells of *A. gemmatalis* have been previously reported for caterpillars exposed to squamocin (Fiaz et al., 2018a), tebufenozide (Fiaz et al., 2018b), and *Bacillus thuringiensis* (Castro et al., 2019), affecting nutrient absorption since these cells are responsible by ionic homeostasis in the midgut of Lepidoptera (Terra and Ferreira, 2020).

The disorganization of the peritrophic matrix of the midgut of caterpillars exposed to chlorantraniliprole also compromises nutrient absorption and exposes epithelial cells to mechanical damage caused by the food bolus. This matrix plays roles as a barrier against toxic products, delaying contact with digestive cells (Wu et al., 2016), but chlorantraniliprole may pass through the peritrophic matrix reaching *A. gemmatalis* midgut epithelial cells such as revealed by histopathology and cytotoxicity.

This study shows that chlorantraniliprole is toxic when ingested by *A. gemmatalis* caterpillars with high mortality, affecting the locomotion behavior, respiration, and causes severe tissue and cell damages in midgut indicating side-effects which may be sufficient to prevent the development and survival of this pest.

Author contribution statement

Bárbara Monteiro de Castro e Castro, Angelica Plata Rueda, Luis Carlos Martinez and José Eduardo Serrão: Conceptualization, Investigation, Validation and writing. Bárbara Monteiro de Castro e Castro, Angelica Plata Rueda, Luis Carlos Martinez, Marcus Alvatenga Soares and Muhammad Fiaz: investigaion and validation. Carlos Frederico Wilcken, Antonio José Vinha Zanuncio, José Cola Zanuncio and José Eduardo Serrão: Validation and writing-reviewing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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