SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Morphometric Study of the Midgut Epithelium in Larvae of *Diatraea saccharalis* Fabricius (Lepidoptera: Pyralidae)

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Estudo Morfométrico do Epitélio do Intestino Médio em Larvas de *Diatraea saccharalis* Fabricius (Lepidoptera: Pyralidae)

RESUMO - A larva de *Diatraea saccharalis* Fabricius (broca-da-cana) tem grande interesse econômico, pois afeta o cultivo e aproveitamento industrial da cana-de-açúcar. Entretanto, poucos são os estudos sobre a morfologia interna desse inseto. O objetivo deste trabalho foi estudar, morfometricamente, o seu epitélio intestinal, ao longo de seu comprimento, visando caracterizar regiões estruturalmente diferentes. O intestino médio de larvas no último instar foi subdividido em três regiões: proximal, mediana e distal e os fragmentos foram processados para observação em microscopia de luz. Os cortes histológicos foram analisados em sistema computadorizado de análise de imagens para medir comprimento, largura e área do epitélio, das diferentes células epiteliais, dos seus respectivos núcleos e do lúmen intestinal. Os dados obtidos foram submetidos ao teste estatístico de Kruskal-Wallis e à análise multivariada. Nossos resultados mostraram que o intestino médio apresentou-se constituído, morfometricamente, por duas diferentes regiões, proximal e distal; a região mediana apresentou valores coincidentes tanto com a região proximal quanto com a distal, sugerindo ser região intermediária. As células epiteliais (colunares, caliciformes e regenerativas), quando avaliadas pela análise estatística multivariada, não apresentaram diferença morfométrica nas diferentes regiões do intestino médio. Entretanto, a análise de variância, realizada para variáveis isoladas, mostrou que as células regenerativas apresentaram maior variabilidade morfométrica.

PALAVRAS-CHAVE: Morfometria, célula epitelial, inseto, broca da cana, mesêntero

ABSTRACT - The sugarcane borer, *Diatraea saccharalis* Fabricius, has great economical interest as it affects the culture and industrial use of the sugarcane. However, there are few studies concerning the internal morphology of this insect. This work aims to study morphometrically the midgut and the epithelium along their lenght, trying to characterize different regions. Midgut of last instar larvae was divided in three regions: anterior, middle and posterior, and the fragments were processed for light microscopic observation. Histological sections were analyzed in a computerized system concerning the length, width and area of the epithelium, their cells, and the midgut lumen. The obtained data were statistically analyzed by the Kruskal-Wallis test and by multivariate analysis. Our results showed that the midgut has two different regions, the anterior and the posterior; the middle region presents values that are coincident with the ones of either the anterior and the posterior portions, suggesting that there is an intermediate region between the other two ones. The epithelial cells (columnar, goblet and regenerative cells), when evaluated by multivariate analysis, do not present significant morphometric differences in the different midgut regions. However, the analysis of variance for separate variables show that the regenerative cells present wide morphometric variability along the midgut.

KEY WORDS: Morphometry, epithelial cell, insect, sugarcane borer, mesenteron

In Lepidoptera four cell types mainly compose the midgut epithelium: columnar, goblet, regenerative and endocrine cells (Lehane & Billingsley 1996). The predominant columnar cells are responsible for processing the diet, secretion of the digestive enzymes and the uptake of the final products (Lehane & Billingsley 1996). The goblet cells co-operate

with the columnar cells in ionic homeostasis and metabolite absorption (Lello *et al.* 1984, Chiang *et al.* 1986). Scattered throughout the epithelium there are relatively undifferentiated regenerative cells found singly, paired or in group (Turbeck 1974, Cavalcante & Cruz-Landim 1999). The scarce endocrine cells are variable in shape being pyramidal, bowl-shaped, oval or fusiform (Andries & Beauvillain 1988, Cavalcante & Cruz-Landim 1999).

Although all cell types can be found in the midgut extension, many works suggest that the distribution, morphology and function of these cells may be variable along the midgut length (Cioffi 1979, Lello *et al.* 1984, Santos *et al.* 1984, Chiang *et al.* 1986, Lehane & Billingsley 1996, Cristofoletti *et al.* 2000). These morphological differences are mainly detected at ultrastructural level for all the cell types (Cioffi 1979, Lello *et al.* 1984, Santos *et al.* 1984, Lehane & Billingsley 1996).

The sugarcane borer, *Diatraea saccharalis* Fabricius, is a serious pest of sugarcane and many others crops including maize, sorghum, corn and rice (Long & Hensley 1972). Preliminary study showed that their columnar epithelial cells present differences in the ultrastructural organization that can be related with their localization in the midgut (Pinheiro & Gregório 2001). This finding has been previously described for other Lepidoptera, such as *Manduca sexta* L. (Cioffi 1979), *Spodoptera frugiperda* Smith (Jordão *et al.* 1999) and *Erinnyis ello* L. (Santos *et al.* 1984).

However, there are few morphometric studies of the epithelial cells along insect midgut, most of them in Diptera (Nopanitaya & Misch 1974, Wood & Lehane 1991, Andrade-Coeho *et al.* 2001), few of them in Heteroptera (Ranjini & Mohamed 2000) and Hemiptera (Billingsley 1988), but none in Lepidoptera.

This work aims to study morphometrically the midgut of the *D. saccharalis* larvae. Measurements of the midgut, their lumen and epithelium, as well as of the columnar, goblet, and regenerative cells were obtained along their lenght, in an attempt to distinguish differential regions in this organ.

Material and Methods

D. saccharalis larvae were reread on artificial diet (Hensley & Hammond 1968) and maintained under controlled temperature (25-27ºC) and humidity (70%). Fifty insects at the beginning the last larval instar (12-17 days of development) were dissected under stereomicroscope and the midgut were immediately fixed in 2% glutaraldehyde-4% paraformaldehyde solution in 0.1 M phosphate buffer (pH 7.3) for 24h.

The midgut was equally divided in three fragments, named anterior, middle and posterior regions. These fragments were processed and embedded in Historesin (JB4-Polysciences); tissue sections were stained either with Toluidine blue (Pearse 1972) or with Schiff's reagent-Bromophenol blue-Ehrlich's haematoxylin (Coello 1989).

Histological cross sections were morphometrically analyzed in computerized system for analysis of images (QWin Lite 2.5 - Leica), adapted in a DMLB light microscope (Leica). The measurement obtained were: 1 - the larger (LAx) and smaller (SAx) axis, and the area (A) of the a) lumen; b) each epithelial cell type and their nucleus; and c) midgut (epithelium + lumen); 2 - the height (H) of the epithelium, expressed as the mean, as well as the maximum (H_{Max}) and the minimum (H_{Min}) values of 20 measurements throughout the midgut perimeter. All measurements were taken in the central region of either the cell or the midgut lumen.

The obtained data were statistically analyzed by the Kruskal-Wallis test, and the difference among the groups was determined by the Dunn's method, using the Sigma-Stat 2.0 software. Significance level was established at 5%. The multivariate analysis, accomplished by the MVSP 3.2 software, was also used to compare the lumen and the different epithelial cell types in the different regions of the midgut.

Results

The *D. saccharalis* midgut is a simple tube composed by a single layered epithelium delimiting the lumen (Fig. 1A); different cell types, mainly the columnar, goblet, and regenerative cells constitute the midgut epithelium (Fig. 1B). The height of the epithelium is quite variable in each of the three studied regions (Table 1); there are differences as much as twice between the H_{Max} and H_{Min} obtained in the same region. However, there is no significant difference among the different regions concerning the H and the H_{Max} values, but the H_{Min} of the anterior and middle regions are significantly different. For the intestinal lumen, the LAx and SAx of the middle and posterior regions are similar amongst themselves, but these measurements of both regions differ significantly from the one of the anterior region; the same differences among the three regions are detected for the midgut (epithelium + lumen).

The multivariate analysis of the data concerning the measurements of the midgut lumen (Fig. 2) shows that the midgut has two different regions, the anterior and the posterior. The middle region presents values that are coincident with the ones of either the anterior region or the posterior region, as well as different values from these two regions.

The analysis of variance (Table 2) shows that some of the separate measurements taken from the different epithelial cells are variable along the midgut. In the columnar cells, most of the variables are not significantly different between the anterior and middle regions; however, the LAx of the cell, and the SAx and the A of their nucleus from both of the anterior and middle regions were different of the ones from the posterior region. The goblet cells show little variability among the midgut regions; the LAx of the cell and their nucleus in the anterior region are significantly different from the ones of both the middle and posterior regions. The regenerative cells are morphometric variable along the midgut; the SAx and the A of the cell and of their nucleus in the anterior and posterior regions are significantly different, and these values for the middle region do not differ from the ones of the anterior and posterior regions.

The multivariate analysis of the data concerning all the measurements of the epithelial cells in the midgut (Figs. 3-5) shows that the columnar, goblet, and regenerative cells do not constitute morphometrically different cellular populations in the three analyzed midgut regions.

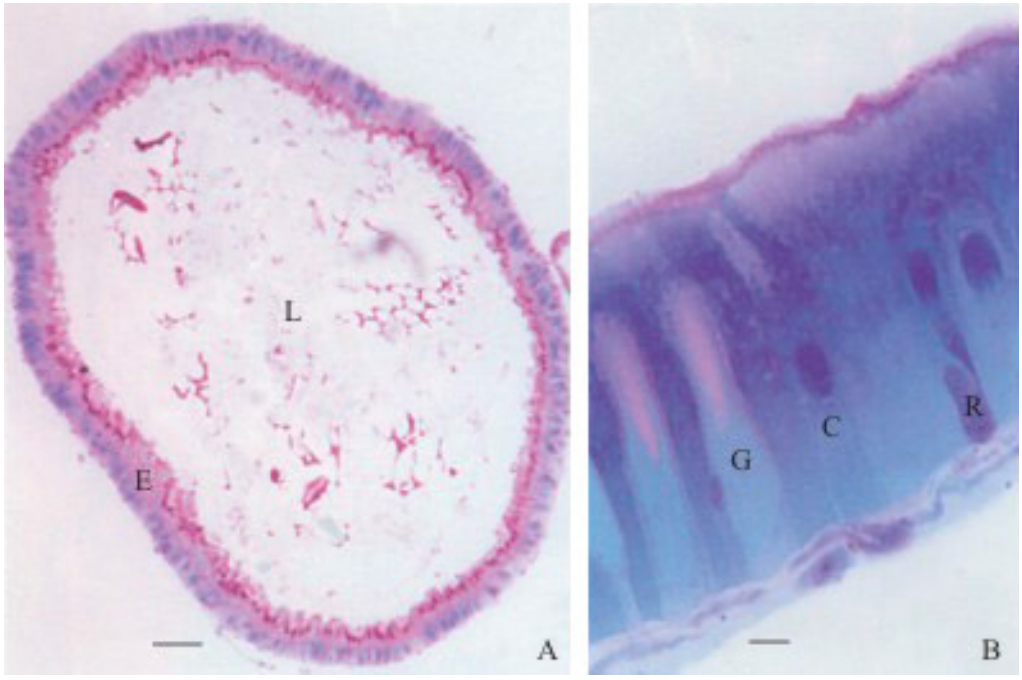

Figure 1. Histological section of *D. saccharalis* midgut: A) lumen (L) and epithelial (E) cells from middle region stained with Schiff's reagent-Bromophenol blue-Ehrlich's haematoxylin (Bar = 100 μm); B) columnar (C), goblet (G) and regenerative (R) cells in the epithelium from the anterior region stained with Toluidine blue (Bar = 10 μm).

Table 1. Morphometric data of lumen and epithelium from anterior, middle, and posterior regions of *D. saccharalis* midgut

Regions	Epithelium			Lumen			Epithelium + Lumen		
	H (μm)	H_{Max} (μm)	H_{Min} (μm)	LAx (mm)	SAx (mm)	A (mm^2)	LAx (mm)	SAx (mm)	A (mm^2)
Anterior (n = 32)	93.1 (4.7)a	124.3 (12.6)a	70.9 (5.6)a	1.5 (0.2)a	0.4 (0.1)a	471.2 (173.7)a	1.6 (0.2)a	0.6 (0.1)a	826.9 (231.3)a
Middle (n = 42)	89.2 (12.0)a	123.8 (18.9)a	62.9 (5.7)b	1.0 (0.2)b	0.5 (0.1)b	374.2 (197.8)a	1.2 (0.2)b	0.7 (0.1)b	671.6 (222.4)a
Posterior (n = 47)	97.1 (23.1)a	130.7 (31.3)a	61.7 (13.6)ab	1.0 (0.5)b	0.7 (0.3)b	607.8 (472.9)a	1.2 (0.5)b	0.9 (0.3)b	843.3 (572.4)a

Note: All values represent the median and the semi-interquartile range of data. Same letters in the columns represent $P > 0.05$ and different letters represent $P < 0.05$. H – height; H_{Max} – maximum height of the epithelium; H_{Min} - minimum height of the epithelium; LAx – larger axis; SAx – smaller axis; A – area.

Discussion

The morphometrical evaluation of the *D. saccharalis* midgut (the epithelium and the lumen) showed that the anterior and the posterior regions are distinct; the middle region present intermediate values between the anterior and the posterior one, suggesting that this portion of the midgut may be considered a transition. Studies on the insect midgut morphometry, at light microscopy level, do not exist in the literature. Our results confirmed the general idea that the long midgut of the insect larvae are structurally differentiate along their lenght, as suggested by ultrastructural observations (Cioffi 1979, Lello *et al.* 1984, Santos *et al.* 1984).

However, when each individual epithelial cell type was morphometrically analyzed in the three midgut regions, it was not possible to characterize differences in the cells along the midgut. The multivariate analysis compares two or more parameters, using as much as possible numeric variables for these entities. Moço *et al.* (2002) used this analysis in the identification and characterization of *Hepatozoon,* a protozoa that parasites snake erythrocytes; measuring cellular and nuclear variables (length, width and area), these authors were able to identify different populations of this blood parasites.

Preliminary report on the ultrastructure of the columnar cells in the sugarcane borer (Pinheiro & Gregório 2001) showed differences in their subcellular organization that could be related with the localization along the midgut lenght; these differences were mainly related with the apical cytoplasmic projections, the amount of mitochondria and of the basal membrane infoldings. However, our present results suggest that these ultrastructural characteristics did not interfere in the morphometric parameters analyzed for this cell and their

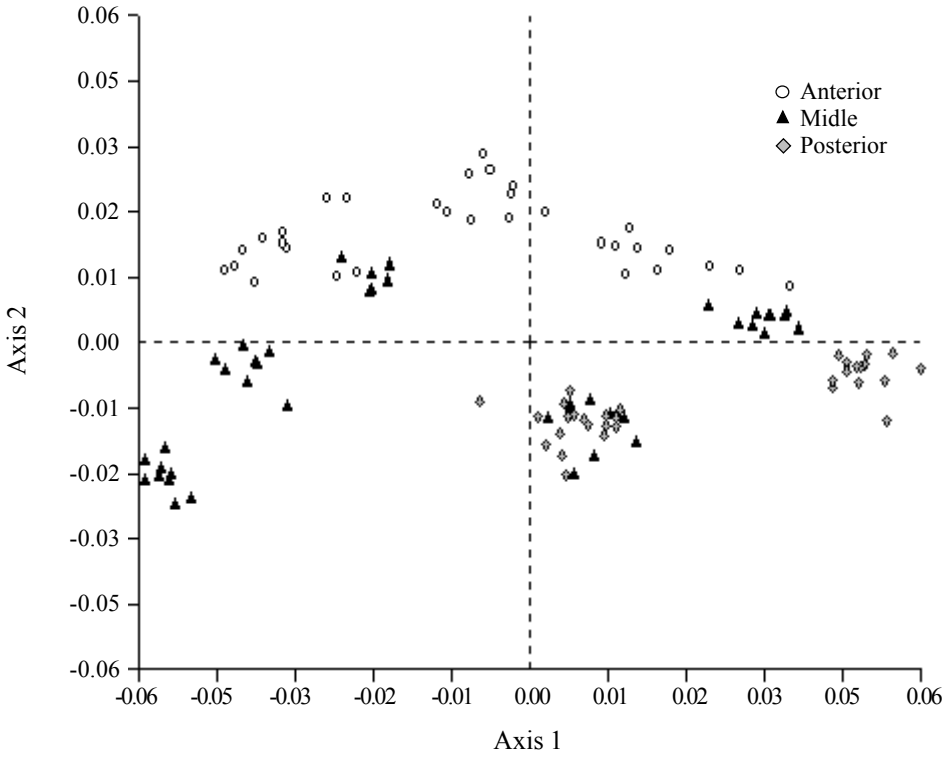

Figure 2. Multivariate analysis of the main components for comparison of the morphometric data of the anterior, middle and posterior regions of *D. saccharalis* midgut. The values presented on the ordinate and abscissa axes represent the largest amount of variation in the data set.

Table 2. Morphometric data of columnar, goblet, and regenerative cells from anterior, middle and posterior regions of *D. saccharalis* midgut.

Cell Type	Cell			Nucleus		
	LAx (μm)	SAx (μm)	A (μm^2)	LAx (μm)	SAx (μm)	A (μm^2)
Columnar						
Anterior (n = 51)	68.4 (5.3)a	8.0 (2.0)a	577.3 (127.7)a	11.3 (0.9)a	4.9 (0.9)a	44.6 (8.9)a
Middle (n = 55)	64.2 (6.0)ab	9.9 (2.3)a	615.1 (124.6)a	11.3 (1.1)a	4.7 (1.0)a	44.8 (11.0)a
Posterior (n = 57)	60.4 (3.1)b	9.1 (1.9)a	565.5 (105.7)a	10.8 (0.9)a	4.0 (0.6)b	38.5 (7.6)b
Goblet						
Anterior (n = 54)	58.4 (4.2)a	7.2 (0.9)a	408.3 (42.7)a	5.4 (0.7)a	3.4 (0.5)a	17.1 (3.0)a
Middle (n = 44)	56.2 (6.8)b	7.7 (1.2)a	421.1 (61.8)a	6.8 (1.2)b	2.9 (0.8)a	17.5 (2.9)a
Posterior (n = 50)	63.2 (4.4)b	7.6 (1.6)a	418.5 (83.7)a	7.2 (1.2)b	3.0 (0.6)a	17.8 (2.9)a
Regenerative						
Anterior (n = 51)	14.1 (5.0)a	5.9 (1.3)a	69.2 (18.3)a	6.0 (1.6)a	4.1 (1.2)a	20.3 (4.9)a
Middle (n = 61)	14.4 (2.4)a	5.4 (0.9)ab	60.5 (16.1)ab	6.3 (0.8)a	3.7 (0.6)ab	19.9 (3.8)ab
Posterior (n = 54)	13.9 (3.6)a	5.4 (0.9)b	58.5 (19.7)b	6.0 (2.5)a	3.4 (0.5)b	17.3 (5.4)b

Note: All values represent the median and the semi-interquartile range of data. Same letters in the columns represent $P > 0.05$ and different letters represent $P < 0.05$. LAx – larger axis; SAx – smaller axis; A – area.

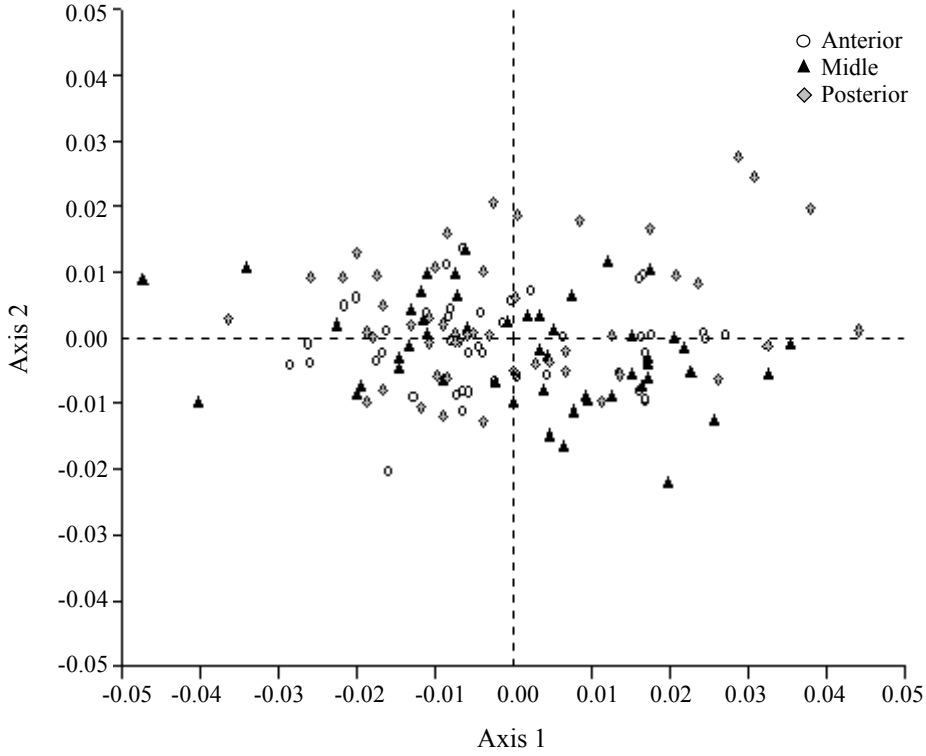

Figure 3. Multivariate analysis of the main components for comparison of the species of morphometric data of columnar cells from the anterior, middle and posterior regions of *D. saccharalis* midgut. The values presented on the ordinate and abscissa axes represent the largest amount of variation in the data set.

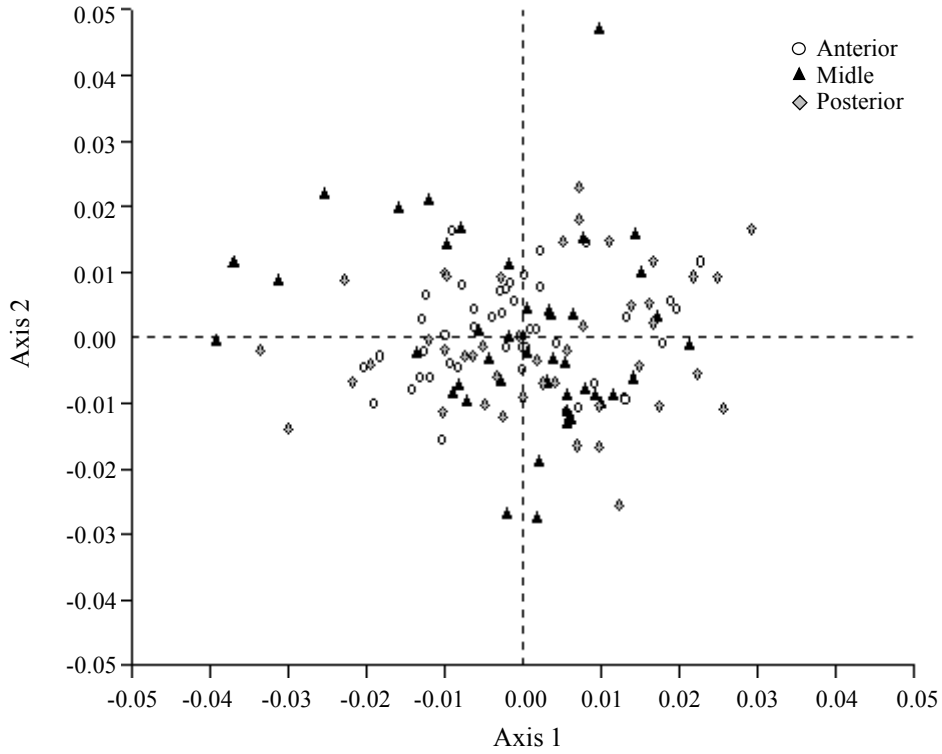

Figure 4. Multivariate analysis of the main components for comparison of the species of morphometric data of goblet cells from the anterior, middle and posterior regions of *D. saccharalis* midgut. The values presented on the ordinate and abscissa axes represent the largest amount of variation in the data set.

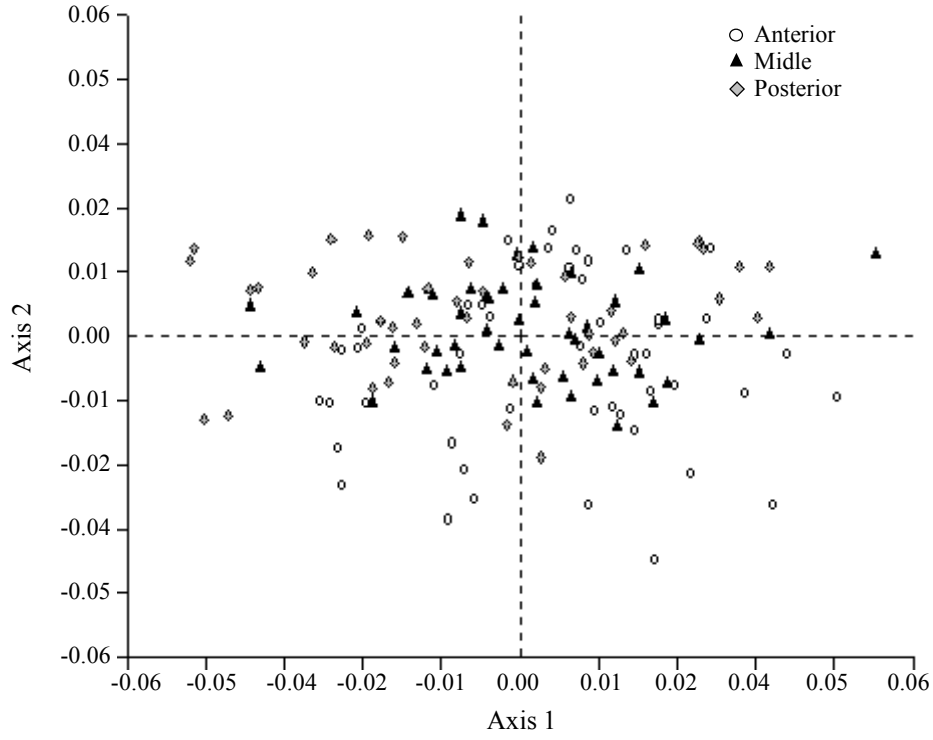

Figure 5. Multivariate analysis of the main components for comparison of the species of morphometric data of regenerative cells from the anterior, middle and posterior regions of *D. saccharalis* midgut. The values presented on the ordinate and abscissa axes represent the largest amount of variation in the data set.

nucleus (LAx, SAx and A) in the same regions.

Morphometric data concerning the ultrastructural organization of the midgut epithelial cells were reports for many insect order, as in Diptera (Nopanitaya & Misch 1974, Wood & Lehane 1991, Andrade-Coeho *et al.* 2001), Orthoptera (Srivastava 1997), Heteroptera (Ranjini & Mohamed 2000), and Hemiptera (Billingsley 1988). However, they did not compare the cells along the midgut; they were mainly related with experimental aspects during the digestion process (Billingsley 1988, Wood & Lehane 1991, Ranjini & Mohamed 2000, Andrade-Coeho *et al.* 2001), sex differences (Rudin & Hecker 1976) and insect development (Nopanitaya & Misch 1974). The application of multivariate analysis, presented in this paper, may also be an important tool for a better interpretation of the physiologic, sexual and ontogenetic differences, as showed by the authors above.

The endocrine cells were not considered in our study, as they are scarce and very difficult to be identified at light microscopy level, mainly using conventional staining techniques (Endo *et al.* 1983, Montuenga *et al.* 1989). The small number of endocrine cells in the midgut epithelium were also described for many insect species (Andries & Beauvillain 1988, Montuenga *et al.* 1989).

The regenerative cells presented wide morphometric variability in the different midgut regions. Our data showed that there are difference among these cells comparing the anterior and the posterior regions. The middle region presented an intermediate pattern for the regenerative cells parameters, supporting the idea that there are a transition portion in the midgut between the anterior and the posterior ones.

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