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LAMBDA-CYHALOTHRIN PROMOTES OXIDATIVE STRESS AND PATHOLOGICAL CHANGES IN THE MIDGUT AND GONADS OF COTTON BOLL WEEVIL

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Abstract: The cotton boll weevil, *Anthonomus grandis*, is the main cotton pest in Brazil and its control is mainly based on the application of conventional insecticides, such as pyrethroids. Insecticides are one of the examples of stressing agents that can promote damage to intestinal epithelial cells, impairing the absorption of nutrients and causing deleterious effects on the life history of these organisms. Therefore, the present study evaluated the effects of lambda-cyhalothrin pyrethroid on oxidative stress, apoptosis, cell proliferation and morphohistochemistry of the midgut and gonads of *A. grandis*. For this, flower buds were immersed in the insecticide solution (200 µL of the active ingredient + 100 mL of distilled water) and offered to adult insects for 24h. All parameters were evaluated 24h after feeding. In all treatments with the insecticide, there was histological damage to the midgut characterized by protrusions, vacuolization and disorganization of columnar cells, in addition to a substantial increase in regenerative cells. grouped in nests. The cell proliferation index revealed positive nuclei above 40%, while the apoptotic index revealed above 80%. It was also observed, from the measurement of lipid peroxidation, an increase in oxidative stress. In the testes there was a higher proportion of cysts in relation to sperm (characterizing reduction) and thickening of the peritoneal covering, while in the ovarioles the seminal vesicle was not identified. In the treated group, the number of pixels was lower, configuring a histochemical decrease in carbohydrates and proteins. Therefore, we conclude that the lambda-cyhalothrin pyrethroid is capable of increasing oxidative stress and promoting morphohistochemical and immunohistochemical pathological changes in the midgut epithelium and gonads, with consequences for gametogenesis in adults of *A. grandis*.

Keywords: Pyrethroid, boll weevil, oxidative

stress, apoptosis, cell proliferation, gonads

INTRODUCTION

Many stressing agents promote adverse effects on insects, such as insecticides. One of the effects, for example, is on digestive physiology, affecting the cells that line the epithelium of the midgut region. As this region acts in the absorption of nutrients, the damage can have consequences in the reproductive processes. In addition, exposure can directly affect the gonads, leading to complications in the gametogenesis of the testes and ovarioles. Therefore, intestinal homeostasis is directly related to nutritional maintenance and, consequently, to the proper functioning of the insect's vital processes (Cunha et al. 2015, Costa et al. 2017). The intestinal epithelium is constantly renewed, especially when the region suffers some damage. Renewal occurs through the proliferation of regenerative cells, also called intestinal stem cells (Park & Takeda 2008, Caccia et al. 2019). Many studies relate epithelial renewal, from stem cell proliferation, during molt in holometabolous insects, as a response to infection to pathogens or exposure to xenobiotics (Buchon et al. 2009, Naszai et al. 2015, Taracena et al. 2018, Janeh et al. 2019). This mechanism often acts as a response to apoptosis, a naturally occurring phenomenon that can be induced (Gregorc & Ellis 2011, Huang et al. 2013). This induction can occur from the accumulation of reactive oxygen species (ROS) during oxidative stress, which leads to the loss of basic functions of cellular constituents. (Lalouette *et al.* 2011, Velki *et al.* 2011).

To investigate the occurrence of these mechanisms in insects of agricultural importance is essential to develop better control tactics, such as the boll weevil. (*Anthonomus grandis* Boheman, Coleoptera: Curculionidae). This beetle is the main pest of cotton cultivation in all regions of Brazil and can cause losses of 75% to 100% in productivity (Godoy & Nakano 2011, Abrapa 2022, MAPA 2022). In order to maintain the viability of cultivation, applications of conventional formulations (such as pyrethroids) are necessary for the control of this beetle. (Rolim *et al.* 2019).

The cellular responses in *A. grandis* due to contamination of pymetrozine (TRPV channel modulators) and lufenuron (chitin biosynthesis inhibitor) and exposure to low-frequency radiation have already been documented (Cunha et al. 2015, Costa et al. 2017, Ferreira et al. 2021). Despite this, these methods are not often used in the field to control this weevil. Thus, what would be the cellular responses involved in contamination by pyrethroids in this pest?

Therefore, the present study evaluated the effects of lambda-cyhalothrin pyrethroid ingestion on oxidative stress, as well as on apoptosis, cell proliferation and morphohistochemistry of the midgut and gonads of *A. grandis*.

MATERIAL AND METHODS

The research was carried out at the Insect Physiology Laboratory of the Department of Animal Morphology and Physiology, at the Research Support Center (Cenapesq) of the Federal Rural University of Pernambuco (UFRPE, Recife, Pernambuco, Brazil) and at the Renal Physiology Laboratory of the Department of of Biochemistry and Physiology at the Federal University of Pernambuco (UFPE, Recife, Pernambuco, Brazil).

CREATING AND OBTAINING *ANTHONOMUS GRANDIS*

Flower buds and cotton bolls infested with larvae and pupae of *A. grandis* were collected in an experimental field at the Agronomy Department of the Federal Rural University of

Pernambuco and sent to the Insect Physiology Laboratory of the Federal Rural University of Pernambuco (UFRPE, Recife, Pernambuco, Brazil). The flower buds and cotton bolls were then housed in plastic emergency cages (42.5 × 30.7 × 30.5 cm) stored in a laboratory maintained at 25 ± 1 °C, with a relative humidity of 65 ± 5 % and photophase 12 hour.

LAMBDA-CYHALOTHRIN TREATMENT

The insects were exposed to the pyrethroid lambda-cyhalothrin at the recommended dose for the control of *A. grandis* at a concentration of 200 µL b.w. + 100 mL of distilled water, based on the label dose (300 mL pc/ha in 100 to 200 L of solution/ha, Karate Zeon 50® 50 g/L encapsulated suspension, Syngenta Crop Protection Ltda, São Paulo / SP-Brazil). Cotton flower buds were immersed in the insecticide solution and dried at room temperature for 1 hour. Distilled water was used for the control. After this period, the treated buds were offered as food for 24 hours to adult insects (24 h of age), separated by sex, individually placed in plastic containers (80 mL) lined internally with moistened filter paper and kept in a chamber. acclimatized under 12 h of photoperiod, 25 ± 0.5 °C and relative humidity of 70%. All evaluations were performed after 24 h of insecticide exposure. The bioassay was conducted in a completely randomized block design with three blocks of 50 insects for each treatment.

OXIDATIVE STRESS

For oxidative stress, lipid peroxidation performed by measuring thiobarbituric acid reactive substances (TBARS) was evaluated, according to Ohkawa et al. (1979). Five adult insects from each treatment were homogenized with 1.15% KCl + 3 mM EDTA in an ice bath. Subsequently, a reaction medium containing 0.3% thiobarbituric acid, 0.4% SDS and 7.5% acetic acid (pH 3.5) was added, and subsequently the mixture was heated to 95 °C for a hour. The samples were centrifuged and the supernatant had its absorbance measured at a wavelength of 535 nm. The data obtained were corrected for the protein concentration of the homogenate, measured according to Lowry et al. (1951).

COLLECTION OF ORGANS AND INCLUSION IN HISTORESIN

Ten digestive tubes, ten testes and ten ovarioles after 24 h were collected. The insects were immobilized at low temperature (4 °C), dissected under a stereoscopic microscope, fixed in 10% formalin for 24h and stored in 70% alcohol. The midguts of the insects were sectioned and, together with the testes and ovarioles, dehydrated in increasing baths of ethanol (70–100%) for 10 minutes each. The samples were impregnated in alcohol + historesin (1:1) for 24h and finally included in pure historesin (Leica®).

MAKING AND STAINING HISTOLOGICAL AND HISTOCHEMICAL SLIDES

In the total: µm thick slices were obtained from a Leica© RM 2035 microtome and subjected to toluidine blue staining techniques for morphological analysis. For histochemical analysis, periodic acid-Schiff (P.A.S.) (neutral carbohydrate detection) and Xylidine Ponceau (total protein detection) were used. All slides were examined with a Leica photomicroscope. ®.

PIXEL QUANTIFICATION

For the histochemical quantification of neutral carbohydrates and total protein from the pixels, the images were submitted to the image editor program GIMP® 2.8 (GNU Image Manipulation Program, UNIX platforms) to convert the digital images

into grayscale (black and white). This color segmentation allows the measurement of the number of pixels in the selected fabric (Temitope 2013). For each treatment, three different insect slides were used, where four sections were evaluated, totaling 12 sections.

PREPARATION OF IMMUNOHISTOCHEMICAL SLIDE

For immunohistochemical analysis (apoptosis and cell proliferation), the midguts were embedded in paraffin and 5 μm thick sections were made using a Minot microtome (LEICA RM 2035), placed in a water bath and collected on silanized slides. The images were captured and digitized using the Leica LAS Image software (Junqueira & Junqueira 1983, Solomon 2009).

MIDGUT IMMUNOHISTOCHEMISTRY

To detect apoptosis by DNA fragmentation, silanized slides containing midgut sections were submitted to the TUNEL test (Terminal Deoxynucleotidyl Transferase Uracil Nick End Labeling) (Gravrieli et al. 1992) following the protocol of the Apoptag Plus kit (Merck®). The sections were initially dewaxed and hydrated and then incubated in PBS for 5 minutes at room temperature. Afterwards, Proteinase K was applied on the slides for 15 minutes. The slides were washed in distilled water and incubated in hydrogen peroxide for 5 minutes at room temperature. The sections were washed in PBS and incubated in equilibrium buffer for 60 minutes at 4°C. Afterwards, the sections were incubated in TdT at 37°C for 1 hour in a humid chamber. The stop solution was applied for 10 minutes at room temperature, then the slides were washed in PBS and incubated in anti-digoxigenin. Slides were rinsed in PBS and sections revealed with diaminobenzidine chromogenic substrate (DAB, DakoCytomationTM) (±20 minutes), and counterstained with hematoxylin for 20 to 30 seconds. After that, the slides were washed in running water, dehydrated in increasing concentrations of alcohol and placed in xylene to be mounted and observed under a light microscope.

To determine cell proliferation, the sections were depafaraffinized, hydrated and subjected to antigenic recovery with citrate buffer (pH=6) in a water bath for 20 minutes at 100 °C, and after a 20-minute rest at room temperature, it was applied over 3% hydrogen peroxide for 30 minutes. Then, the sections were washed in Tris buffer and with PCNA (cellular proliferation nuclear antigen) antibody (Spring) at 1:100 dilution for 1 hour at room temperature. The sections were washed in Tris buffer and incubated with histofine for 30 minutes, subjected to diaminobenzidine chromogen (DAB, DakoCytomation™) and counterstained with hematoxylin.

The apoptotic index and cell proliferation were determined by the percentage of positive cells from the count of at least 500 nuclei/ treatment subdivided into 10 randomly chosen fields using the 40x objective (Losa et al. 2000, Wu et al, 2013).

QUANTIFICATION OF REGENERATIVE CELLS IN MIDGUT

Quantification was performed using two midline sections of the intestine of three insects per treatment (control and lambda-cyhalothrin 24 h), stained with toluidine blue. The images were captured using a Sony® video camera attached to an Olympus BX50 microscope. In each repetition, (blade with a cut section) regenerative cells were counted throughout the midgut.

STATISTICAL ANALYSIS

Data were compared by parametric t-test for two independent means at the 5% probability level in the SAS Institute program.

RESULTS

OXIDATIVE STRESS

Boll weevils treated with the insecticide for 24 hours showed increased lipid peroxidation compared to the control group, suggesting the occurrence of oxidative stress caused by the insecticide lambda-cyhalothrin (t value = |16.43|; P< 0.0001) (Table 1).

HISTOLOGY AND HISTOCHEMISTRY OF THE MIDGUT

Control adults, after 24 hours, had a midgut coated externally by two layers of striated muscle tissue, one internal, arranged circularly, and the other external, longitudinally. The epithelium is simple, composed of two types of cells: (1) the columnar digestive cells, with central spherical nuclei and a brush border of microvilli at their apex, and (2) stem or regenerative cells with basophilic cytoplasm, which occur individually. or grouped in nests, and in contact with the basal lamina. In addition, the presence of a peritrophic membrane in the intestinal lumen was observed, separating the food bolus from the epithelial cell layer (Fig. 1 A and B).

The midgut of adults treated with lambda-cyhalothrin showed, after 24 hours, preserved muscle tissue layers. However, there was a substantial increase in the number of regenerative and nesting cells (t value = |16.92|; P<0.0001) (Table 1). In the intestinal epithelium it is possible to identify mainly columnar cell disorganization, formation of cytoplasmic protrusions and vacuolization (Fig. 1 C and D).

The histochemical analysis of the epithelial cells for detection of neutral carbohydrates revealed a greater positive reaction to the PAS dye.In the control, the presence and uniform distribution of glycogen granules was observed throughout the cytoplasm of the columnar cells, in addition to the peritrophic membrane (Fig. 2 A). In the treatment with lambda-

Treatment	Oxidative Stress (Lipid Peroxidation)		
	mean (±EP)	t value	p value
Control	0,123 ± 0,006b	|16,43|	<0,0001
lambda-cyhalothrin	0,555 ± 0,025a		

Treatment	Regenerative Cell Nest (Number)		
	mean (±EP)	t value	p value
Control	8,4 ± 0,5b	|16,92|	<0,0001
lambda-cyhalothrin	20,6 ± 0,5a		

EP= average standard error;

t value= test T;

Table 1. Oxidative stress and number of regenerative cell nests in adults of *Anthonomus grandis* treated with lambda-cyhalothrin after 24 hours.

cyhalothrin, absence of the peritrophic membrane and distribution of glycogen granules throughout the epithelium was observed (Fig. 2 B). However, pixel analysis revealed a significant difference, with smaller amounts in the treated group compared to the control (t value= 13.72; P<0.0001) (Fig. 2 E).

For total proteins, the results revealed positive staining for Xylidine Ponceau (Fig. 2 C and D) and the analysis of the number of pixels showed a statistical difference, with lower amounts in the treatment compared to the control (t value=13.46; P<0.0001) (Fig. 2 E).

MIDGUT IMMUNOHISTOCHEMISTRY

Also in the midgut, positive staining (or brown nuclei) was observed in columnar epithelium cells of boll weevils treated with the insecticide, thus indicating the induction of cell death by apoptosis in this organ (Fig. 3 A and B). The apoptotic index revealed that in the treatments, more than 80% of the nuclei were positive, while in the control it was less than 5% (t value= |22.20|; P=0.0016) (Fig. 3 E).

Likewise, PCNA expression revealed positive staining (or brown nuclei), which

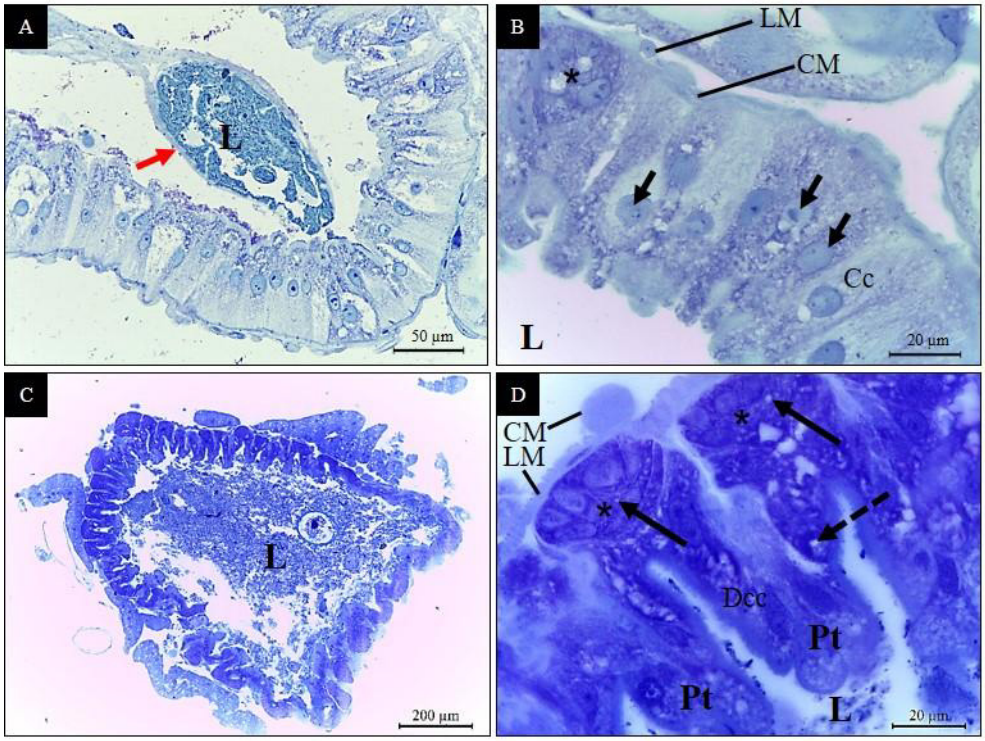

Figure 1. Cross section of the midgut of Anthonomus grandis adults. A and B - control. C and D - treatment with lambda-cyhalothrin after 24 hours. Toluidine Blue Stain. A – general view of the midgut with peritrophic membrane (red arrow) delimiting the food ingested in the lumen (L). B – midgut highlighted with columnar cells (Cc) and nuclei (arrow) in the median region; nests of regenerative cells (asterisk) and circular (CM) and longitudinal (LM) muscles lining the organ externally. C – general view of the intestine; observe a lot of material in the lumen region. D – Identify desquamation, disorganization (Dcc) and protrusions (Pt) of columnar cells, as well as cytoplasmic vacuoles (dashed arrow) and presence of vacuoles in the nests of regenerative cells (long arrow).

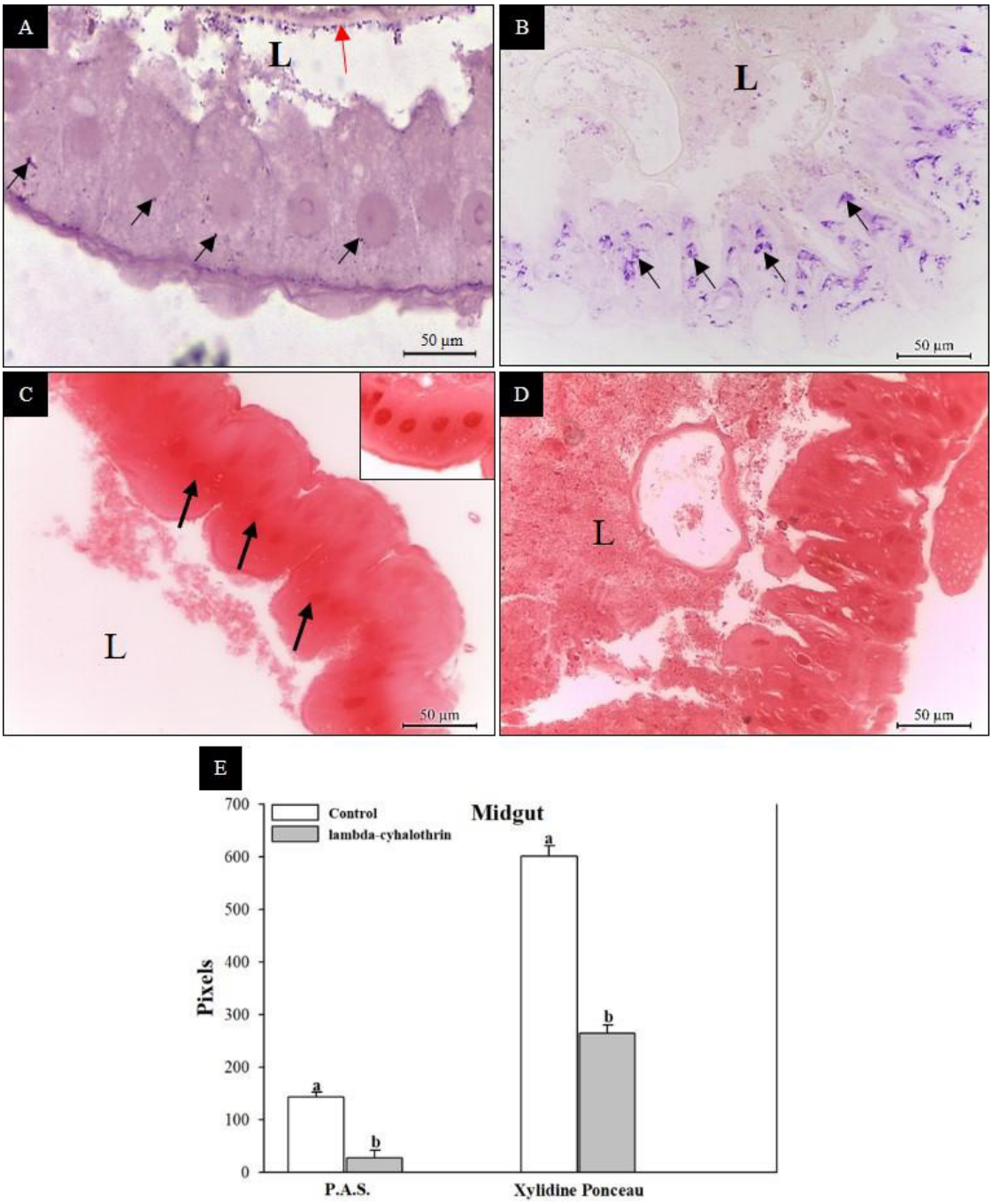

Figure 2. Histochemistry of the midgut of adults of *Anthonomus grandis* for neutral carbohydrates and total proteins. A - control. B - treatment with lambda-cyhalothrin after 24 hours. Periodic acid-Schiff stain (P.A.S.). – Identify the presence and uniform distribution of glycogen granules in the cytoplasm of cells (arrows) and peritrophic membrane (red arrow). B – Observe the uniform distribution of the glycogen granules and the absence of the peritrophic membrane. C - control. D - treatment with lambda-cyhalothrin after 24 hours. Xylidine Ponceau stain. L - lumen; Arrows – cores. E – Average number of pixels. Bars followed by different letters differ significantly by the t test at the 5% probability level.

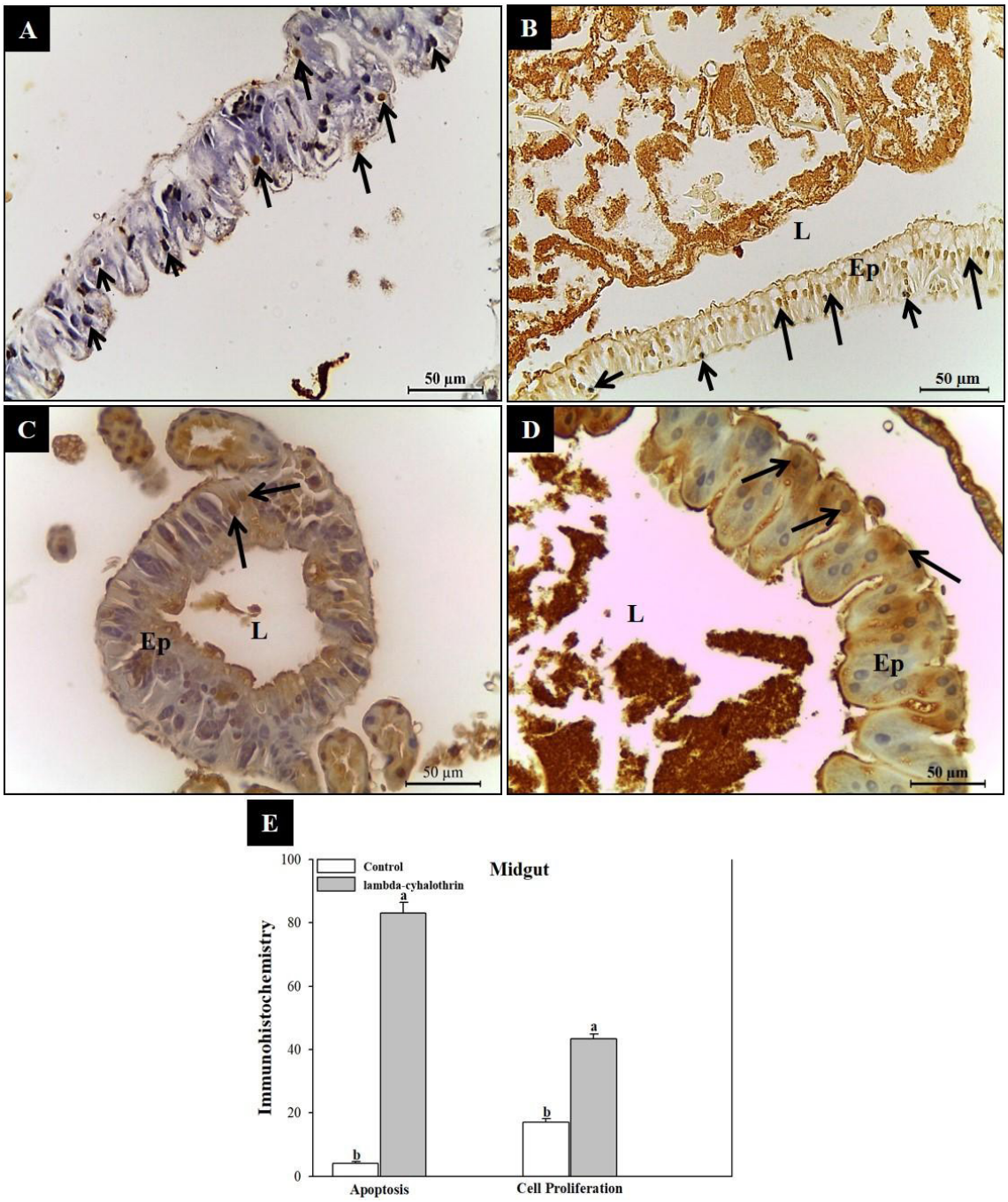

Figure 3. Immunohistochemistry of the midgut of adults of *Anthonomus grandis* for apoptosis and cell proliferation. A and B - TUNEL assay for detection of apoptosis. A - Control. B - treatment with lambda-cyhalothrin after 24 hours. C and D - Expression of the cell proliferation marker PCNA. C - control. D - treatment with lambda-cyhalothrin after 24 hours. E - Apoptotic index and cell proliferation. Bars followed by different letters differ significantly by the t test at the 5% probability level. Positive nucleus (long arrow), normal nucleus (short arrow), epithelium (Ep), Lumen (L).

indicates cell proliferation in the midgut (Fig. 3 C and D), which corroborates the PCNA index, which showed a higher percentage of nuclei positive in the treated group (>4'0%) compared to the control (<20%) (t value=|14.19|; P<0.0001) (Fig. 3 E).

HISTOLOGY AND HISTOCHEMISTRY OF THE TESTICLES

The control group presented testis covered by connective tissue (peritoneal covering) throughout, with formation of several testicular follicles from septate invaginations (Fig. 4 A). In addition, the presence of many spermatozoa and some cysts (containing sperm lineage cells) was observed. Testicles treated with lambda-cyhalothrin revealed a greater proportion of cysts and fewer sperm bundles when compared to the control, in addition to a thicker peritoneal covering (Fig. 4 B).

The histochemistry of the testes showed a positive reaction to the dyes (Fig. 4 C–F), however, the quantification of pixels revealed a significant difference, being lower in the group treated with the insecticide for carbohydrates (t value=|171.97|; P<0.0001) and proteins (t value=|102.21|; P<0.0001) when compared to the control (Fig. 4 G).

HISTOLOGY AND HISTOCHEMISTRY OF OVARIOLES

Histology revealed that the ovarioles are lined by a connective tissue sheath, have a well-developed yolk region, a layer of follicular cells surrounding each forming oocyte and germinal vesicle (Fig. 5 A). The ovarioles after treatment with lambda-cyhalothrin were morphologically similar to the control, however, the germinal vesicle was not identified in the oocytes (Fig. 5 B).

Histochemically, there was positive staining for P.A.S. and Xylidine Ponceau in both the control and treatment groups (Fig. 5 C–F). However, the analysis of pixels revealed statistical differences, in which there were reductions in the treatment with the insecticide of carbohydrates (t value =|213.33|; P<0.0001) and proteins (t value=105.25; P<0.0001) (Fig. 5 G).

DISCUSSION

The midgut damage observed in this research indicates that the pyrethroid lambda-cyhalothrin overcame the physical barrier promoted by the peritrophic membrane. Hegedus (2009), emphasizes the protective characteristics played by this membrane to xenobiotics. This way, the protrusions, disorganizations and vacuolizations in the columnar cells show the adverse effect of the insecticide. Spies & Spence (1985) stated that alterations in columnar cells stimulate factors related to epithelial renewal through proliferation and differentiation of regenerative cells. This corroborates the data of this research, in which a substantial increase in the number of regenerative cells with nest formation was observed in several areas of the intestine. The formation of nests is an insect response to cellular damage and the grouping of regenerative cells in nests aims to maintain the characteristics of stem cells capable of proliferation and differentiation, as stated by Illa-Bochaca & Montuenga (2006).

The damage to the epithelium can still be proven by the cell proliferation index. More than 40% of the nuclei were positive after treatment with lambda-cyhalothrin, while in the control group this value was less than 20%. Therefore, it is evident that the damage promoted by the pyrethroid induces the proliferation of intestinal cells. Despite this, the attempt at epithelial cell repair would possibly not be successful, because the apoptotic index showed that more than 80% of the cells were positive, making the entire process unfeasible. According to Chiang (1986), in order to recover

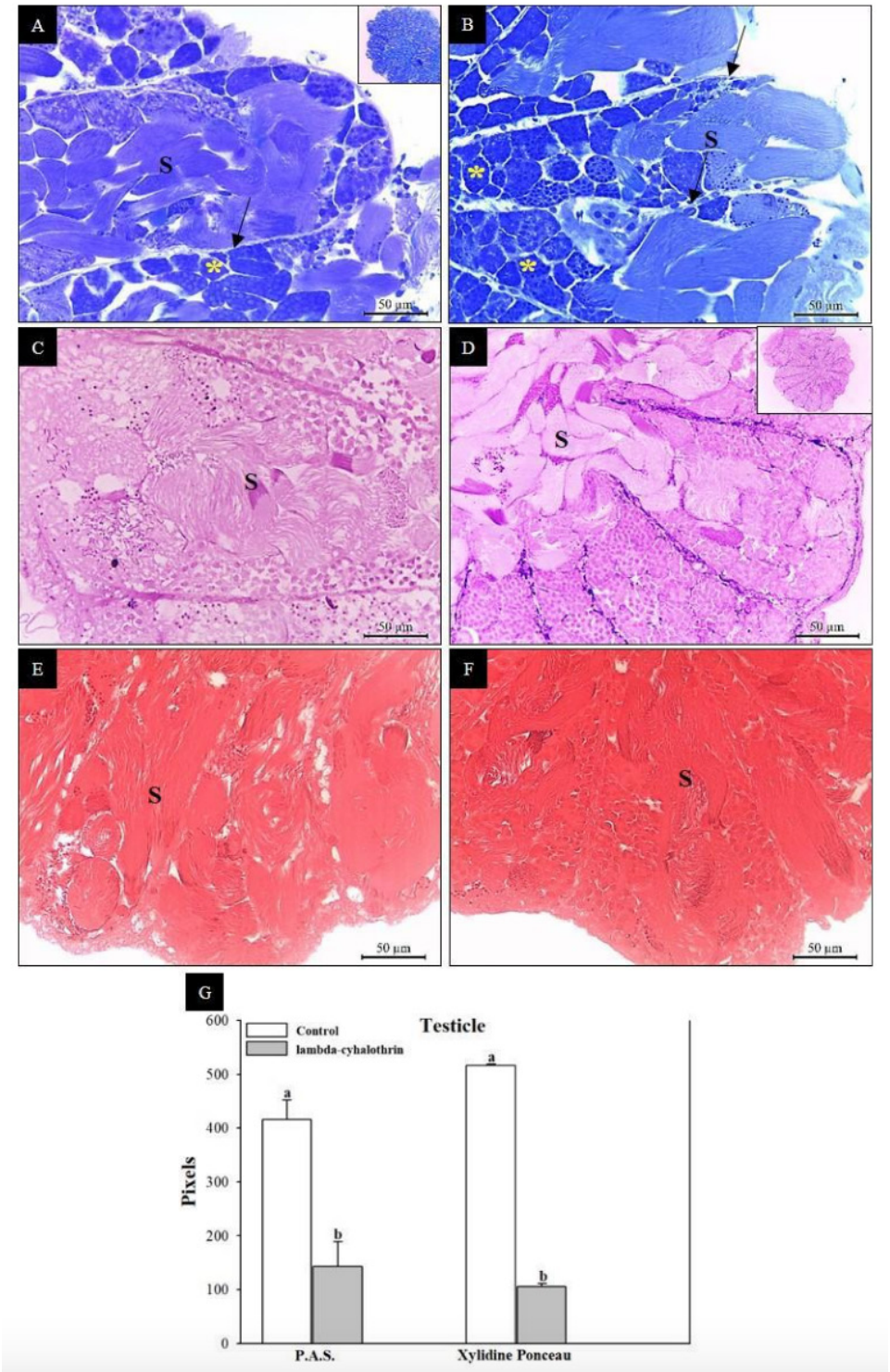

Figure 4. Testicles of adults *Anthonomus grandis.* A, C and E - control. B, D and F - treatment with lambda-cyhalothrin after 24 hours. A – observe follicle with the presence of more sperm in relation to the number of cysts. B – identify follicles with fewer sperm compared to the number of cysts and with a thick peritoneal covering (arrow). Toluidine Blue Stain. Testis histochemistry for detection of neutral carbohydrates (C and D) and total proteins (E and F), Periodic Acid Schiff (P.A.S.) and Xylidine Ponceau stain, respectively. S, sperm; Arrow, peritoneal coverage; Asterisk, cysts. G – average number of pixels. Bars followed by different letters differ significantly by the T test at the 5% probability level.

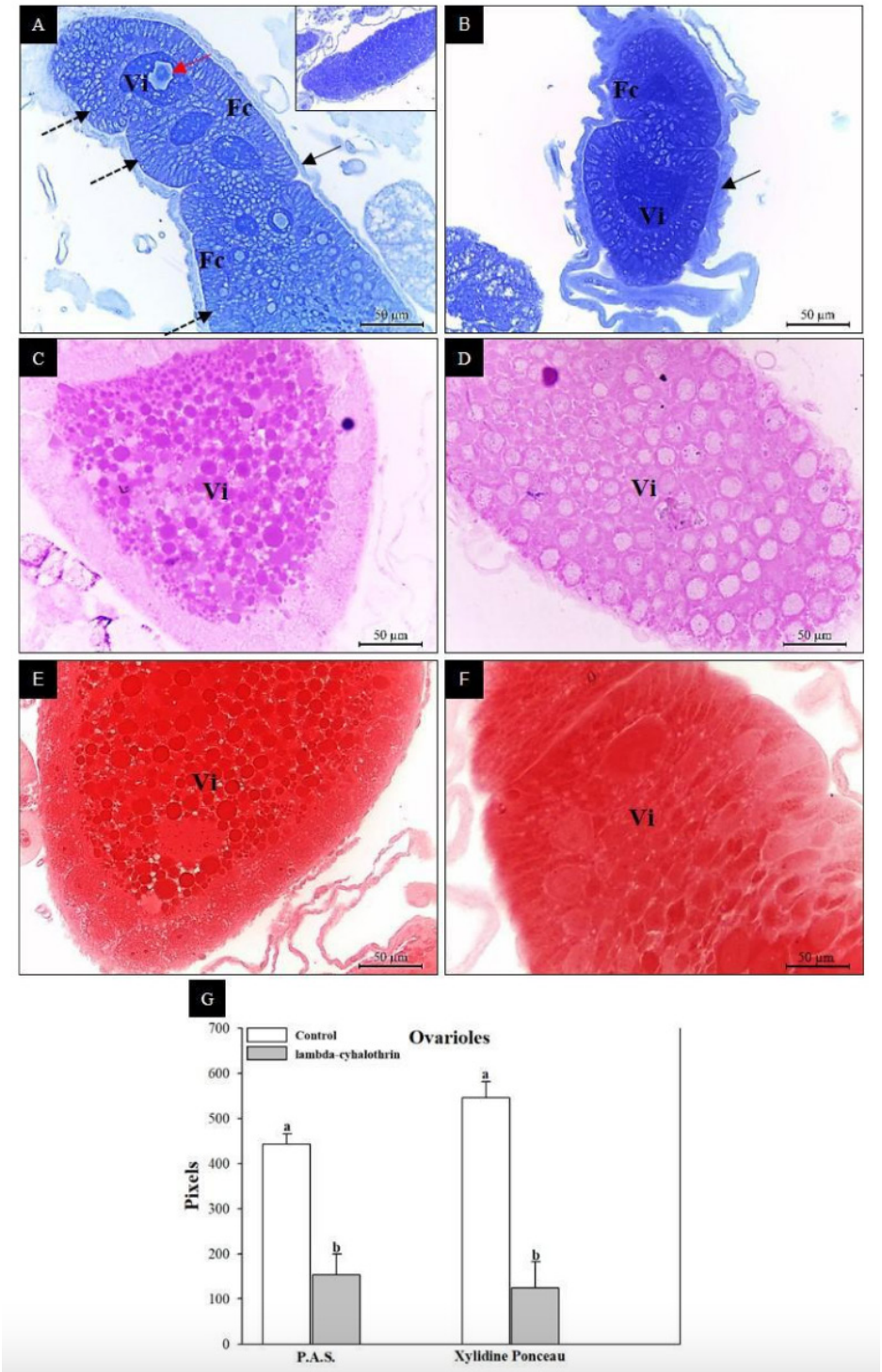

Figure 5. Ovarioles of adults of *Anthonomus grandis.* A, C and E - control. B, D and F - treatment with lambda-cyhalothrin after 24 hours. A – observe follicle with formation of oocytes filled with yolk and coated with follicular cells. B – analyze oocytes with smaller amounts of yolk and without the germinal vesicle. Toluidine Blue Stain. Ovariole histochemistry for detection of neutral carbohydrates (C and D) and total proteins (E and F), Periodic Acid Schiff (P.A.S.) and Xylidine Ponceau stain, respectively. Vi, calf; Fc, follicular cells; Arrow, connective tissue sheath; Dotted arrow, oocytes in formation; Red arrow, germinal vesicle. G – average number of pixels. Bars followed by different letters differ significantly by the T test at the 5% probability level.

the injured epithelium, desquamation of cells in the intestine may occur, which, although considered harmful, favors proliferation.

Regeneration indicates that the damage is transient and reversible, otherwise, in severe situations, it may not be accompanied by cellular repair and cause the organism to die (Miller & Zachary 2017). This can be explained by the large percentage of nuclei in apoptosis and by the increase in oxidative stress, since both are related. Damage to digestive cells can increase this stress and cause accumulation of reactive oxygen species (ROS), leading to loss of basic functions of cellular constituents such as DNA, proteins, carbohydrates and lipids (Bi & Felton 1995, Velki et al. 2011). On the caterpillar: *Helicoverpa armígera,* the pyrethroids permethrin and fenvalarate also increased oxidative stress (Akbar et al.2012), showing the potential of this class of insecticides to increase this phenomenon.

After contamination by insecticides, the organism needs to maintain the physiological balance between repair and the use of energy that maintains vital processes. The above mentioned effects impair the absorption of ingested nutrients (Bi & Felton 1995, Velki *et al.* 2011) and tissue regeneration in the intestine serves precisely to prevent the digestive processes from collapsing. The reductions in carbohydrates and proteins in the intestine show the critical situation that the insecticide promoted in this beetle, since carbohydrates act as the main source of energy, being necessary in many processes, including protection against xenobiotics. (Arrese *et al.* 2010, Rosas-Mejía *et al.* 2015). Carbohydrate reductions were also reported in all wasp stages and sexes: *Piimpla turionellae* (Hymenoptera: Ichneumonidae) exposed to various sublethal doses of the pyrethroid cypermethrin (Sak et al. 2006).

For Costa et al. (2004) and Milano et al. (2010), the decrease in reproduction rates is usually associated with eating disorders and nutritional deficiency. The higher proportion of cysts in testes in relation to sperm bundles, after treatment with lambda-cyhalothrin, suggests an adverse effect on spermiogenesis. In addition, the thicker peritoneal covering lining each testicular follicle indicates compromised integrity in the gonad structure or may represent difficulties in absorbing nutrients from the hemolymph.

In ovarioles, no yolk reduction or changes in follicular cells were identified, but absence of the germinal vesicle. The maturation of oocytes in the ovarioles encompasses several stages and one of them is the transformation of the nuclei into a germinal vesicle that is positioned in the central region (Ullmann 1973, Parthasarathy et al. 2010). Furthermore, Chapman (2013) says that yolk deposition for oogenesis is directly related to the germinal vesicle. Therefore, the non-formation of this vesicle indicates the possibility of complications in the formation of the egg at some point.

The reduction in the levels of neutral carbohydrates and total proteins in the testes and ovarioles after treatment with lambda-cyhalothrin supports the idea that the absorption process in the intestine was affected. In addition, it may also be related to the impairment of the direct absorption of nutrients from the hemolymph by the gonads as a result of the aforementioned morphological alterations. Decreased protein levels in insect ovarioles can be attributed to increased activity of follicular cells, which, in addition to protecting and nourishing oocyte growth, are also involved in vitellogenesis (McKearin et al. 2005, Swevers et al. 2005). The respective histochemical results are of paramount importance, as carbohydrates and proteins are metabolic precursors of many substances, and their reduction leads to effects on various physiological processes.

The results of this research corroborate those reported in others for the same species. Cunha et al. (2015) showed that pymetrozine promotes histological changes in the intestine after 48 and 144 hours, such as protrusions and detachment of columnar cells, vacuolated regenerative cells and removal of the epithelial lamina in some areas of the muscle layer, in addition to reductions in carbohydrates and lipids. Costa et al. (2017), proved that lufenuron after 24 and 120 hours caused histological changes such as disorganization, detachment and vacuolization of columnar cells and an increase in regenerative cells forming nests. In addition, the authors reported histochemical changes in carbohydrate and protein levels in both the intestine and the gonads, as well as reduced yolk and sperm. Cruz et al. (2021) reported that, in females, lufenuron, after 48 hours, caused disorganization of columnar cells, reduction of yolk and ovariole proteins and deregulation of vitellogenin expression, culminating in egg inviability.

These studies, as well as the present research, show that the intestine and gonads of this insect are sensitive to different types of stress and proves that they can be used as a target organ for management strategies for this pest. The reflection of nutrient absorption efficiency is very important in holometabolous insects, as they need adequate nutritional acquisition to maintain their reproductive potential (Behmer 2008, Cruz et al. 2017), essential for the establishment of a pest in the agroecosystem. Engleman (1998) says that the quantity and quality of nutrients available to ovarioles during differentiation can lead to changes in vitellogenesis, egg maturation and egg production. From this perspective, nutrients are of fundamental importance for the development, defense capacity and, above all, reproduction of insects (Klowden 2007, Gullan & Cranston 2012, Chapman 2013).

Thus, it is concluded that the pyrethroid lambda-cyhalothrin increases oxidative stress and promotes morpho-histochemical and immunohistochemical pathological changes in the midgut epithelium and gonads, with consequences for gametogenesis in adults of: *A. grandis*.

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