Cytotoxic Effects of Thiamethoxam in the Midgut and Malpighian Tubules of Africanized *Apis mellifera* (Hymenoptera: Apidae)

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ABSTRACT   Due to its expansion, agriculture has become increasingly dependent on the use of pesticides. However, the indiscriminate use of insecticides has had additional effects on the environment. These products have a broad spectrum of action, and therefore the insecticide affects not only the pests but also non-target insects such as bees, which are important pollinators of agricultural crops and natural environments. Among the most used pesticides, the neonicotinoids are particularly harmful. One of the neonicotinoids of specific concern is thiamethoxam, which is used on a wide variety of crops and is toxic to bees. Thus, this study aimed to analyze the effects of this insecticide in the midgut and Malpighian tubule cells of Africanized *Apis mellifera*. Newly emerged workers were exposed until 8 days to a diet containing a sublethal dose of thiamethoxam equal to 1/10 of LC_{50} (0.0428 ng a.i./l L of diet). The bees were dissected and the organs were processed for transmission electron microscopy. The results showed that thiamethoxam is cytotoxic to midgut and Malpighian tubules. In the midgut, the damage was more evident in bees exposed to the insecticide on the first day. On the eighth day, the cells were ultrastructurally intact suggesting a recovery of this organ. The Malpighian tubules showed pronounced alterations on the eighth day of exposure of bees to the insecticide. This study demonstrates that the continuous exposure to a sublethal dose of thiamethoxam can impair organs that are used during the metabolism of the insecticide. *Microsc. Res. Tech. 77:274–281, 2014.*   © 2014 Wiley Periodicals, Inc.

INTRODUCTION

The honeybee *Apis mellifera* was introduced in Brazil in 1839, and today, the Africanized *A. mellifera* is the result of a cross between *A. mellifera scutellata* (originally from Africa), *A. mellifera mellifera* (originally from Northern Europe), *A. mellifera ligustica* (originally from Italy and Northern Yugoslavia), and *A. mellifera iberica* (from Western Europe) (Ruttner, 1988).

These bees have great economic importance because 90% of fruit plants directly depend on bee pollination for their evolutionary success. In total, 40,000 of the 170,000 species of plants pollinated by bees need *A. mellifera* to aid in their self-perpetuation (Tautz, 2008). Approximately one-third of the feed used in the world depends on pollination by bees, and in the United States, the species *A. mellifera* alone generates about $14 billion per year due to its importance in pollination (Morse and Calderone, 2000; Willians, 1994).

The reduction in the number of pollinators, especially bee species, has become a major problem for beekeepers. In recent years, scientists have identified a phenomenon called "colony collapse disorder" (CCD) in the United States that caused beekeepers to lose 80–100% of their hives. In many cases, the forager workers, which are responsible for collecting nectar and pollen, lose the ability to fly or become disoriented and cannot return to the hive (Oldroyd, 2007). The causes of this high mortality are not well understood. Many stress factors, acting alone or together, can weak the colony. Some possible explanations for CCD include radiation emitted by cellular phones, genetically modified crops, pests, diseases caused by viruses and bacteria, environmental factors, and the excessive use of pesticides (Ratnieks and Carreck, 2010).

Mullin et al. (2010) reported that there is a direct association between exposure of bees to pesticides, the occurrence of CCD, and the decline of the pollinators.

According to many authors one of the hypotheses for the emergence of CCD is the intoxication of bees by insecticides that are increasingly used in agriculture (Croft, 1990; Desneux et al., 2007; Malaspina and Silva-Zacarin, 2006; Thompson, 2003; Tremolada et al., 2010).

Among the different types of insecticides, the neonicotinoids, including imidacloprid, acetamiprid, thiacloprid, dinotefuran, and thiamethoxam, represent an important group of neurotoxins that act as an agonist of the nicotinic acetylcholine receptors of insects (Elbert et al., 2008; Matsuda et al., 2001). Neonicotinoid insecticides are widely used in agriculture, especially against sucking insects. However, these insecticides eventually also affect non-target insects such as bees. Neonicotinoid use is widespread because these compounds have a high selectivity for the nicotinic receptors of insects (El Hassani et al., 2008).

According to the IUPAC (International Union of Pure and Applied Chemistry), the chemical name of thiamethoxam is 3-(2-chloro-thiazol-5-yl-methyl)-5-methyl [1,3,5] oxadiazinan-4-ylidene-*N*-nitroamine. Antunes-Kenyon and Kennedy (2001) found that, independent of the form of contamination, thiamethoxam was extremely toxic to bees, causing the death of more than 80% of the specimens after 3 days. Additional studies also confirmed the high toxicity of this compound and showed that regardless of the exposure mode (spray, intake, or residue on the surface of the culture), thiamethoxam (37.5 ng a.i./µL) is extremely toxic to bees. This chemical had a LT_{50} on average of 3.57 h (Carvalho et al., 2009).

In a previous study, Oliveira et al. (2013) assessed the toxic effects of thiamethoxam on newly emerged worker bees of the Africanized honeybee. This study determined the lethal concentration 50 (LC_{50}) of thiamethoxam (4.28 ng a.i./µL of diet) and showed that when the bees were exposed to 1/10 of the LC_{50}, they had a 41.2% reduction of lifespan. Additionally, this study used morphological analysis to show that some brain structures (mushroom bodies and optical lobes) and the digestive and regenerative cells of the midgut from exposed bees showed morphological and histochemical alterations.

This study demonstrates that, although the action of thiamethoxam occurs in the nervous system, secondary targets may also be affected by this compound. Therefore, it is important to analyze the cytotoxicity of thiamethoxam in tissues reached via the metabolism of contaminated food containing insecticides.

Thus, one important organ for toxicity analysis is the midgut, since it is responsible for digestion and absorption of ingested food. Moreover, it is one of the primary sources of contact when the insect comes in contact with the insecticide orally.

The alimentary canal of bees is divided into three regions: stomodeum or foregut, midgut or ventriculus, and proctodeum or hindgut. The midgut is the portion of the digestive tract where most of the digestion and absorption of the food and products of digestion occurs (Cavalcante and Cruz-Landim, 1999).

Another important organ for toxicity analysis is the Malpighian tubules, responsible for the excretion of substances. Thus, this organ comes into contact with the thiamethoxam and its metabolites.

The excretory system is the primarily responsible for maintaining homeostasis. In insects, it consists, in most cases, of a variable number of Malpighian tubules, which have their point of confluence in the intestine, but are free in the hemolymph, where they end in a blind bottom. The tubules produce a filtrate from the hemolymph, called the primary urine, which initially presents as an iso-osmotic fluid, which serves as a carrier of waste products, such as toxic compounds and excess of ions. As it passes through the interior of the tubule, it is modified by reabsorption of water and other essential substances to the body, even turn into the urine that will be eliminated (Cruz-Landim, 2009; Habib, 2003).

Some authors showed the alterations caused by insecticides on midgut cells and Malpighian tubules cells (Cruz et al., 2010; Ferreira et al., 2013; Jesus et al., 2005; Oliveira et al., 2013, Rossi et al., 2013). Kakamand et al. (2008) in a study that aimed to analyze the effects of acute oral toxicity of insecticides including thiamethoxam showed that 0.125 ng a.i./µL caused high mortality (96.67%) of *A. mellifera* and causes disruption in midgut cells.

The aim of this study was to analyze the cytotoxic effects of thiamethoxam in the midgut, which is responsible for the absorption of nutrients, and the Malpighian tubules, which constitutes the excretory and osmoregulatory system of the Africanized *A. mellifera*.

MATERIAL AND METHODS
Honeybee Collection

To obtain the newly emerged Africanized honeybee, three frames with sealed broods (near of adult emergence) were collected on a queen-right colony and kept in a controlled climate room ($34 \pm 2°C$, relative humidity (RH) of $80 \pm 10\%$ and in darkness). Using this procedure, we obtained specimens of a known age (0–24 h). For all experiments, adults were put into disposable cages ($11 \times 11 \times 7$ cm^3), fed a sucrose + H_2O solution (1:1), and maintained at $32 \pm 2°C$ with a $70 \pm 10\%$ of RH in darkness.

Bioassays of Intoxication with Thiamethoxam

The analytical standard thiamethoxam (92.5% of purity) was obtained from Syngenta Crop Protection (Brazil). The honeybee intoxication assay with a sublethal dose of thiamethoxam was performed using the LC_{50} of 4.28 ng a.i/µL diet obtained from our previous study (Oliveira et al., 2013). From the stock solution (1,000 ng a.i./l L acetone), we prepared a diet with thiamethoxam final solutions of 1/10 LC_{50}. A total of 75 newly emerged honeybees were equally divided into three disposable cages (250 mL). The bees were collectively fed the contaminated syrup, with the total volume adjusted so that each bee could daily consume 10 µL of sucrose solution containing 0.0428 ng/µL thiamethoxam per bee (i.e., 250 µL of enriched diet per cage). Therefore, each bee ingested 0.428 ng/µL thiamethoxam per day (1/10 of LC_{50}) (CEB, 2003). Every day, the number of dead bees was counted, and the total volume of syrup was adjusted to the number of remaining live bees. An experimental control without acetone was used in these experiments, where the bees were fed only with sucrose and H_2O (1:1).
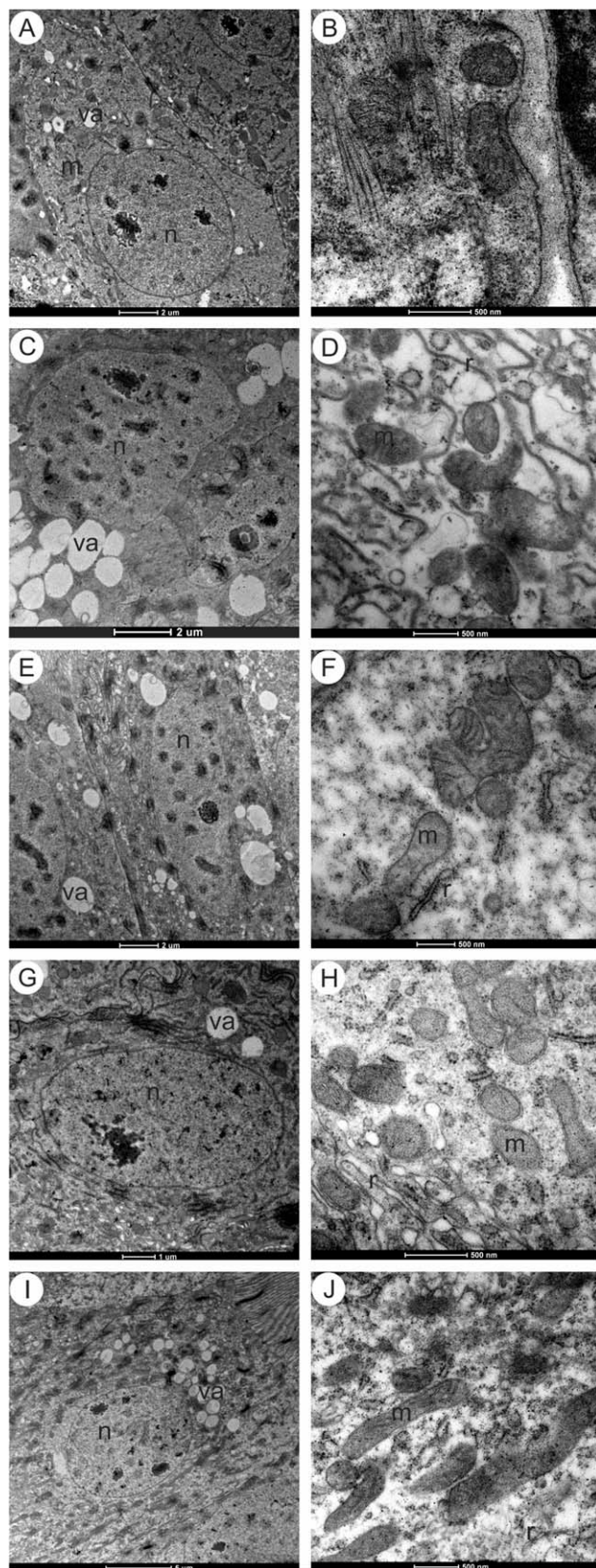
Fig. .1.

Transmission Electron Microscopy

Honeybees were collected at intervals of 1, 3, 5, and 8 days after the start of the bioassay. Five samples were taken per treatment/time. Midguts and Malpighian tubules of exposed and control bees were removed in buffer solution containing 20 mM Na_2HPO_4/KH_2PO_4, pH 7.4 + 130 mM of NaCl (modified of Dade, 2009) and fixed in modified Karnovsky (4% paraformaldehyde, 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2), during 2 h at 4°C. Once fixed, the organs were washed in the same buffer and postfixed in 1% osmium tetroxide in the same buffer for 2 h at room temperature and again washed in the buffer. The post-fixed organs were contrasted *en bloc* in 2% uranyl acetate in 10% acetone for 2 h and then dehydrated in a standard acetone series. The material was embedded in Epon–Araldite resin. Ultrathin sections cut on a Porter Blum ultramicrotome MT2 were stained with lead citrate for 15 min and examined under the transmission electron microscopy (TEM; Philips CM100).

RESULTS
Midguts

The midguts of the Africanized honeybee from different control groups for all periods analyzed in this study showed typical characteristics of this structure: preserved digestive cells usually with nuclei of spherical shape, organelles with no alterations, especially mitochondria with preserved double membranes and evident cristae, and rough endoplasmic reticules (Figs. 1A and 1B and 3A). Vacuoles were also observed in the cytoplasm. The control groups that were treated with acetone also showed these same characteristics.

The ultrastructural analysis of midguts from honeybees exposed to a concentration corresponding to the $CL_{50/10}$ of thiamethoxam showed that the effect of this chemical was most evident in honeybees that were exposed for 1 day (Fig. 3B). In the digestive cells, mitochondria exhibited decreased mitochondrial cristae (Fig. 1D). The rough endoplasmic reticulum contained dilated cisterns that were disorganized and had a lower amount of attached ribosomes (Fig. 1D). Additionally, the nuclei of these cells were irregularly shaped (Fig. 1C).

Fig. 1. TEM of digestive cells from the midgut of *A. mellifera* exposed or not exposed to a sublethal dose of thiamethoxam. **A,B:** Digestive cells of a honeybee from the control group without solvent after 5 days and with solvent after 8 days, respectively, showing digestive cells with typical ultrastructural morphology: regular nuclei (n) with decondensed chromatin, cytoplasmic vacuolation (va), and well defined mitochondria (m) with cristae. **C,D:** Digestive cells of a honeybee exposed to $LC_{50/10}$ for 1 day. Note the irregular shape of the nuclei (n), the presence of vacuoles (va), mitochondria (m) exhibiting decreased mitochondrial cristae and the rough endoplasmic reticulum (r) with fewer ribosomes attached. **E,F:** Digestive cells of a honeybee exposed to $LC_{50/10}$ for 3 days, showing the presence of a greater amount of vacuoles (va) in cytoplasm. The nuclei (n) and the mitochondria (m) show no alterations. **G,H:** Digestive cells of a honeybee exposed to $LC_{50/10}$ for 5 days. Note the regular nuclei (n) with decondensed chromatin, mitochondria (m) exhibiting cristae and double membrane, rough endoplasmic reticulum (r) with ribosomes and vacuoles (va) in cytoplasm. **I,J:** Digestive cells of a honeybee exposed to $LC_{50/10}$ for 8 days. Observe in (I) that the cell is ultrastructurally intact, showing mitochondria with cristae that are well defined and the rough endoplasmic reticulum with no alterations.

Digestive cells of bees exposed to the insecticide for 3 days had nuclei with a more regular shape (Fig. 1E) and mitochondria (Fig. 1F) with better defined cristae than the cells in bees exposed for 1 day (see Fig. 1C for nucleus comparison and 1D for mitochondria comparison of bees exposed for 1 day). There were vacuoles in the digestive cells of individuals exposed to the insecticide (Fig. 1E).

After 5 days of continuous exposure to the insecticide, the digestive cells contained nuclei with decondensed chromatin and no irregularities in their shapes (Fig. 1G). The mitochondria of all groups at this time point had intact morphology, which included the presence of mitochondrial cristae and a double membrane (Fig. 1H). The rough endoplasmic reticulum appeared to be reorganized and the associated ribosomes were visualized in this structure (Fig. 1H). Cytoplasmic vacuoles were also visible in both the control and $CL_{50/10}$ group at 5 days (Fig. 1G).

On the eighth day of analysis, the digestive cells of the exposed group showed intact morphology (Figs. 1I and 3C). The nuclei were similar to the nuclei of the control group (Fig. 1A) and showed no signs of chromatin compaction. The mitochondria exhibited cristae that were clearly defined and an organized rough endoplasmic reticulum was observed (Fig. 1J).

Malpighian Tubules

As described for the midguts, typical ultrastructural characteristics were observed in the Malpighian tubules for the control groups at each time point (Fig. 3D). The epithelium appeared to be well preserved and showed no alterations in the basal labyrinth (Fig. 2A) and the normal presence of microvilli in the apical portion (Fig. 2C). Many mitochondria with cristae could be visualized in the cells (Fig. 2B). Similar to the midgut, the control with acetone showed no ultrastructural alterations and had the same characteristics as the control with no solvent.

On the first day of exposure, a distinct disorganization of the basal labyrinth (Figs. 2D and 3E) was observed when compared with the control group (Fig. 2A). The mitochondria remained well defined and the cristae were visible (Fig. 2E) and the microvilli showed no alterations (Fig. 2F).

On the third day, the most pronounced effect resulting from exposure to $CL_{50/10}$ was the increase in the amount of smooth endoplasmic reticulum (Figs. 2H and 3F) in the cytoplasm. As observed on the first day of exposure, the basal labyrinth showed characteristics of degeneration (Fig. 2G). The mitochondria and microvilli exhibited the same characteristics as the control group that was not exposed to the insecticide (Figs. 2I and 2J).

The fifth day of observation revealed the disruption of the cytoplasm and the basal labyrinth as well as the loss of cytoplasmic organelles (Fig. 2K). At this exposure time, mitochondrial alterations were also observed. The mitochondria were greatly dilated (Fig. 2L) compared with the control group and with the other groups previously exposed to thiamethoxam. Additionally, small alterations could be seen in the microvilli of the Malpighian tubules with 5 days of exposure to $CL_{50/10}$ of the insecticide (Fig. 2M).

On the eighth day of exposure, the ultrastructural analysis showed a near complete loss of the basal labyrinth of the Malpighian tubules, and spaces (electron lucid regions) could be observed in the cytoplasm, which may have resulted from the disruption of the mitochondria (Fig. 2N). In addition, the nuclei contained condensed chromatin (Fig. 2O), and the microvilli were dilated at their apical portion (Figs. 2P and 3G).

Figure 3 shows a schematic representation summarizing the alterations observed in the midgut and Malpighian tubules cells during the exposure of Africanized honey bees to thiamethoxam.

DISCUSSION

The results in the present work showed that exposure to a sublethal dose of thiamethoxam is cytotoxic to the midgut and to the Malpighian tubules of the bee *A. mellifera*. Thiamethoxam residue counts in pollen have been measured to be as high as 53 mg/kg (Mullin et al., 2010). Therefore, it is highly probable that non-target insects such as honey bees come in contact with thiamethoxam at different levels of exposure.

The cytotoxicity of thiamethoxam to these organs can be explained because this insecticide is a neonicotinoid that acts systemically on the plant. Therefore, bees may feed on nectar and pollen containing this insecticide. Although the main target of this insecticide is the nicotinic acetylcholine receptors (nAChR) that are present in the nervous system of insects (Tan et al., 2007; Tomizawa and Casida, 2003), secondary targets may also be affected, such as the organs involved in the metabolism of the compound. Moreover, ultrastructural analysis showed the cellular structures that are affected after exposure of Africanized bees to a sublethal dose of thiamethoxam. Knowledge of these structures allows a better understanding about the mode of action of thiamethoxam in the cell and provides evidence that toxins can affect tissues in pesticide-exposed organisms.

The ultrastructural changes observed in the midgut of honeybees exposed to a sublethal dose of thiamethoxam included alterations of the nuclei, mitochondria, and rough endoplasmic reticulum. The alterations of these structures indicate that the midgut epithelium suffers damage that compromises the cellular energy and protein production organelles and thereby changes the physiology of the cells. Other studies also showed morphological changes in the midguts of adults and larvae of *A. mellifera* exposed to insecticides. Oliveira et al. (2013), in a morphological study of the midgut of Africanized honeybee with a dose of 0.428 ng/μL of thiamethoxam per day, showed that thiamethoxam causes a reduction in the number of regenerative cells in the epithelium in addition to inducing cytoplasmic vacuolization. Honeybee larvae exposed to an insecticide (diet with 400 ppm of imidacloprid) showed significant apoptotic cell death in the midgut (Gregorc and Ellis, 2011). Fipronil and acid boric also increase cytoplasmic vacuolization, chromatin compaction, and cellular elimination in the midgut of larvae of *A. mellifera* (Cruz et al., 2010).

The ultrastructural changes observed in midgut cells in this study were more evident in the individuals exposed for 1 day to the insecticide. The midguts of
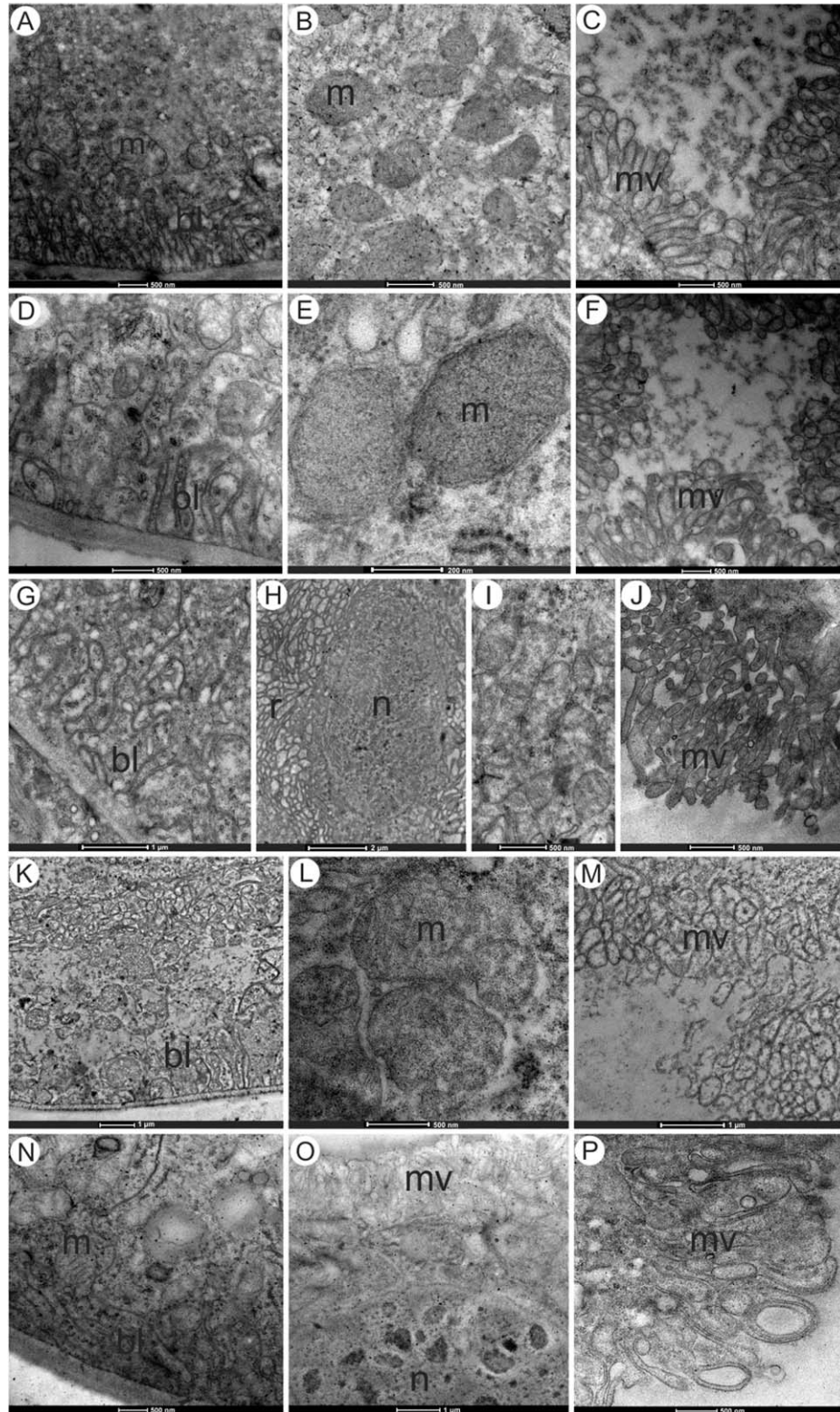
Fig. 2. TEM of Malpighian tubules of *A. mellifera* exposed or not exposed to a sublethal dose of thiamethoxam. **A–C:** Malpighian tubules of a honeybee from the control group without solvent after 1 and 5 days and with solvent after 1 day, respectively, showing cells with typical ultrastructural morphology. Note the invaginations of the plasmatic membrane constituting the basal labyrinth (bl), well defined microvilli (mv), and mitochondria (m) with cristae. **D–F:** Malpighian tubules of a honeybee exposed to $LC_{50/10}$ for 1 day. Observe in (D) the disorganization of the basal labyrinth (bl). Mitochondria (m) appeared with cristae and microvilli with no alterations. **G–J:** Malpighian tubules of a honeybee exposed to $LC_{50/10}$ for 3 days. Note the abundance of smooth endoplasmic reticulum (ser). **K–M:** Malpighian tubules of a honeybee exposed to $LC_{50/10}$ for 5 days. Observe the disruption of the cytoplasm and of the basal labyrinth in K and the dilated mitochondria (m) in (L). **N–P:** Malpighian tubules of a honeybee exposed to $LC_{50/10}$ for 8 days, showing the loss of the basal labyrinth (bl), the condensed chromatin (n), the disruption of mitochondria (m), and the dilated microvilli (mv).
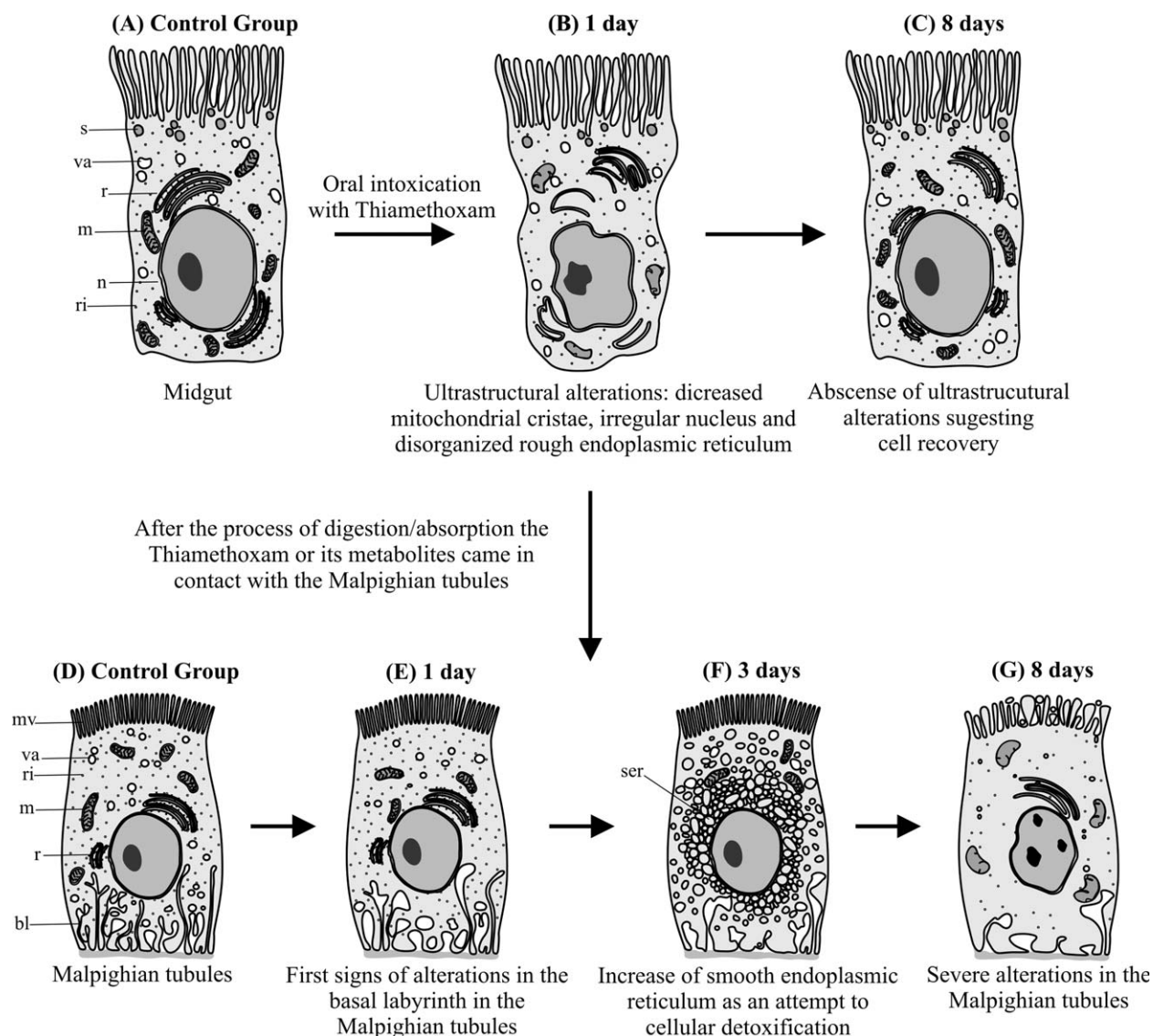
Fig. 3. Schematic representation showing the ultrastructural alterations in the midgut and Malpighian tubules cells of *A. mellifera* exposed to thiamethoxam. **A–C**: Digestive cells of a honeybee of control group and exposed to thiamethoxam for 1 and 8 days, respectively. **D–G**: Malpighian tubules of a honeybee of control group and bees exposed to thiamethoxam for 1, 3, and 8 days, respectively. s = secretory vesicle, va = vacuole, r = rough endoplasmic reticulum, m = mitochondria, n = nucleus, ri = ribosome, mv = microvilli, bl = basal labyrinth, ser = smooth endoplasmic reticulum.

bees exposed to $Cl_{50/10}$ for three or more days showed more subtle ultrastructural alterations. On the last day of the exposure, digestive cells appeared ultrastructurally intact and organized and had characteristics similar to the control group. According to Cruz-Landim (2009), the midgut is the portion of the digestive tract that is responsible for most of the digestion and absorption of food and is considered the functional stomach in insects. Because the insecticide was administered orally, the midgut was one of the first sites to come in contact with the sublethal dose, and this organ suffered immediate effects at the beginning of the exposure. In the midgut of *A. mellifera*, there are three families of enzymes that play a role in the detoxification process: glutathione-S-transferases, cytochrome P450s, and carboxylesterases (Claudianos et al., 2006). These enzymes carry out the process of biotransformation of the chemical compounds (Yu et al., 1984).

The process of phase I biotransformation (oxidation reactions) of organic compounds is performed by the system of mixed-function oxygenases, which consists of P450 cytochrome (Niyogi et al., 2001). This system plays a central role in the metabolism of many xenobiotics and catalyzes both detoxification reactions and bioactivation (Teramitsu et al., 2000). After the reactions mediated by P450 cytochrome, the lipophilic products can be conjugated with the endogenous tripeptide reduced glutathione by the activity of glutathione S-transferase. This reaction forms products that are more soluble and thus more easily excreted (Fitzpatrick et al., 1997).

The digestive cells are mainly responsible for the synthesis of these enzymes. Thus, the results show that the midgut epithelium suffers the initial damage from exposure to the insecticide. It is possible that because of the action of the enzymes of the detoxification system, the epithelium can recover itself. Therefore, there is little evidence of ultrastructural alterations in bees exposed for three or more days to the insecticide.

In addition to the action of the detoxification enzymes present in the midgut, the absorptive and secretory activity of this tissue causes digestive cells to be discharged into the lumen. This activity can be enhanced when midgut cells are exposed to pathological or toxicological situation. Therefore, the worn out cells are eliminated in the lumen and then replaced by differentiation of regenerative cells (Bowen et al., 1998; Cavalcante and Cruz-Landim, 1999). Thus, this characteristic cell replacement process may have contributed to the adaptation and recovery of the midgut after exposure to the insecticide and may explain the integrity of the organelles that was observed in the digestive cells of bees exposed for 8 days. The slight effects observed in the midgut cells also suggest that thiamethoxam may have just been absorbed through its wall without being digested.

Following the metabolism route of thiamethoxam in the body of the bee, the digested products (or the molecule if it was not digested by the midgut) will next reach the hemolymph and subsequently the Malpighian tubules, which are also analyzed in this study. The Malpighian tubules form the excretory system of insects, and this organ is primarily responsible for the maintenance of homeostasis and plays an important role in the detoxification process by actively eliminating excess or unmetabolized substances from the body (Habib, 2003).

The data obtained in this study suggest that during the process of digestion of contaminated food in the midgut of bees, thiamethoxam excess that were not metabolized or its metabolites, may have caused the ultrastructural changes observed later in the Malpighian tubules and to the degeneration of the cells by the insecticide.

The ultrastructural results showed that the cytotoxicity in the Malpighian tubules begins in bees after exposure for 1 day to thiamethoxam. The first noticeable alteration was the loss of the basal labyrinth, which is the structure responsible for promoting greater contact with the hemolymph and optimizing the uptake of metabolized substances (Cruz-Landim, 1998).

With the function of the basal labyrinth already compromised by the effects of the sublethal dose of thiamethoxam, the next evidence of cytotoxicity was found in bees after 3 days of intake of contaminated diet. At this time, there was an increase in the smooth endoplasmic reticulum, which may have been an attempt to detoxify the organ. However, due the high toxicity of thiamethoxam or its metabolite, this attempt at detoxification was ineffective because bees exposed for 5 and 8 days to the insecticide had Malpighian tubule cells with severe ultrastructural alterations. These changes included the degeneration of the mitochondria and of the microvilli of the Malpighian

tubules. Thus, this damage would likely be debilitating to the excretion activity, which is the main function of this structure.

Thus, the damage in Malpighian tubules can cause the non-excretion of the neonicotinoid (or its metabolite) most likely results in subsequent action of this substance in the nervous system of the insect, which is the final target of this type of insecticide and can result in the death of the bee. Oliveira et al. (2013) exposed newly emerged Africanized bees to the same dose used in this study (0.428 ng/μL thiamethoxam per day) and found the presence of condensed cells in the mushroom bodies and optical lobes in exposed honeybees. This group concluded that the intoxication with a sublethal dose of thiamethoxam can cause impairment in the brain of Africanized honeybee. Ferreira et al. (2013) observed a reduction in survival of the bee *Scaptotrigona postica* when they were subjected to a diet contaminated with fipronil (0.1 μg/kg of food) and boric acid (0.75% wt/wt). The same authors also analyzed the effects of these compounds on the Malpighian tubules of the insect and observed that these chemicals caused dilation of the microvilli, loss of ribosomes from the rough endoplasmic reticulum, and an increase of the electron dense matrix of the mitochondria. Rossi et al. (2013) showed that very low doses of imidacloprid (0.809 ng/bee) caused cytotoxic effects in the Malpighian tubules of Africanized *A. mellifera* such as the loss of the basal labyrinth, the increase of pyknotic nuclei, and the vacuolization of the cells.

The present work demonstrates the negative effects of thiamethoxam in the midguts and in the Malpighian tubules. These data strengthen the case that ultrastructural damage to these organs results from sublethal doses of insecticide, and this damage greatly affects the absorption and excretion functions, which are essential for the survival of individuals. These effects of thiamethoxam in non-target organ show that low dose of this compound even when it is administrated for a short period (8 days) can impair the physiology of Africanized honeybee.

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