Chemosphere 212 (2018) 629–637

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Permethrin induces histological and cytological changes in the midgut of the predatory bug, *Podisus nigrispinus*

Luis Carlos Martínez [a], Angelica Plata-Rueda [b], Guilherme da Silva Neves [a], Wagner Gonzaga Gonçalves [a], José Cola Zanuncio [c], Hakan Bozdoğan [d], José Eduardo Serrão [a, *]

[a] Departamento de Biologia Geral, Universidade Federal de Viçosa, 36570-000, Viçosa, Minas Gerais, Brazil
[b] Instituto de Ciências Agrárias, Universidade Federal de Viçosa, 38810-000, Viçosa, Minas Gerais, Brazil
[c] Departamento de Entomologia, Universidade Federal de Viçosa, 36570-000, Viçosa, Minas Gerais, Brazil
[d] Department of Plant and Animal Production, Kirsehir Vocational School of Technicial Sciences, Ahi Evran University, 40100, Kirsehir, Turkey

HIGHLIGHTS

- The predatory bug, *Podisus nigrispinus* is used to control agricultural and forestry pests.
- Toxicity, histopathology, and cytotoxic effects by permethrin were evaluated in the midgut.
- Cytotoxic effects were cytoplasm vacuolization, autophagy and apocrine secretion.
- Permethrin induces apoptosis on midgut cells via ingestion after 24 h exposure.
- Permethrin has side-effect in the midgut and can't be used simultaneously with this predator.

ARTICLE INFO

Article history:
Received 7 May 2018
Received in revised form
24 August 2018
Accepted 26 August 2018
Available online 27 August 2018

Handling Editor: David Volz

Keywords:
Apoptosis
Cytotoxicity
Digestive epithelial cells
Insecticide
Non-target organism
Toxicity

ABSTRACT

Insecticides used in the agriculture and forestry have side effects on non-target organisms used as natural enemies. This study evaluated the histopathology and cytotoxicity of permethrin on the midgut of the non-target predatory bug, *Podisus nigrispinus* (Heteroptera: Pentatomidae) used in the biological control of pest insects. The toxicity and survival of this insect were determined using six concentrations of permethrin via ingestion. Histological and ultraestutural changes of the midgut of *P. nigrispinus* were analyzed after exposure to permethrin. The insecticide caused toxicity in *P. nigrispinus* with $LC_{50} = 0.46\ \mu g\ L^{-1}$ and survival of 47% after 72 h of exposure. The histological changes in the midgut were irregularly bordered epithelium, cytoplasmic vacuolization and apocrine secretions in the lumen after 6 h following exposure to the insecticide. Cytotoxic effects such as granules and vacuoles secreted into the lumen, presence of autophagosomes, and dilatation of infolds of the basal plasma membrane were observed in the three regions of the midgut. Cells of the midgut in apoptosis occurred after 12 h of exposure. Permethrin causes toxic effects, inhibits survival, and produces changes in the histology and cytology of the midgut in *P. nigrispinus,* suggesting that the cell stress induced by this insecticide can disrupt physiological processes such as digestion, compromising the potential of the predator as a biological control agent of pests. The low selectivity of permethrin to a non-target organism such as the predatory bug, *P. nigrispinus* indicates that the associated use of this insecticide in biological control should be better evaluated.

1. Introduction

Predator insects play an important role in invertebrate communities, as variation in the availability of food or prey has an effect on the survival, dispersion and population dynamics of these organisms (Cohen, 1990; Coll and Guershon, 2002). Predators can be

* Corresponding author.
E-mail addresses: lc.martinez@outlook.com (L.C. Martínez), angelicaplata@yahoo.com.mx (A. Plata-Rueda), zanuncio@ufv.br (J.C. Zanuncio), hakan.bozdogan@ahievran.edu.tr (H. Bozdoğan), jeserrao@ufv.br (J.E. Serrão).

https://doi.org/10.1016/j.chemosphere.2018.08.134
0045-6535/

classified as opportunistic, obligatory, or optional, depending on the importance of the material ingested as animal, vegetable or other organic and inorganic sources (Coll and Guershon, 2002). Among the opportunistic predators, phytozoophagous are herbivores that eventually feed on prey, and zoophytophagus are carnivores that eventually feed on plants (Memmott et al., 2000; Coll and Guershon, 2002). The predatory species of Miridae, Pentatomidae, and Reduviidae (Heteroptera) are zoophytophagous and used in biological control in agriculture and forestry (Zanuncio et al., 2008; De Bortoli et al., 2011).

Among the predatory bugs, the zoophytophagus *Podisus nigrispinus* Dallas (Hemiptera: Pentatomidae) has been used in the control of Lepidoptera defoliating larvae and Coleoptera (Ferreira et al., 2008; Torres et al., 2010; Martínez et al., 2016). Several studies have been conducted on the biology and ecology of *P. nigrispinus*, including its development, morphology (Martínez et al., 2014a,b, 2017), predator-prey interaction (Ferreira et al., 2008), and feeding strategies such as extraoral digestion (Fialho et al., 2012; Martínez et al., 2016). As *P. nigrispinus* is used in agricultural and forest pest control, it may be exposed to insecticides used in pest control.

Insecticide selectivity and impact on natural enemies is key in Integrated Pest Management (IPM) programs (Metcalf, 1980; Hardin et al., 1995; Desneux et al., 2007). Chemical control is the most commonly used method to control pest insects, and its use has increased in several crops (Song and Swinton, 2009; Meissle et al., 2010; Pedlowski et al., 2012). In this sense, the search for safer insecticides for human health and the environment has resulted in the development of specific compounds for pests and selective for non-target organisms (Matsumura, 2004; Nicholson, 2007; Biondi et al., 2012). Exposure to insecticide can cause adverse effects without the death of individuals (Desneux et al., 2007; Martínez et al., 2014a,b). These effects may be physiological ones, affecting development, longevity, and fecundity. Besides, they may alter behaviors such as mobility and feeding (Desneux et al., 2004; Kim et al., 2006; He et al., 2012).

Ingestion organs such as the digestive tract and salivary glands participate in feeding strategies in predatory bugs. The digestive tract of those insects is divided into three main regions: the foregut and hindgut that are derived from the ectoderm, and the midgut that originates from the endoderm is the main organ where digestion and absorption occur (Chapman, 2013; Terra and Ferreira, 2012). The midgut of Pentatomidae predatory bugs is anatomically divided into three regions: anterior, middle and posterior (Fig. 1), which perform different functions in digestion (Fialho et al., 2012, 2013). As in other Hemiptera, the midgut has lumen and is lined by a lipoprotein membrane called the perimicrovilar membrane (Terra, 1990; Teixeira et al., 2013). This contributes to subdivision of digestion, optimization of amino acid absorption, immobilization of some digestive enzymes, and protection of intestinal cells against mechanical hazards (Terra and Ferreira, 2012).

Predatory insects have insecticide tolerance, which emphasizes the importance of the potential success of IPM programs (Kim et al., 2006; Cordeiro et al., 2010; Zanuncio et al., 2011). In different studies, permethrin was demonstrated to possess toxic effect against lepidopteran pest as *Anticarsia gemmatalis* Hübner (Yu, 1988), *Helicoverpa zea* Boddie (Usmani and Knowles, 2001), and *Spodoptera frugiperda* (J.E. Smith) (Noctuidae) (Zanuncio et al., 1998) and, these insects are naturally preys of *P. nigrispinus* in Brazilian agricultural crops (Zanuncio et al., 2013). In contrast, the lethal and sublethal effects caused by permethrin have been demostrated on predatory bugs (Zanuncio et al., 2003; Guedes et al., 2009). Permethrin and other pyrethroids are known to cause rapid paralysis in invertebrates by disrupting nerve conduction; their primary site of action is the voltage-gated sodium channels (Martínez et al., 2014a,b; Antwi and Reddy, 2015; Sparks and Nauen, 2015). However, there is little information on the histopathological and cytotoxic effects on the midgut in predatory insects exposed to permethrin used to control their preys.

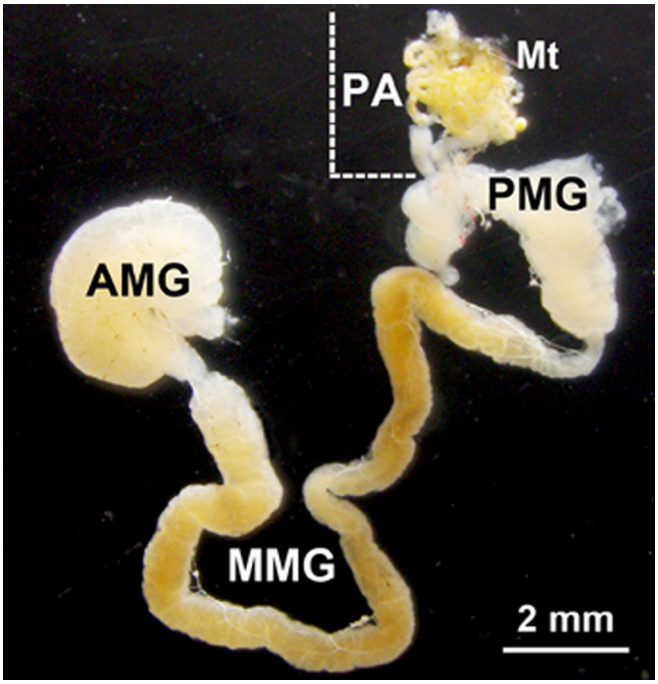

Fig. 1. Macrophotography of the midgut of *Podisus nigrispinus* showing the anterior (AMG), middle (MMG) and posterior (PMG) regions. Posterior intestine (PA) and Malphigi tubules (Mt).

The objective was to evaluate the acute toxicity, histopathology and cytotoxicity in the midgut of *P. nigrispinus* exposed to permethrin, an insecticide used to control preys of this insect.

2. Materials and methods

2.1. Insects

Adults of *P. nigrispinus* were obtained from the mass rearing in the Laboratory of Cellular and Structural Biology (Federal University of Viçosa, Minas Gerais, Brazil), maintained at 27 ± 2 °C, relative humidity of 75 ± 5% and 12 h photophase. These insects were fed pupae of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) and *Eucalyptus grandis* (W. Hill ex. Maiden) *ad libitum* (Neves et al., 2010). Pupae of *T. molitor* were obtained from the mass rearing in the Laboratory of Biological Control of Insects (LCBI) of the Institute of Applied Biotechnology for Agriculture (BIOAGRO) of the Federal University of Viçosa (UFV) in Viçosa, State of Minas Gerais, Brazil. Pupae of *T. molitor* were kept in plastic trays (60 cm long × 40 cm wide × 12 cm high) with a temperature of 25 ± 1 °C, relative humidity of 70 ± 10% and 12:12 h of photoperiod. Adults of *P. nigrispinus* and *T. molitor* pupae, without apparent amputations or malformations, were used in the bioassays.

2.2. Toxicity

The insecticide, permethrin (Permethrin® EC, 384 g L^{-1} active ingredient (ia), Fersol Indústria e Comércio Ltda., Mainrique, São Paulo, Brazil) was used in acute toxicity tests and diluted in 1 L of water to produce a stock solution by adjusting 100 g L^{-1} of insecticide to the required concentrations. The insecticidal efficacy was

determined by calculating the lethal concentration values (LC_{25}, LC_{50}, LC_{75} and LC_{90}) under laboratory conditions. Six concentrations of permethrin besides the control (distilled water) were adjusted in 1 mL stock solution (treatments and distilled water): 0.312, 0.625, 0.125, 0.25, 0.5 and 10 μg L^{-1} (w/v). Pupae of *T. molitor* were soaked for 5 s at each concentration and allowed to dry at room temperature. In the treatments, a pupa of *T. molitor* exposed to the insecticide was placed in a glass vial (2 × 10 cm) as a food source with an adult of *P. nigrispinus*. Fifty adults of *P. nigrispinus* were used by concentration and the number of dead insects was counted after exposure of the *T. molitor* pupae with the insecticide up to 72 h.

2.3. Survivorship

Newly emerged adults of *P. nigrispinus* were exposed to four concentrations of permethrin (LC_{25}, LC_{50}, LC_{75} and LC_{90}) determined by the toxicity and control bioassay with distilled water. All insecticide exposure procedures in insects followed the same procedures described above for the toxicity test. The number of dead insects was quantified every 6 h by 72 h.

2.4. Light microscopy

Adults of *P. nigrispinus* were exposed to the estimated lethal concentration LC_{50} of permethrin at different time periods (30 min, 1, 3 and 6 h) and cryoanesthetized at −4 °C. The midgut was dissected in insect saline solution (0.1 M NaCl + 0.1 M KH_2PO_4 + 0.1 M Na_2HPO_4), divided into the anterior, middle and posterior regions, and transferred to Zamboni's fixative solution (Stefanini et al., 1967) for 12 h at 5 °C. The samples were then dehydrated in a grade ethanol series (70°, 80°, 90° and 95°) and embedded in historesin (Leica Biosystem Nussloch GmbH, Wetzlar, Germany). Sections 3 μm thick were obtained, stained with hematoxylin and eosin, and analyzed under an Olympus BX-60 light microscope (Olympus Corporation, Tokyo, Japan).

2.5. Transmission electron microscopy

Adults of *P. nigrispinus* were exposed to the estimated lethal concentration LC_{50} of permethrin for 6 h and cryosanesthetized at −4 °C. The three midgut regions (anterior, middle and posterior) of *P. nigrispinus* were dissected in insect saline solution and transferred to 2.5% glutaraldehyde in 0.2 M sodium cacodylate buffer, pH 7.2 containing 0.2 M of sucrose for 4 h at room temperature. The samples were then post-fixed in 1% osmium tetroxide in the same buffer for 2 h, followed by washing in the buffer and dehydrated in a grade ethanol series (70°, 80°, 90° and 99°). The samples were embedded in LR White resin (London Resin Company Ltd.) and ultra-thin sections (80–90 nm thick), obtained with PowerTomes PT-X ultramicrotome glass razor (RMC Boeckeler Instruments Inc., Tucson, AZ, USA), were compared with 1% aqueous uranyl acetate and lead citrate (Reynolds, 1963) and examined on Zeiss EM 109 transmission electron microscope (Carl Zeiss, Jena, Germany).

2.6. Immunofluorescence

The three regions of the midgut of *P. nigrispinus* exposed by 12, 24 and 36 h to estimated lethal concentration LC_{50} of permethrin were dissected in 0.1 M sodium phosphate buffer (PBS) and transferred to Zamboni fixative solution for 2 h. Next, the samples were washed with PBS containing 1% Triton X-100 (PBST) and incubated with 1.5% bovine serum albumin in PBST for 2 h. Samples were incubated with anti-cleaved caspase-3 antibody (Cell Signaling Technology, Danvers, MA, USA) at 1:500 in PBS for 3 day at −4 °C. After incubation, the samples were washed in PBS and incubated with fluorescein isotiosinate (FITC) conjugated anti-rabbit IgG secondary antibody (Sigma-Aldrich, St. Louis, MO, USA) diluted 1:500 in PBS for 24 h in the dark at −4 °C. The medium intestines were washed, and the cell nuclei were stained with TO-PRO-3 propidium iodide (Life Technologies, Carlsbad, CA, USA) for 1 h. The samples were mounted on 50% sucrose glass slides and examined on Zeiss LSM510 META (Carl Zeiss, Jena, Germany) laser scanning confocal microscope.

2.7. Statistic

The lethal concentrations (LC_{25}, LC_{50}, LC_{75} and LC_{90}) and confidence limits were determined by regression based on probit-mortality concentration (Finney, 1964) with PROC PROBIT procedure of SAS User v. 9.0 for Windows (SAS Institute, 2002). Survival bioassay data were submitted to survival analysis using the Kaplan-Meier estimator (Log-rank method) with the Origin Pro v 9.1 program (Originlab Corporation, 2013). Data from adults that survived until the end of the experiment were treated as censored data.

3. Results

3.1. Toxicity and survivorship

The toxicity of insecticide permethrin obtained in *P. nigrispinus* was estimated by Probit (Chi-square; $P < 0.0001$) and evaluated at different concentrations (Table 1; Fig. 2A). Dose–response bioassays showed results with $LC_{25} = 0.38$ μg L^{-1}, $LC_{50} = 0.46$ μg L^{-1}, $LC_{75} = 0.55$ μg L^{-1}, and $LC_{90} = 0.64$ μg L^{-1}. Mortality was always <1% in the control.

The survival analysis of adults of *P. nigrispinus* exposed to permethrin showed differences between the concentrations (Log-rank test, chi-square = 66.34, df = 4, $P < 0.001$) (Fig. 2B). Survival was greater than 95.6% in adults that were not exposed to permethrin after 72 h, decreasing to 61.9% with LC_{25}, followed by 47.1% with LC_{50}, 25.3% with LC_{75} and 1.4% with LC_{90}.

3.2. Histopathology

The anterior midgut (AMG) of *P. nigrispinus* showed an epithelium with high columnar cells when it was not exposed to permethrin (Fig. 3A). The apical portion had striated border and the epithelial cells presented cytoplasm with little stained vesicles, small colored granules and nucleus with clusters of condensed chromatin. After 30 min and 1 h exposure of the insect to permethrin LC_{50}, the AMG showed epithelium with irregular apical surface, and vacuolization in the cytoplasm (Fig. 3B). The apical surface of the epithelium was irregular, with projections of the cytoplasm towards the lumen and with apocrine secretion (Fig. 3C). Vacuolization of the cytoplasm decreased from 3 to 6 h exposure of the insect to permethrin LC_{50} (Fig. 3D–E) and the apical surface of

Table 1
Lethal concentration of the insecticide permethrin in *Podisus nigrispinus* for 72 h after feeding with pupae of *Tenebrio molitor*. Insecticide concentrations were applied using a topical solution on the prey. X^2, chi-square for lethal concentration and fiducial limits based on a logarithmic scale with significance level at P < 0.0001.

Concentration (df = 5)	Estimated value (μg L^{-1})	Fiducial Limits		X^2
		Inferior	Superior	
CL_{25}	0.381	0.233	0.453	20.01
CL_{50}	0.468	0.376	0.535	
CL_{75}	0.555	0.489	0.650	
CL_{90}	0.642	0.570	0.795	
CL_{99}	0.832	0.713	1.146	

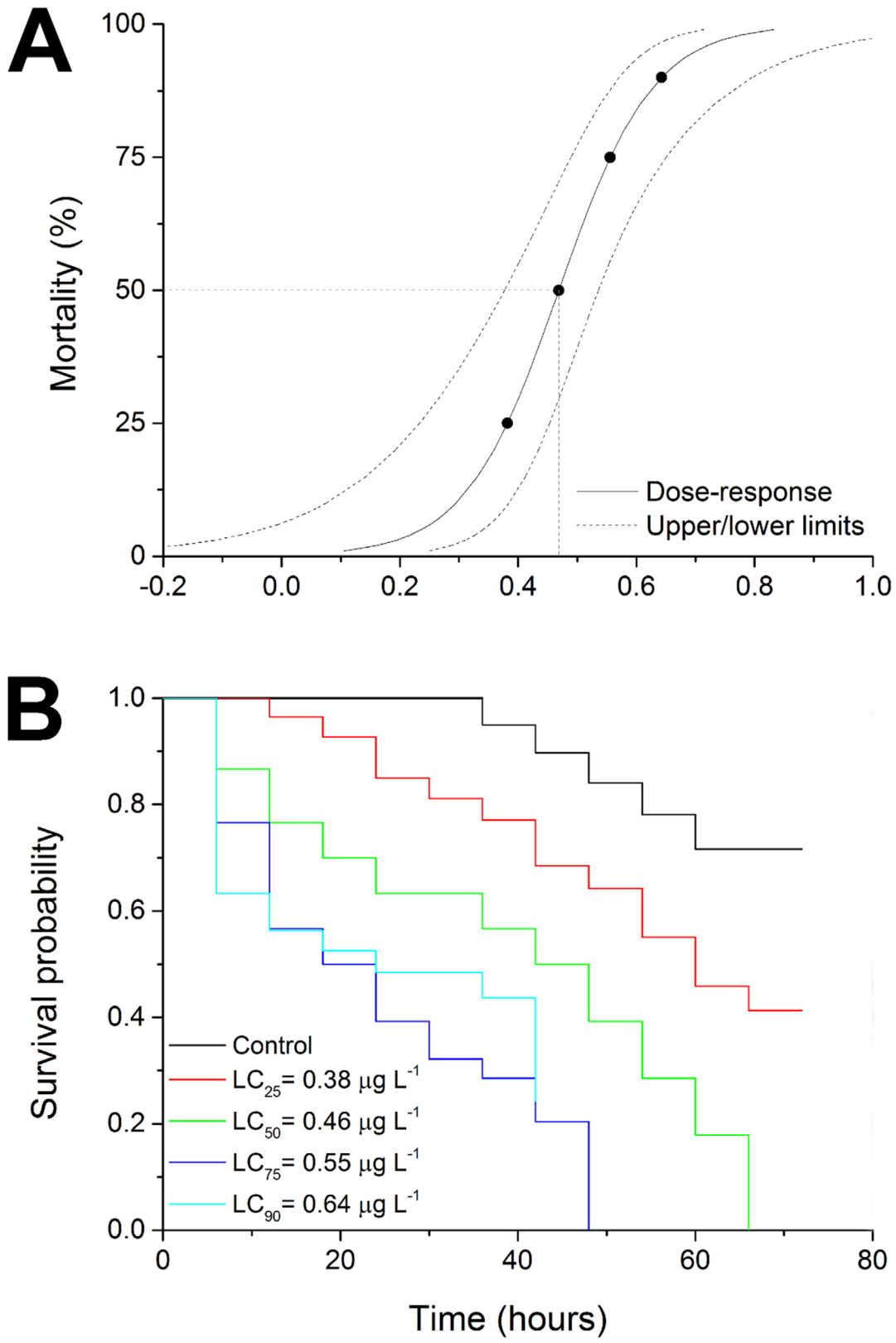

Fig. 2. Toxic effects of insecticide permethrin on *Podisus nigrispinus*. (A) Adult mortality caused by permethrin at different lethal concentrations (LC_{25}, LC_{50}, LC_{75} and LC_{90}) (Chi-square = 20.01, df = 5, $P < 0.001$). Dotted lines denote 95% confidence intervals. Black point represents the LC_{50} concentration selected to evaluate morphological changes. (B) Survival curves of adults exposed to different concentrations of permethrin for 72 h using the Kaplan-Meier method and compared using the log-rank test (Chi-square = 66.34; $P < 0.001$).

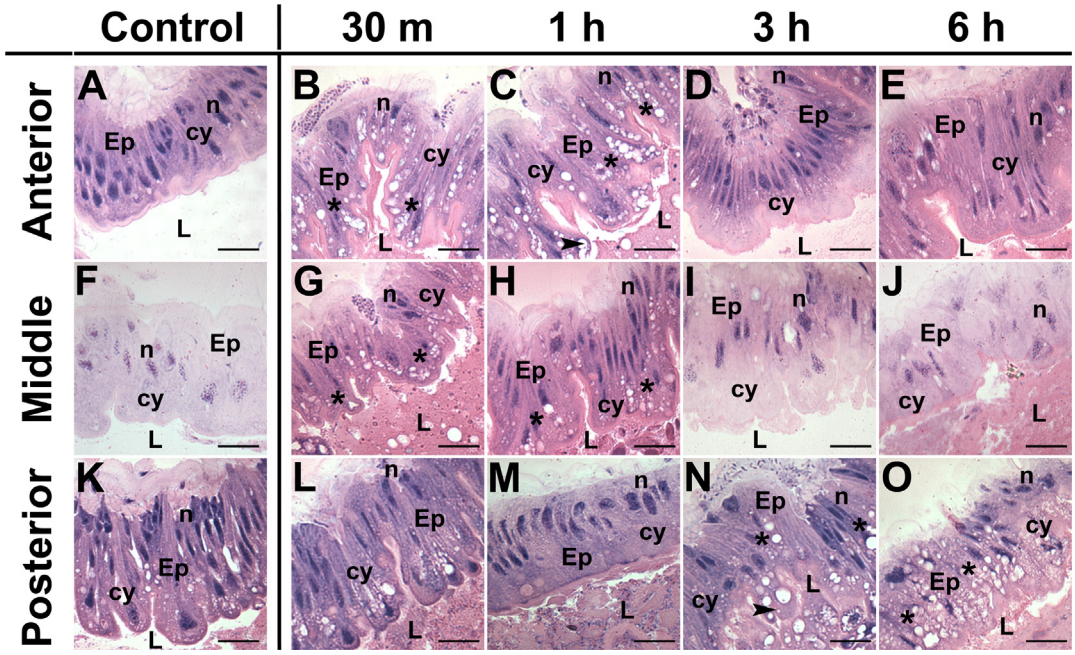

Fig. 3. Histological changes in the midgut of *Podisus nigrispinus* exposed to permethrin for 30 m, 1, 3 and 6 h (A–E) Anterior midgut showing sequential effects for 6 h after exposure of the insecticide. (F–J) Middle midgut showing sequential effects for 6 h after exposure of the insecticide. (K–O) Posterior midgut showing sequential effects for 6 h after exposure of the insecticide. Epithelium (Ep), lumen (L), cytoplasm (cy), nucleus (n); rupture of the plasma membrane and release of cellular content in midgut lumen (arrows); cytoplasmic vacuolization (*). Control (A, F, K). (Bars = 20 μm).

the epithelium was similar to that of the insect not exposed to permethrin.

In the control, the median region of the midgut (MMG) showed epithelium with columnar cells and apical portion with striated border (Fig. 3F). These epithelial cells presented cytoplasm with vesicles and the spherical nucleus with predominance of decondensed chromatin containing some clumps of condensed chromatin (Fig. 3F). After 30 min of exposure of *P. nigrispinus* to the LC_{50} of permethrin, the apical striated border of the MMG epithelium was irregular, the cytoplasm showed many vacuoles and high apocrine secretion (Fig. 3G). Similar effects occurred in the MMG of exposed insects LC_{50} from permethrin for 1 h (Fig. 3H). In the insects exposed for 3 h at the LC_{50} of permethrin, the amount and size of vacuoles as well as the rate of apocrine secretion decreased (Fig. 3I). After 6 h of exposure, MMG was similar to the control (Fig. 3J).

The posterior midgut (PMG) of *P. nigrispinus* not exposed to permethrin showed epithelium with high columnar cells with rich cytoplasm granules and vesicles and nucleus with clumps of condensed chromatin (Fig. 3K). In insects exposed for 30 min and 1 h LC_{50} of permethrin, no changes in PMG were observed in relation to the control (Fig. 3L–M). After 3 h of LC_{50} exposure of permethrin, PMG of *P. nigrispinus* showed epithelium with vacuolization and lumen with apocrine secretion (Fig. 3N). In those insects with 6 h exposure to permethrin LC_{50}, there was an increase in cytoplasmic vacuolization, the nuclei had irregular sizes with decondensed chromatin, and there was a decrease in apocrine secretion (Fig. 3O).

3.3. Cytotoxicity

In the control, the digestive cells of the AMG of *P. nigrispinus* showed short microvilli associated with perimicrovilar membrane on the apical surface (Fig. 4A). The apical cytoplasm was rich in electron-dense vesicles, lysosomes and mitochondria, the medial

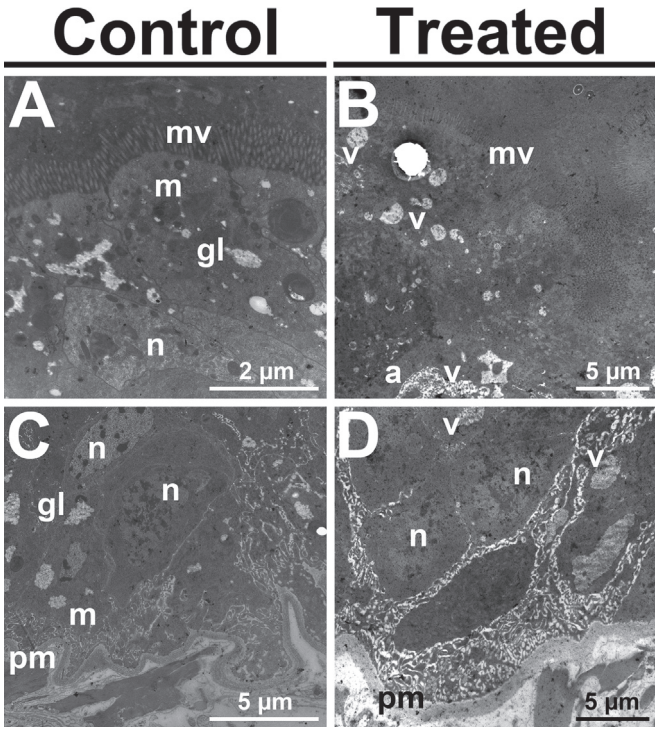

Fig. 4. Cytotoxic changes in the anterior midgut of *Podisus nigrispinus* caused by the insecticide permethrin. (A and C) Digestive cells without exposure to insecticide showing short microvilli (mv) associated with the perimicrovilar membrane in the apical portion, cytoplasm rich in electron-dense vesicles and mitochondria (m), and some glycogen islands (gl). The nucleus (n) with decondensed chromatin and folds of the plasma membrane (pm) in the basal portion. (B and D) Digestive cells exposed to the insecticide showing a high number of vacuoles (v), autophagosomes (a) and granules in the apical portion, in addition to many vacuoles secreted into the lumen. The basal portion of the cells showed dilatation of the folds of the plasma membrane (pm).

portion contained the nucleus and cytoplasm some glycogen islands, while the basal portion of the cells showed mitochondria associated with infolds of the plasma membrane (Fig. 4C). In the AMG exposed LC_{50} of permethrin, the cells showed apical cytoplasm with vacuoles, autophagosomes and granules, in addition to many vacuoles secreted into the lumen (Fig. 4B), whereas in the basal portion of the cells, the infolds of the plasma membrane were dilated and in larger quantity (Fig. 4D) that in the control.

In insects not exposed to permethrin, MMG digestive cells were columnar with short microvilli associated with perimicrovilar membrane (Fig. 5A). The cytoplasm showed many mitochondria, electron-dense vesicles and lysosomes (Fig. 5A). The basal portion of the digestive cells was rich in mitochondria, secretory vesicles, and the basal plasma membrane showed few short folds (Fig. 5C). In the MMG of *P. nigrispinus* exposed to permethrin, the digestive cells showed large vacuoles, autophagosomes with membrane remnants and few electron-dense granules, and some vesicles were released into the lumen (Fig. 5B). In the basal portion of the digestive cells, there was an increase in the folds of the basal plasma membrane (Fig. 5D).

The PMG of *P. nigrispinus* not exposed to permethrin had columnar cells with short microvilli and perimicrovilar membrane (Fig. 6A). In the apical portion, the cytoplasm had many mitochondria, secretory vesicles, and electron-dense granules (Fig. 6A), and the basal portion had some glycogen islands and basal plasma membrane with long folds associated with mitochondria (Fig. 6C). In the insects exposed to permethrin LC_{50}, the PMG had the digestive cells with many autophagosomes and granules in the apical portion, in addition to vesicles released in the lumen (Fig. 6B), while the basal portion of the cell showed autophagosomes and the basal plasma membrane with elongated infolds (Fig. 6D).

In all the analyzed insects, the nuclei presented a predominance of decondensed chromatin with some clusters of condensed chromatin and evident nucleolus. The regerative cells were found in the three regions of the midgut and organized in nests with developed nuclei and cytoplasm with few organelles in all the insects evaluated.

3.4. Apoptosis

In the insects of the control group, there were few digestive cells with a positive reaction to cleaved caspase-3 (Fig. 7A, E, 7I). However, in *P. nigrispinus*, LC_{50} of permethrin was exposed after 12, 24 and 48 h, there was an increase in digestive cells with a positive reaction to cleaved caspase-3 AMG (Fig. 7B–D), MMG (Fig. 7F–H), and PMG (Fig. 7J–L).

4. Discussion

Podisus nigrispinus was susceptible to permethrin with $LC_{50} = 0.46$ (0.37–0.53) μg L^{-1} and showed survival of 47% after 72 h of exposure. The lethality and low survival caused by permethrin show that *P. nigrispinus* is sensitive to this pyrethroid. The susceptibility of stink bugs may vary with the exposure of insecticides applied by contact or ingestion (Tillman and Mullinix, 2004; Snodgrass et al., 2005; Zanuncio et al., 2016). Predatory bugs (Hemiptera) as *Geocoris punctipes* (Say) (Geocoridae), *Nabis*

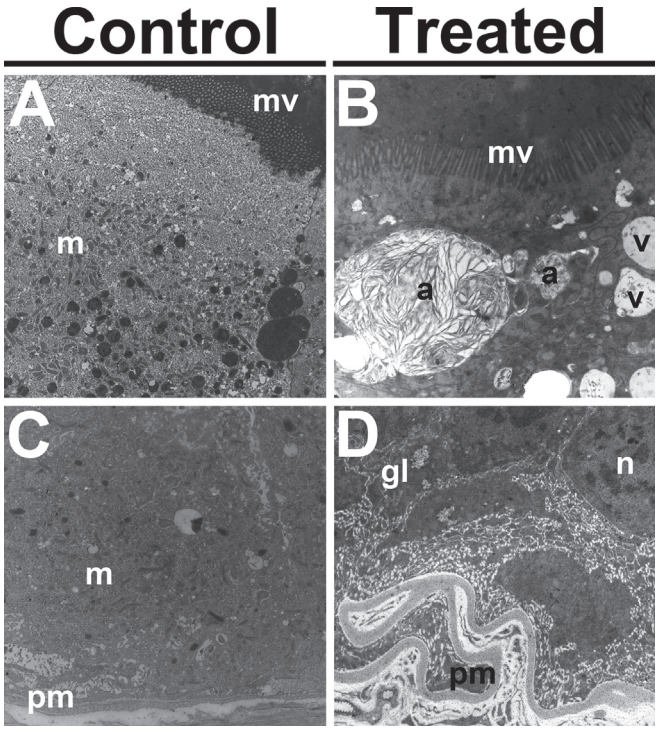

Fig. 5. Cytotoxic changes in the middle midgut of *Podisus nigrispinus* caused by the insecticide permethrin. (A and C) Digestive cells without exposure to insecticide showing short microvilli (mv) and associated with perimicrovilar membranewith the perimicrovilar membrane, cytoplasm with many mitochondria (m), electron-dense vesicles and lysosomes (ly) and plasma membrane (pm) with few and short folds. (B and D) Digestive cells exposed to the insecticide showing large vacuoles (v), autophagosomes (a) with membrane remnants, few electron-dense granules, and vesicles released into the lumen. Basal portion of the digestive cells with increase in the extension of the folds of the plasma membrane (pm).

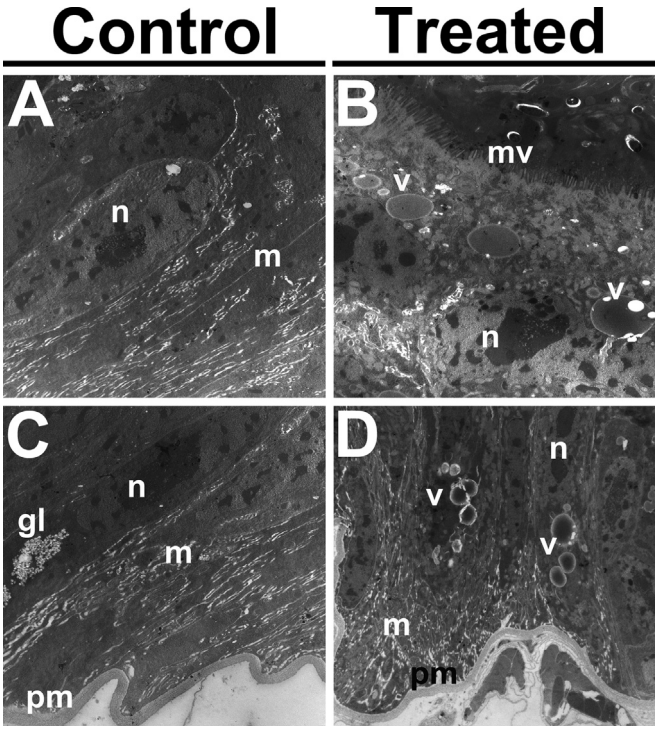

Fig. 6. Cytotoxic changes in the posterior midgut of *Podisus nigrispinus* caused by the insecticide permethrin. (A and C) Digestive cells without exposure to insecticide showing short microvilli (mv) in the apical portion, the cytoplasm with many mitochondria (m), secretory vesicles and electron-dense granules. The basal portion with glycogen islands (gl) and plasma membrane (pm) with long folds associated with mitochondria (m). (B and D) Digestive cells exposed to the insecticide with many autophagosomes and granules (V) in the apical portion, in addition to vesicles released into the lumen. Basal portion of the cell showing autophagosomes (V) and the basal plasma membrane with elongated folds.

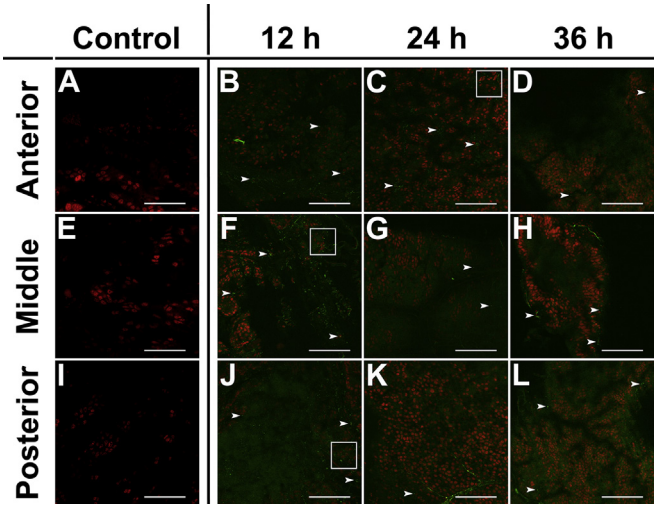

Fig. 7. Immunofluorescence for detection apoptosis of *Podisus nigrispinus* midgut exposed to permethrin for 12, 24 and 36 h using anti-cleaved caspase-3 antibody (green). (A–D) Anterior midgut. (E–H) Middle midgut. (I–L) Posterior midgut. Control without exposure to insecticide (A, E, I); active cleaved caspase-3 in cells (arrowheads); regions with high activity of cleaved caspase-3 (white square). (Bars = 100 μm).

roseipennis Reuter (Nabidae) and *Podisus maculiventris* (Say) (Pentatomidae) are sensitive to permethrin to different concentration ranges (Boyd and Boethel, 1998). Our study suggests that, despite being highly effective against pest insects, permethrin is not selective for non-target predator *P. nigrispinus* used as a pest control agent.

Permethrin ingestion exposure causes histological changes in the midgut of *P. nigrispinus* in short periods of time, characterized by cell degeneration, such as cytoplasmic vacuolization and widening of the space between the epithelium and the basal membrane of the insect. Cell degeneration in the midgut is described in non-target insects such as *Apis mellifera* L. (Hymenoptera: Apidae) in response to exposure to thiamethoxam (Catae et al., 2014), *Calliabaetis radiatus* Navas (Ephemeroptera: Baetidae) exposed to deltamethrin (Gutiérrez et al., 2016), the predator *Ceraeochrysa claveri* Navas (Neuroptera: Chrysopidae) exposed to azadirachthin (Scudeler and dos Santos, 2013), and *Chironomus calligraphus* Goeldi (Diptera: Chironomidae) exposed to cypermethrin (Lavarías et al., 2017).

In *P. nigrispinus*, the histopathological effects were more intense in the AMG and MMG between 30 min and 1 h after exposure to permethrin was reduced after 3 h. In PMG, the degenerative effects were observed after 3 h and increased in 6 h of exposure of the insect to the permethrin. A possible explanation for the faster intense insecticide effect on AMG and MMG may be the fact that the insecticide passes through the food and reaches AMG first, followed by MMG and PMG during digestion. The midgut of *P. nigrispinus* is divided into three anatomic regions, with AMG acting on rapid fluid uptake and carbohydrate digestion, MMG on protein digestion of nutrient uptake, and PMG on water transport from hemolymph to lumen (Fialho et al., 2012, 2013). In this sense, the histological damages of permethrin can affect the processes of digestion and absorption of nutrients in this predator. Thus, some histological changes decreased on 6 h, which suggests that midgut cells undergo some detoxification processes and reduce the permethrin effects. The occurrence of detoxification processes in the insect midgut is not unexpected since the primary functions of the midgut include digestive enzyme production (Xu et al., 2015).

Permethrin causes immediate toxic effects on the midgut of *P. nigrispinus* during the first 6 h with increased apocrine secretion, suggesting that digestive cells are in the process of detoxification, thus reducing the effects of the insecticide. The occurrence of detoxification processes in the midgut of insects is expected, because in insects the midgut also produces enzymes to protect cells from physical or chemical effects during digestion (Terra and Ferreira, 2012; Esther et al., 2015). In insects, enzymes play a role in the detoxification process as glycosyltransferases (GTSs), cytochrome P450s (P450s) and carboxylases involved in the metabolism of insecticides (Feyereisen, 1999; Li et al., 2006; Meech et al., 2012). Digestive enzymes are found in the vesicles of the digestive cell cytoplasms and released via apocrine secretion (Ferreira et al., 1990; Aumüller et al., 1999). This type of secretion occurs more intensely when the midgut is exposed to xenobiotic agents (Ferreira et al., 1990; Cristofoletti et al., 2001). This suggests that the increase in apocrine secretion in the midgut of *P. nigrispinus* may be a response to neutralize the effects of permethrin during the first few hours of exposure.

The cytotoxicity caused by permethrin was observed in three regions of the midgut of *P. nigrispinus*. Cytotoxic effects such as granules and vacuoles secreted into the lumen, presence of autophagosomes and dilatation of infolds of the plasma membrane were observed in the digestive cells. These characteristics correspond to degenerative cellular events also described in epithelial cells of the midgut of *A. mellifera* exposed to fipronil (Cruz et al., 2010) and spinosade (Lopes et al., 2018) and *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae) exposed to squamocin (Fiaz et al., 2018). In this context, the effect caused by permethrin may in turn affect the protection of the midgut epithelium against mechanical and chemical damage, nutrient absorption, signaling pathways and transport processes, which leaves the midgut more vulnerable to pathogens and may lead to insect death (Wang and Granados, 2000; Berini et al., 2016; Lopes et al., 2018).

In *P. nigrispinus*, cytotoxic effects first occurred in AMG followed by MMG and PMG. The differences in cellular responses caused by permethrin in different regions of the midgut are mainly influenced by the location of these regions in the alimentary canal. The AMG shows a more severe cellular degeneration probably because they come into contact with the contaminated food of the insecticide in the first stages of the digestion (Catae et al., 2014; Oliveira et al., 2014), and they consequently compromise the digestion, absorption and secretion of digestive enzymes (Terra, 1990; Fialho et al., 2012, 2013; Torres and Boyd, 2009). However, the increased secretion observed in the midgut lumen of *P. nigrispinus* can be attributed to the continued production of detoxifying enzymes that play a role in the metabolism of pesticides (Yu and Hsu, 1993; Enayati et al., 2005; Zhu et al., 2011). This seems to be supplanted by insecticide due to cell damage encountered. Cytoplasmic vacuolation suggests that the cell may be in autophagy of damaged cellular components as a response to chemical/physiological stress (Clarke, 1990; Lockshin and Zakeri, 2004). Autophagy does not necessarily culminate in cell death, as it is a normal physiological process of recycling proteins and organelles (Ferreira et al., 2013; Rossi et al., 2013; Yoshimori, 2004). However, if chemical stress prevails, the cell may be completely degenerated by autophagy or trigger death by apoptosis (Lockshin and Zakeri, 2004; Rossi et al., 2013). Thus, the cytotoxic effects caused by permethrin show that the digestive cells of *P. nigrispinus* can not maintain the digestion and absorption processes resulting in energy cost in the detoxification of the insecticide.

Permethrin induces a progressive increase of cell death by apoptosis in the midgut of *P. nigrispinus* as demonstrated by the presence of cleaved caspase-3. Activation by caspases during apoptosis consists of mitochondrial membrane permeabilization, chromatin condensation and DNA fragmentation, resulting in cell death (Green, 2005; Nikoletopoulou et al., 2013). Morphological

changes such as chromatin condensation, nucleus fragmentation and vacuolization are typical signs of apoptosis (Häcker, 2000; Ziegler and Groscurth, 2004; Rost-Roszkowska et al., 2008; Nikoletopoulou et al., 2013). Our results suggest that permethrin induces apoptosis in the three regions of the midgut of *P. nigrispinus*, as demonstrated by the measurement of both cleaved caspase-3, as observed in histological and ultrastructural analyzes.

Our data show that permethrin is toxic when ingested by the predatory bug, *P. nigrispinus,* reducing its survival between 48 and 72 h, and when the insect is exposed to $LC_{50,}$ cellular damage occurs in the midgut that can affect the digestion and absorption. Permethrin reduces the predation abilities and survival of this predator, indicating that the joint use of this insecticide and the predator should be better evaluated during the management of agricultural and forest pests.

Acknowledgments

The Brazilian agencies “Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)”, “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)”, and “Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG)” for the financial contribution. To Núcleo de Microscopia e Microanálise of the Universidade Federal de Viçosa for technical support.

References

Antwi, F.B., Reddy, G.V., 2015. Toxicological effects of pyrethroids on non-target aquatic insects. Environ. Toxicol. Pharmacol. 40, 915–923.
Aumüller, G., Wilhelm, B., Seitz, J., 1999. Apocrine secretion—factor artifact? Ann. Anat. 181, 437–446.
Berini, F., Caccia, S., Franzetti, E., Congiu, T., Marinelli, F., Casartelli, M., Tettamantu, G., 2016. Effects of *Trichoderma viride chitinases* on the peritrophic matrix of Lepidoptera. Pest Manag. Sci. 72, 980–989.
Biondi, A., Mommaerts, V., Smagghe, G., Viñuela, E., Zappalà, L., Desneux, N., 2012. The non-target impact of spinosyns on beneficial arthropods. Pest Manag. Sci. 68, 1523–1536.
Boyd, M.L., Boethel, D.J., 1998. Residual toxicity of selected insecticides to Heteropteran predaceous species (Heteroptera: Lygaeidae, Nabidae, Pentatomidae) on soybean. Environ. Entomol. 27, 154–160.
Catae, A.F., Roat, T.C., Oliveira, R.A., Ferreira Nocelli, R.C., Malaspina, O., 2014. Cytotoxic effects of thiamethoxam in the midgut and malpighian tubules of Africanized *Apis mellifera* (Hymenoptera: Apidae). Microsc. Res. Tech. 77, 274–281.
Chapman, R.F., 2013. The Insects: Structure and Function, fifth ed. Cambridge University Press, Cambridge, UK.
Clarke, P.G., 1990. Developmental cell death: morphological diversity and multiple mechanisms. Anat. Embryol. 181, 195–213.
Cohen, A.C., 1990. Feeding adaptations of some predaceous Heteroptera. Ann. Entomol. Soc. Am. 83, 1215–1223.
Coll, M., Guershon, M., 2002. Omnivory in terrestrial arthropods: mixing plant and prey diets. Annu. Rev. Entomol. 47, 267–297.
Cordeiro, E.M.G., Corrêa, A.S., Venzon, M., Guedes, R.N.C., 2010. Insecticide survival and behavioral avoidance in the lacewings *Chrysoperla externa* and *Ceraeochrysa cubana*. Chemosphere 81, 1352–1357.
Cristofoletti, P.T., Ribeiro, A.F., Terra, W.T., 2001. Apocrine secretion of amylase and exocytosis of trypsin along the midgut of *Tenebrio molitor* larvae. J. Insect Physiol. 47, 143–155.
Cruz, S.A., Silva-Zacarin, E.C., Bueno, O.C., Malaspina, O., 2010. Morphological alterations induced by boric acid and fipronil in the midgut of worker honeybee (*Apis mellifera* L.) larvae. Cell Biol. Toxicol. 26, 165–176.
De Bortoli, S.A., Otuka, A.K., Vacari, A.M., Martins, M.I.E.G., Volpe, H.L.X., 2011. Comparative biology and production costs of *Podisus nigrispinus* (Hemiptera: Pentatomidae) when fed different types of prey. Biol. Contr. 58, 127–132.
Desneux, N., Pham-Delègue, M.H., Kaiser, L., 2004. Effects of sublethal and lethal doses of lambda-cyhalothrin on oviposition experience and host searching behaviour of a parasitic wasp *Aphidius ervi*. Pest Manag. Sci. 60, 381–389.
Desneux, N., Decourtye, A., Delpuech, J.M., 2007. The sublethal effects of pesticides on beneficial arthropods. Annu. Rev. Entomol. 52, 81–106.
Enayati, A.A., Ranson, H., Hemingway, J., 2005. Insect glutathione transferases and insecticide resistance. Insect Mol. Biol. 14, 3–8.
Esther, E., Smit, S., Beukes, M., Apostolides, Z., Pirk, C.W., Nicolson, S.W., 2015. Detoxification mechanisms of honey bees (*Apis mellifera*) resulting in tolerance of dietary nicotine. Sci. Rep. 5, 11779.
Ferreira, C., Bellinello, G.L., Ribeiro, A.F., Terra, W.R., 1990. Digestive enzymes associated with the glycocalyx, microvillar membranes and secretory vesicles from midgut cells of *Tenebrio molitor* larvae. Insect Biochem. 20, 839–847.
Ferreira, J.A.M., Zanuncio, J.C., Torres, J.B., Molina-Rugama, A.J., 2008. Predatory behaviour of *Podisus nigrispinus* (Heteroptera: Pentatomidae) on different densities of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) larvae. Biocontr. Sci. Technol. 18, 711–719.
Ferreira, R.A.C., Zacarin, E.C.M.S., Malaspina, O., Bueno, O.C., Tomotake, M.E.M., Pereira, A.M., 2013. Cellular responses in the Malpighian tubules of *Scaptotrigona postica* (Latreille, 1807) exposed to low doses of fipronil and boric acid. Micron 46, 57–65.
Feyereisen, R., 1999. Insect P450 enzymes. Annu. Rev. Entomol. 44, 507–533.
Fialho, M.C.Q., Zanuncio, J.C., Neves, C.A., Ramalho, F.S., Serrão, J.E., 2012. Prey digestion in the midgut of the predatory bug *Podisus nigrispinus* (Hemiptera: Pentatomidae). J. Insect Physiol. 58, 850–856.
Fialho, M.C.Q., Terra, W.R., Moreira, N.R., Zanuncio, J.C., Serrão, J.E., 2013. Ultrastructure and immunolocalization of digestive enzymes in the midgut of *Podisus nigrispinus* (Heteroptera: Pentatomidae). Arthropod Struct. Dev. 42, 277–285.
Fiaz, M., Martínez, L.C., da Silva Costa, M., Cossolin, J.F.S., Plata-Rueda, A., Gonçalves, W.G., Sant'Ana, A.E.G., Zanuncio, J.C., Serrão, J.E., 2018. Squamocin induce histological and ultrastructural changes in the midgut cells of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae). Ecotoxicol. Environ. Saf. 156, 1–8.
Finney, D.J., 1964. Probit Analysis. Cambridge University Press, Cambridge, UK.
Green, D.R., 2005. Apoptotic pathways: ten minutes to dead. Cell 121, 671–674.
Guedes, R.N.C., Magalhaes, L.C., Cosme, L.V., 2009. Stimulatory sublethal response of a generalist predator to permethrin: hormesis, hormoligosis, or homeostatic regulation? J. Econ. Entomol. 102, 170–176.
Gutiérrez, Y., Santos, H.P., Serrão, J.E., Oliveira, E.E., 2016. Deltamethrin-mediated toxicity and cytomorphological changes in the midgut and nervous system of the mayfly Callibaetis radiatus. PLoS One 11 (3), e0152383.
Häcker, G., 2000. The morphology of apoptosis. Cell Tissue Res. 301, 5–17.
Hardin, M.R., Benrey, B., Coll, M., Lamp, W.O., Roderick, G.K., Barbosa, P., 1995. Arthropod pest resurgence: an overview of potential mechanisms. Crop Protect. 14, 3–18.
He, Y., Zhao, J., Zheng, Y., Desneux, N., Wu, K., 2012. Lethal effect of imidacloprid on the coccinellid predator *Serangium japonicum* and sublethal effects on predator voracity and on functional response to the whitefly *Bemisia tabaci*. Ecotoxicology 21, 1291–1300.
Kim, D.S., Brooks, D.J., Riedl, H., 2006. Lethal and sublethal effects of abamectin, spinosad, methoxyfenozide and acetamiprid on the predaceous plant bug *Deraeocoris brevis* in the laboratory. Biocontrol 51, 465–484.
Lavarías, S., Arrighetti, F., Siri, A., 2017. Histopathological effects of cypermethrin and *Bacillus thuringiensis* var. israelensis on midgut of *Chironomus calligraphus* larvae (Diptera: Chironomidae). Pestic. Biochem. Physiol. 139, 9–16.
Li, X., Schuler, M.A., Berenbaum, M.R., 2006. Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. Annu. Rev. Entomol. 52, 231–253.
Lockshin, R.A., Zakeri, Z., 2004. Apoptosis, autophagy, and more. Int. J. Biochem. Cell Biol. 36, 2405–2419.
Lopes, M.P., Fernandes, K.M., Tomé, H.V.V., Gonçalves, W.G., Miranda, F.R., Serrão, J.E., Martins, G.F., 2018. Spinosad-mediated effects on the walking abilities, midgut, and Malpighian tubules of Africanized honey bee workers. Pest Manag. Sci. 74, 1311–1318.
Martínez, L.C., Fialho, M.D.C.Q., Zanuncio, J.C., Serrão, J.E., 2014a. Ultrastructure and cytochemistry of salivary glands of the predator *Podisus nigrispinus* (Hemiptera: Pentatomidae). Protoplasma 251, 535–543.
Martínez, L.C., Plata-Rueda, A., Zanuncio, J.C., Serrao, J.E., 2014b. Comparative toxicity of six insecticides on the rhinoceros beetle (Coleoptera: Scarabaeidae). Fla. Entomol. 97, 1056–1062.
Martínez, L.C., Fialho, M.C.Q., Barbosa, L.C.A., Oliveira, L.L., Zanuncio, J.C., Serrão, J.E., 2016. Stink bug predator kill prey with salivary non-proteinaceous compounds. Insect Biochem. Mol. Biol. 68, 71–78.
Martínez, L.C., Plata-Rueda, A., Zanuncio, J.C., de Souza Tavares, W., Serrão, J.E., 2017. Comparative morphology of the odoriferous system in three predatory stink bugs (Heteroptera: Asopinae). Protoplasma 254, 1965–1972.
Matsumura, F., 2004. Contemporary issues on pesticide safety. J. Pestic. Sci. 29, 299–303.
Meech, R., Miners, J.O., Lewis, B.C., Mackenzie, P.I., 2012. The glycosidation of xenobiotics and endogenous compounds: versatility and redundancy in the UDP glycosyltransferase superfamily. Pharmacol. Ther. 134, 200–218.
Meissle, M., Mouron, P., Musa, T., Bigler, F., Pons, X., Vasileiadis, V.P., Otto, S., Antichi, D., Kiss, J., Pálinkás, Z., Dorner, Z., van der Weide, R., Groten, J., Czembor, E., Adamczyk, J., Thibord, J.B., Melander, B., Cordsen Nielsen, G., Poulsen, R.T., Zimmermann, O., Vershwele, A., Oldenburg, E., 2010. Pest, pesticides use and alternative options in European maize production: current status and future prospects. J. Appl. Entomol. 134, 357–375.
Memmott, J., Martinez, N.D., Cohen, J.E., 2000. Predators, parasitoids and pathogens: species richness, trophic generality and body sizes in a natural food web. J. Anim. Ecol. 69, 1–15.
Metcalf, R.L., 1980. Changing role of insecticides in crop protection. Annu. Rev. Entomol. 25, 219–256.
Neves, R.C.S., Torres, J.B., Zanuncio, J.C., 2010. Production and storage of mealworm beetle as prey for predatory stinkbug. Biocontr. Sci. Technol. 20, 1013–1025.
Nicholson, G.M., 2007. Fighting the global pest problem: preface to the special Toxicon issue on insecticidal toxins and their potential for insect pest control. Toxicon 49, 413–422.

Nikoletopoulou, V., Markaki, M., Palikaras, K., Tavernarakis, N., 2013. Crosstalk between apoptosis, necrosis and autophagy. Biochim. Biophys. Acta Mol. Cell Res. 1833, 3448–3459.
Oliveira, R.A., Roat, T.C., Carvalho, S.M., Malaspina, O., 2014. Side-effects of thiamethoxam on the brain andmidgut of the africanized honeybee *Apis mellifera* (Hymenopptera: Apidae). Environ. Toxicol. 29, 1122–1133.
Originlab Corporation, 2013. OriginPro V. 9.0.0 SR2 B87. Originlab Corporation. Originlab Corporation, Northampton, MA. http://www.OriginLab.com.
Pedlowski, A.M., Canela, M.C., Terra, M.A.C., Faria, R.M.R., 2012. Modes of pesticides utilization by Brazilian smallholders and their implications for human health and the environment. Crop Protect. 31, 113–118.
Reynolds, E.S., 1963. The use of lead citrate at high pH as an electronopaque stain in electron microscopy. J. Cell Biol. 17, 208–2012.
Rossi, A.C., Roat, T.C., Tavares, D.A., Cintra-Socolowski, P., Malaspina, O., 2013. Brain morphophysiology of Africanized bee *Apis mellifera* exposed to sublethal doses of imidacloprid. Arch. Environ. Contam. Toxicol. 65, 234–243.
Rost-Roszkowska, M.M., Poprawa, I., Klag, J., Migula, P., Mesjasz-Przybylowicz, W., 2008. Degeneration of the midgut epithelium in *Epilachna* cf. *nylanderi* (Insecta, Coccinellidae): apoptosis, autophagy, and necrosis. Can. J. Zool. 86, 1179–1188.
SAS Institute, 2002. The SAS System for Windows, Release 9.0. SAS Institute, Cary, N.C. http://www.sas.com.
Scudeler, E.L., dos Santos, D.C., 2013. Effects of neem oil (*Azadirachta indica* A. Juss) on midgut cells of predatory larvae *Ceraeochrysa claveri* (Navás, 1911) (Neuroptera: Chrysopidae). Micron 44, 125–132.
Snodgrass, G.L., Adamczyk, J.J., Gore, J., 2005. Toxicity of insecticides in a glass-vial bioassay to adult brown, green, and southern green stink bugs (Heteroptera: Pentatomidae). J. Econ. Entomol. 98, 177–181.
Song, F., Swinton, S.M., 2009. Returns to integrated pest management research and outreach for soybean aphid. J. Econ. Entomol. 102, 2116–2125.
Sparks, T.C., Nauen, R., 2015. IRAC: mode of action classification and insecticide resistance management. Pestic. Biochem. Physiol. 121, 122–128.
Stefanini, M., De Martino, C., Zamboni, L., 1967. Fixation of ejaculated spermatozoa for electron microscopy. Nature 216, 173–174.
Teixeira, A.D., Fialho, M.C.Q., Zanuncio, J.C., Ramalho, F.S., Serrão, J.E., 2013. Degeneration and cell regeneration in the midgut of *Podisus nigrispinus* (Heteroptera: Pentatomidae) during post-embryonic development. Arthropod Struct. Dev. 42, 237–246.
Terra, W.R., 1990. Evolution of digestive systems of insects. Annu. Rev. Entomol. 35, 181–200.
Terra, W.R., Ferreira, C., 2012. Biochemistry and Molecular Biology of Digestion. Insect Molecular Biology and Biochemistry. Academic Press, pp. 365–418.
Tillman, P.G., Mullinix, B.G., 2004. Comparison of susceptibility of pest *Euschistus servus* and predator *Podisus maculiventris* (Heteroptera: Pentatomidae) to selected insecticides. J. Econ. Entomol. 9, 800–806.
Torres, J.B., Boyd, D.W., 2009. Zoophytophagy in predatory Hemiptera. Braz. Arch. Biol. Technol. 52, 1199–1208.
Torres, J.B., Barros, E.M., Coelho, R.R., Pimentel, R.M.M., 2010. Zoophytophagous pentatomids feeding on plants and implications for biological control. Arthropod Plant Interact. 4, 219–227.
Usmani, K.A., Knowles, C.O., 2001. Toxicity of pyrethroids and effect of synergists to larval and adult *Helicoverpa zea*, *Spodoptera frugiperda*, and *Agrotis ipsilon* (Lepidoptera: Noctuidae). J. Econ. Entomol. 9, 868–873.
Wang, P., Granados, R.R., 2000. Calcofluor disrupts the midgut defense system in insects. Insect Biochem. Mol. Biol. 30, 135–143.
Xu, Z.B., Zou, X.P., Zhang, N., Feng, Q.L., Zheng, S.C., 2015. Detoxification of insecticides, allechemicals and heavy metals by glutathione S-transferase SlGSTE1 in the gut of *Spodoptera litura*. Insect Sci. 22, 503–511.
Yoshimori, T., 2004. Autophagy: a regulated bulk degradation process inside cells. Biochem. Biophys. Res. Commun. 313, 453–458.
Yu, S.J., Hsu, E.L., 1993. Induction of detoxification enzymes in phytophagous insects: role of insecticide synergists, larval age, and species. Arch. Insect Biochem. Physiol. 24, 21–32.
Yu, S.J., 1988. Selectivity of insecticides to the spined soldier bug (Heteroptera: Pentatornidae) and its Lepidopterous prey. J. Econ. Entomol. 81, 119–122.
Zanuncio, J.C., Batalha, V.C., Guedes, R.N.C., Picanço, M.C., 1998. Insecticide selectivity to *Supputius cincticeps* (stal) (Het., Pentatomidae) and its prey *Spodoptera frugiperda* (JE Smith) (Lep., Noctuidae). J. Appl. Entomol. 122, 457–460.
Zanuncio, T.V., Serrão, J.E., Zanuncio, J.C., Guedes, R.N.C., 2003. Permethrin-induced hormesis on the predator *Supputius cincticeps* (Stål, 1860) (Heteroptera: Pentatomidae). Crop Protect. 22, 941–947.
Zanuncio, J.C., Silva, C.A.D., Lima, E.R., Pereira, F.F., Ramalho, F.S., Serrão, J.E., 2008. Predation rate of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae with and without defense by *Podisus nigrispinus* (Heteroptera: Pentatomidae). Braz. Arch. Biol. Technol. 51, 121–125.
Zanuncio, J.C., Jusselino-Fillho, P., Ribeiro, R.C., Zanuncio, T.V., Ramalho, F.S., Serrão, J.E., 2011. Hormetic responses of a stinkbug predator to sublethal doses of pyrethroid. Bull. Environ. Contam. Toxicol. 87, 608–614.
Zanuncio, J.C., Jusselino-Filho, P., Ribeiro, R.C., Castro, A.A., Zanuncio, T.V., Serrao, J.E., 2013. Fertility and life expectancy of a predatory stinkbug to sublethal doses of a pyrethroid. Bull. Environ. Contam. Toxicol. 90, 39–45.
Zanuncio, J.C., Mourão, S.A., Martínez, L.C., Wilcken, C.F., Ramalho, F.S., Plata-Rueda, A., Serrão, J.E., 2016. Toxic effects of the neem oil (*Azadirachta indica*) formulation on the stink bug predator, *Podisus nigrispinus* (Heteroptera: Pentatomidae). Sci. Rep. 6, 30261.
Zhu, Y.C., Guo, Z., Chen, M.-S., Zhu, K.Y., Liu, X.F., Scheffler, B., 2011. Major putative pesticide receptors, detoxification enzymes, and transcriptional profile of the midgut of the tobacco budworm, *Heliothis virescens* (Lepidoptera: Noctuidae). J. Invertebr. Pathol. 106, 296–307.
Ziegler, U., Groscurth, P., 2004. Morphological features of cell death. Physiology 19, 124–128.