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Midgut morphophysiology in *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera: Curculionidae)

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ABSTRACT

Sitophilus zeamais is one of the most aggressive pests of stored grains, causing a significant decrease in the nutritional quality of the grains and major losses in economic trade. The foraging capacity of this pest is assigned to its highly efficient digestive system. Investigations on the morphofunctional features of the midgut, which is the most active region of the alimentary canal, are fundamental to understand the feeding habits of this species. In this study, the midgut of adult insects was isolated, processed, and analyzed on light microscopy, scanning and transmission electron microscopy, protein and enzymatic activities determination, including analyses of the starch hydrolysis products. In *S. zeamais*, the midgut was differentiated into anterior midgut and posterior midgut, and consisted of digestive, regenerative and endocrine cells. The anterior midgut showed high density of regenerative crypts. Cells containing organelles associated with protein synthesis and presence of amylases and lipases indicated that majority of the digestion process occurred in the anterior midgut. The posterior midgut exhibited numerous gastric caeca and peritrophic membrane. Cells with poorly differentiated cytoplasmic into organelles, elongated microvilli, and low enzymatic activities indicated that the posterior midgut was mainly involved in absorption.

1. Introduction

In the insect, the alimentary canal is differentiated into foregut or stomodeum, midgut or mesenteron and hindgut or proctodeum (Cruz-Landim, 2009). In curculionids, the midgut begins at the stomodeal valve, differentiates into anterior midgut and posterior midgut, and terminates at the pyloric valve (Baker et al., 1984; Bu and Chen, 2009; Rubio et al., 2008; Sánchez et al., 2000).

Digestive, regenerative and endocrine cells have been previously identified in the midgut of Hemiptera (Billingsley and Downe, 1986), Hymenoptera (Cruz-Landim, 2009), Diptera (Billingsley, 1990; Priester, 1971), and Neuroptera (Scudeler and Santos, 2013). Goblet cells are commonly found in the midgut of Lepidoptera (Snodgrass, 1993). Digestive and regenerative cells have been earlier identified in Coleoptera, however, no endocrine cells have been observed in this insect (Silva-Olivares et al., 2003). Together, these cells are responsible for the synthesis and secretion of enzymes and peptide hormones, digestion and absorption of food, epithelial regeneration and secretion of potassium (Snodgrass, 1993). Thus, the midgut is the most active region of the alimentary canal (Balogun, 1969; Cruz-Landim, 2009; Priester, 1971; Silva-Olivares et al., 2003).

The food ingested by insects is mainly composed of polymers, which undergo three stages of digestion. Initial digestion involves the breakdown of polymeric chains into oligomers, which are further broken down into dimmers during intermediary digestion, and into monomers in the final digestion (Terra and Ferreira, 1994). Digestive enzymes are required for the hydrolysis of food, and they act in different regions of the alimentary canal. In the midgut, Baker et al. (1984) determined amylase and protease activities in *Sitophilus granarius*; Balogun (1969) identified carbohydrases, proteases and lipases in *Ips cembrae* (Coleoptera: Scolytidae); and Fialho et al. (2012) measured activities of carbohydrases and proteases in *Podisus nigrispinus* (Hemiptera: Pentatomidae).

Sitophilus zeamais, popularly known as beetle or weevil, is an insect pest of economical importance, because it infests stored grains and damages crops of apples, grapes, and peaches (Boneti et al., 1999; Botton et al., 2005; Giacobbo et al., 2005). According to Ramalho et al. (1976), the economic damages associated with maize infestation may reach 60% in six months, with decrease in grain weight of above 15%.

There are several studies related to the morphological characterization of the midgut in Coleoptera, or on the properties of synthesized enzymes in the midgut. However, few studies emphasize the interaction between morphology and identification of

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enzymatic activities. Thus, this study was conducted to establish a characterization of the morphological and functional interactions of the midgut in *S. zeamais* adults. The information generated in this study may be utilized for future studies on the digestive physiology in Coleoptera.

2. Materials and methods

2.1. Insects

Adults of *S. zeamais* (14 days old) were obtained from breeding stocks of the Laboratory of Insect Morphology and Cytogenetics of the State University of Maringa, Paraná, Brazil. The insects were kept under the following conditions: 30 ± 1 °C, relative humidity of 70 ± 10%, 12 h photoperiod, and fed on maize grains (*Zea mays*, AG 9010 PRO).

2.2. Light microscopy

Male and female adults of *S. zeamais* were anesthetized with ether vapor and dissected in insect saline solution (0.1 M NaCl, 0.1 M Na_2HPO_4 and 0.1 M KH_2PO_4). The midguts of 30 specimens were collected and fixed in Bouin solution for 7 h. The tissues were dehydrated in ethanol progressive series (70%, 80%, 90% and 100%), cleared in xylol, embedded in histological paraffin, and cut into 6 μm thick sections on Leica RM 2250 microtome. The sections were stained with hematoxylin–eosin (Junqueira and Junqueira, 1983), Periodic Acid Schiff (Junqueira and Junqueira, 1983), xylidine ponceau (Mello and Vidal, 1980), bromophenol blue (Pearse, 1961) and picrosirius–hematoxylin trichrome (Junqueira and Junqueira, 1983). The material was examined on Olympus microscope, and photographs were obtained using Samsung PL 120 digital camera.

2.3. Scanning electron microscopy (SEM)

Male and female adults of *S. zeamais* were anesthetized with ether vapor and dissected in insect saline solution (0.1 M NaCl, 0.1 M Na_2HPO_4 and 0.1 M KH_2PO_4). The midguts of 10 specimens were isolated and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) for 48 h at room temperature, and washed with distilled water. The fragments were post fixed in 1% osmium tetroxide in distilled water for 30 min, and dehydrated in ethanol crescent series (7.5%, 15%, 30%, 50%, 70%, 90% and 100%). The tissues were dried on Leica CPD 030 critical point dryer and coated with gold in Baltec SCD 050 sputter coater. The material was examined and imaged on Fei Company Quanta 200 scanning electron microscope at the Electron Microscopy Center of the Biosciences Institute, UNESP, Botucatu-SP.

2.4. Transmission electron microscopy (TEM)

The midguts of 10 male and female adults of *S. zeamais* were fixed in 2.5% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3) for 24 h at room temperature, and post fixed in 1% osmium tetroxide in the same buffer for 2 h. After washing in distilled water, the tissues were contrasted in aqueous solution of 0.5% uranyl acetate for 2 h, dehydrated using acetone progressive series (50%, 70%, 90% and 100%), and embedded in Araldite® resin. Ultrafine sections were contrasted using lead citrate and uranyl acetate, and then examined and imaged in Fei Company Tecnai Spirit and Phillips CM100 transmission electron microscope at the Electron Microscopy Center of the Biosciences Institute, UNESP, Botucatu-SP.

2.5. Protein determination and enzyme assays

It has recently been reported that the enzymatic activities between males and females vary (Fialho et al., 2012), and thus, this study focused on female adults of *S. zeamais*, which were anesthetized by freeze and dissected in insect saline solution (0.1 M NaCl, 0.1 M Na_2HPO_4 and 0.1 M KH_2PO_4). The midguts of 36 specimens were isolated, divided into anterior midgut and posterior midgut (in the constriction that differentiates the organ), and kept in 1 mL of 50 mM sodium phosphate buffer (pH 6.0) at −20 °C until use. The fragments were macerated and centrifuged at 4 °C, 6000 rpm for 20 min, and the supernatant was used for analysis. Initially, protein content of the extracts was determined according to Bradford (1976), using bovine serum albumin (BSA) as a standard. The absorbance reading was conducted at 595 nm, and the results were expressed in milligrams of protein per milliliter in a sample (mg/mL). Amylase activity was performed by determining the appearance of reducing groups in 50 mM sodium phosphate buffer (pH 6.0) using 1% starch as substrate (Noelting and Bernfeld, 1948). Absorbance was measured at 550 nm. Proteolytic activity was determined using 1% casein in 50 mM Tris HCl buffer (pH 7.0) as substrate, and absorbance measured at 660 nm (Sumantha et al., 2006). Lipolytic activity was accomplished using *p*-nitrophenyl palmitate (3 mg/mL) substrate diluted in isopropanol and homogenized in 0.1 M sodium phosphate buffer solution (pH 7.0), X-100 Triton and Arabic gum (Pimentel et al., 1997). Absorbance was measured at 410 nm. The enzymatic activities were expressed in milliunits of enzyme per millimeter in a sample (mU/mL), and the specific activity in milliunits of enzyme per milligram in a sample (mU/mg).

2.6. Silica thin layer chromatography (TLC)

The foregut, anterior midgut and posterior midgut of 10 male and female adults of *S. zeamais* were isolated and kept in 40 μL of 50 mM sodium phosphate buffer (pH 6.0) at −20 °C until use. The tissues were macerated and centrifuged at 4 °C, 6000 rpm for 20 min, and the supernatant was used to identify the hydrolysis products of amylase through ascending chromatography using a silica layer, according to Fontana et al. (1988). The running solvent was comprised of ethyl acetate, acetic acid, formic acid, and distilled water (9:3:1:1) (v/v), whereas the revelation solution consisted of 0.2% orcinol in solution of sulfuric acid and methanol (1:9) (v/v). Maltose and glucose (1 mg/mL) were used as standards. The plate was incubated in 100 °C oven until the appearance of bands, and then imaged using a Samsung PL 120 digital camera.

3. Results

3.1. Morphological and ultrastructural characterization

The midgut of *S. zeamais* showed a constriction that differentiated the organ into anterior midgut and posterior midgut (Fig. 1A). A thin musculature, consisting of longitudinal external and internal circular muscular fibers (Fig. 1B and C), coated a layer of connective tissue known as the basal lamina. The lamina was acidophilic and glycoproteic, and showed fibrous proteins such as collagen (Fig. 2A–F) On the basal lamina, the simple epithelial was composed of digestive, regenerative and endocrine cells. Only the digestive cells showed differences between the anterior and posterior midgut.

3.1.1. Anterior midgut

The epithelium showed high density of regenerative crypts (Fig. 1B), in which regenerative, endocrine and digestive cells were observed. The regenerative cells only occurred in the crypts, whereas the digestive and endocrine cells were also found in the epithelial layer.

The digestive cells were columnar and basophilic (Fig. 2A and B). The basal cytoplasm showed a poorly developed basal labyrinth, lipid droplets, vacuoles, rough endoplasmic reticulum and electron dense mitochondria (Fig. 3A and B). The middle region of the digestive cells exhibited rounded nucleus and rough endoplasmic reticulum. In the apical region, were found rough endoplasmic reticulum, Golgi,