ANATOMY AND HISTOLOGY OF THE ALIMENTARY CANAL OF THE COLORADO POTATO BEETLE LARVAE*

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In this study, anatomy and histology of the alimentary canal of Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) larvae, were investigated. The alimentary canal is divided into three main regions: foregut (stomodeum), midgut (mesodeum) and hindgut (proctodeum). The foregut forms the smallest part of the alimentary canal. The foregut consists of a thick cuticle (intima), a thin and simple epithelium, a basement membrane and muscle layer. The midgut forms the longest part of the alimentary canal. The midgut epithelium is characterized by a striated border of microvilli. The epithelium is surrounded by muscle layers and comprised of three different types of cells: columnar, endocrine and regenerative cells (or nidi). The hindgut is made up of the same layers as the foregut, but the epithelial cells are larger and the cuticle is thinner. The epithelium of all parts of the gut consists of a single layer of cells. Six Malpighian tubules are located at the beginning of the ileum and join into the rectum independently.

Leptinotarsa decemlineata; foregut; midgut; hindgut

INTRODUCTION

Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is one of the most widespread and destructive pests of potato. Both adults and larvae feed on this plant often cause their complete defoliation with considerable yield losses, up to 50% of the crop (Rasocha, 2003). In the Czech Republic, it belongs to the most abundant pest of potato that is difficult to be controlled by chemical insecticides due to resistance. This is why new strategies based on the expression of toxins produced by genes from bacterium *Bacillus thuringiensis* (Costa et al., 2000; Loseva et al., 2002), *Photorhabdus luminescens* (Blackburn et al., 2005) or from plants (e.g. enzyme inhibitors) (Bolter, Jongsma, 1995; Gruden et al., 1998; Visal et al., 1998) against this pest are being developed. To understand the effect of insecticidal proteins on the gut we studied and described the structure of the alimentary canal of *L. decemlineata* larvae. We compared our results with structures of the alimentary canal of the other Coleoptera larvae.

MATERIAL AND METHODS

Standard strain of Colorado potato beetle was obtained from Department of Entomology, Crop Research Institute in Prague. Larvae and beetles were fed with potato plants. Light period was 16 h light and 8 h dark. The temperature was 22 °C (± 2 °C), r. h. 50–60%.

Larvae in the last instar, approximately three weeks old, were dissected in Ringer's solution. Anatomy of the alimentary canal of 20 larvae was described using a stereomicroscope. Larvae were fixed in a Bouin solution for 24 , dehydrated in an alcohol series, and embedded in paraffin at 58 °C for 24 h. Histological sections were prepared from the whole length of the alimentary canal, from the mouth to the anus with rotary microtome Microm HM 200. 6 μm thick sections were deparaffinized and stained in Masson's trichrome stain or periodic acid – Schiff (PAS) reaction (Vacek, 1988). The histological preparations were examined under Axioskop Zeiss DCS-S 75 microscope.

RESULTS

Anatomy. The alimentary canal of *Leptinotarsa decemlineata* larvae is a long tube. As in other insects, it consists of the foregut, midgut and hindgut. Two valvelike folds separate these three regions. One valve is located between the foregut and the midgut (cardia), and the other between the midgut and the hindgut (pylorus). Six Malpighian tubules are attached to the alimentary canal and mark the transition to the hindgut, which is a simple straight and narrow tube. The hindgut connects with the exterior through the anus.

* The study was supported by the Ministry of Agriculture of the Czech Republic, Project No. MZE 000-2700063 and MSM 6046070901.

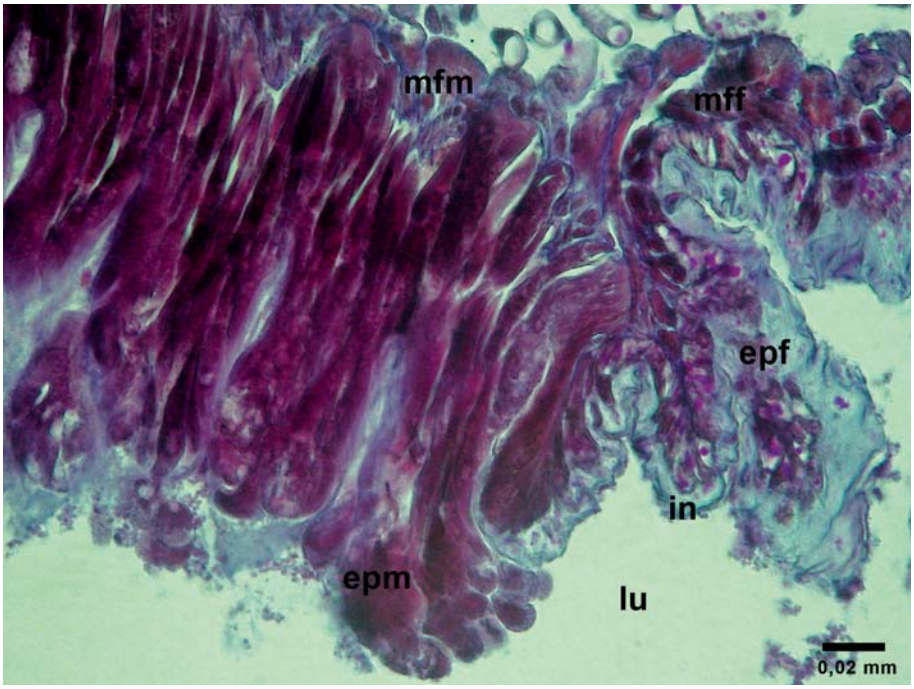

Fig. 1. *Leptinotarsa decemlineata*, posterior end of foregut and anterior end of midgut: epf – epithelium of foregut, epm – epithelium of midgut, in – intima, lu – lumen, mff – muscle fibers of foregut, mfm – muscle fibers of midgut, PAS reaction

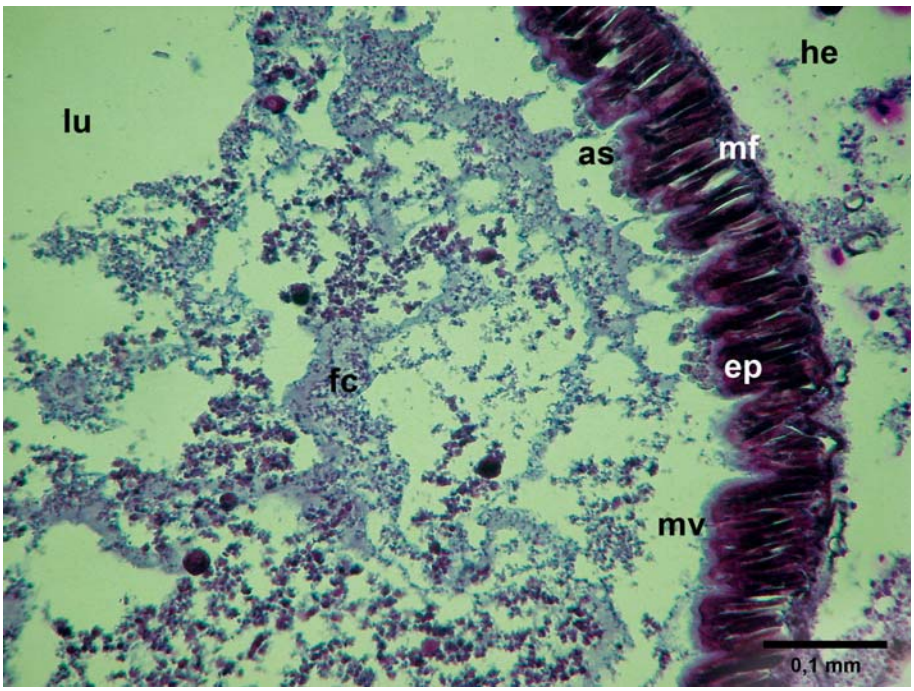

Fig. 2. *Leptinotarsa decemlineata*, part of midgut showing secretory extrusions: as – apical secretory extrusion, ep – epithelium, fc – food contents within gut lumen, he – haemocoel, lu lumen, mf – muscle fibers, mv – microvilli, PAS reaction

Histology. The foregut, midgut, and hindgut are different in their histological organization.

Light micrograph crosssection (Fig. 1) shows that the foregut is surrounded by a layer of muscle. Its epithelium is cubic and simple. A cuticle (intima) is present on the lumen side of the gut.

The midgut, the longest part of the alimentary canal, is situated between the foregut and the hindgut. The structure of the midgut (Figs 2 and 3) is composed of a single layer epithelium resting on the basement membrane. The apical area of the epithelium is characterized by a striated border of microvilli instead of an intima. The epithelium and the basement membrane are surrounded on the haemocoel side by layer of muscles. Collagen fibers occur among muscle cells.

Columnar cells comprise the majority in the epithelium and are of cylindrical shape. These tall cells contain microvilli projecting into the lumen, and their nuclei are ovoid, large, and generally located in the center of cells. The cells are strongly vacuolized indicating strong secretory activity. The apical extrusions at the apical parts of the cells release into gut lumen. On the external surface of the microvilli the presence of mucopolysaccharides or glycocalix can be observed (Figs 2 and 3).

Besides the columnar cells there are small clusters of regenerative cells. Regenerative cells are undifferentiated

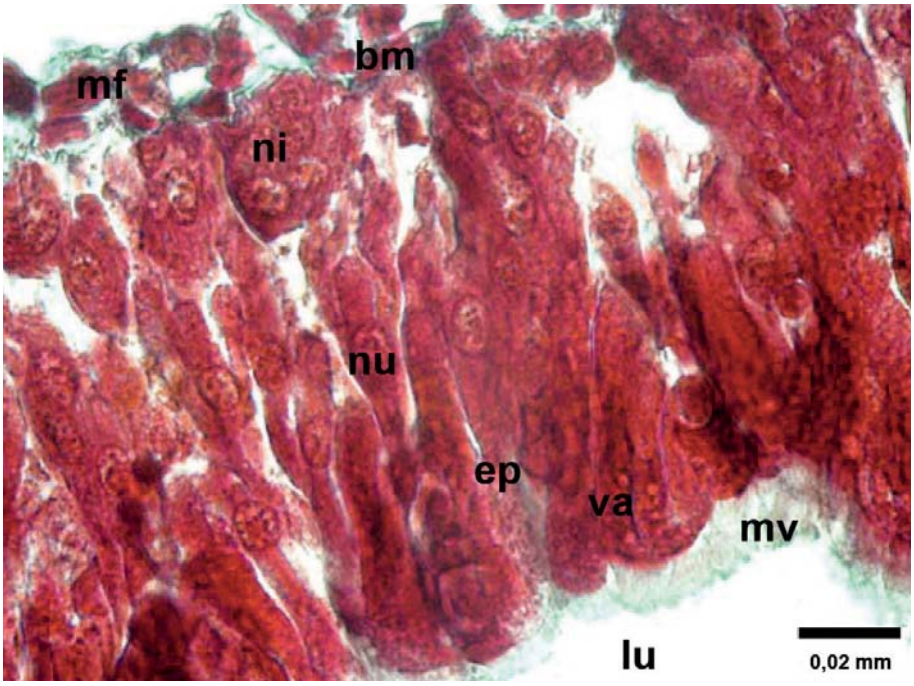

Fig. 3. *Leptinotarsa decemlineata*, part of midgut showing the well-developed microvillar border on the apical side of the epithelium: bm – basement membrane, ep – epithelium, lu – lumen, mf – muscle fibers, mv – microvilli, ni – nidus, nu – nucleus, va – vacuole, Masson's trichrome stain

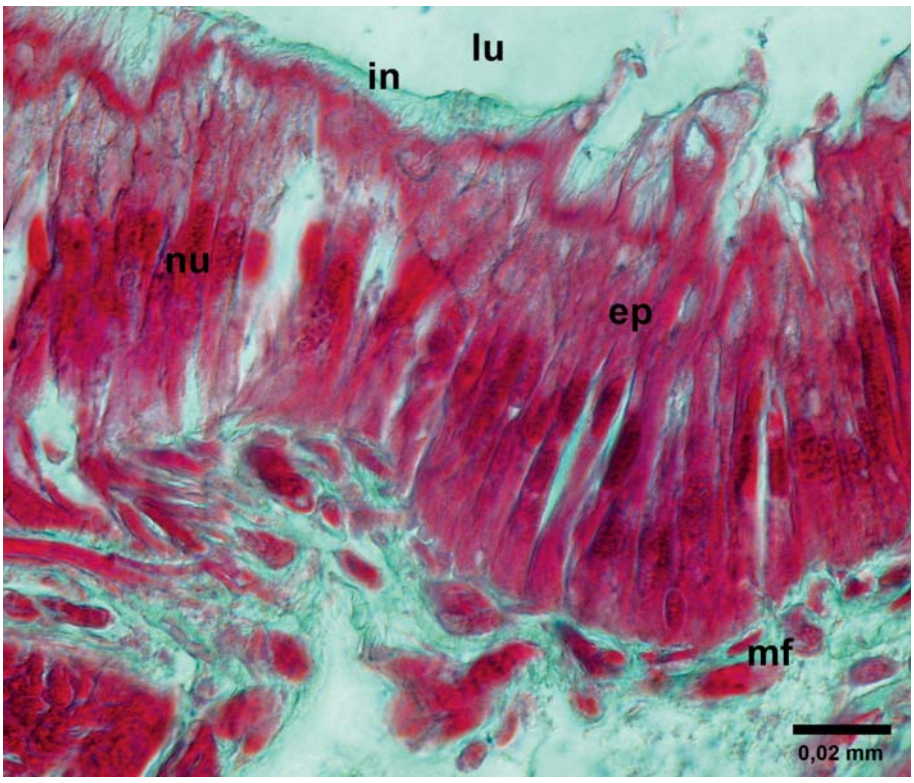

Fig. 4. *Leptinotarsa decemlineata*, hindgut: ep – epithelium, in – intima, lu – lumen, mf – muscle fibers, nu – nucleus, Masson's trichrome stain

replacement cells (cellular nidi) scattered between the epithelial cells. The innermost cell of the nidus is small and conical with a round nucleus in the centre. The surrounding cells grow gradually from the inner to the outer side and are often collected together. These cells are shorter than the normal epithelial cells and lack microvilli. They do not make contact with the gut lumen. (Fig. 3).

The third type of the midgut epithelium cells of *L. decemlineata* are endocrine cells distributed throughout the epithelium, although midgut endocrine cells are usually more common in the posterior midgut. These cells are of variable shape and are localized in the epithelial base.

Hindgut has a structure similar to that of the foregut. Above a layer of muscles there is a simple cylindrical epithelium covered by a thin cuticle absent in the midgut´s epithelium (Fig. 4). Towards the hind end the epithelial cells tend to increase in size and are arranged in six longitudinal folds. This wider part is termed the rectum (Fig. 5).

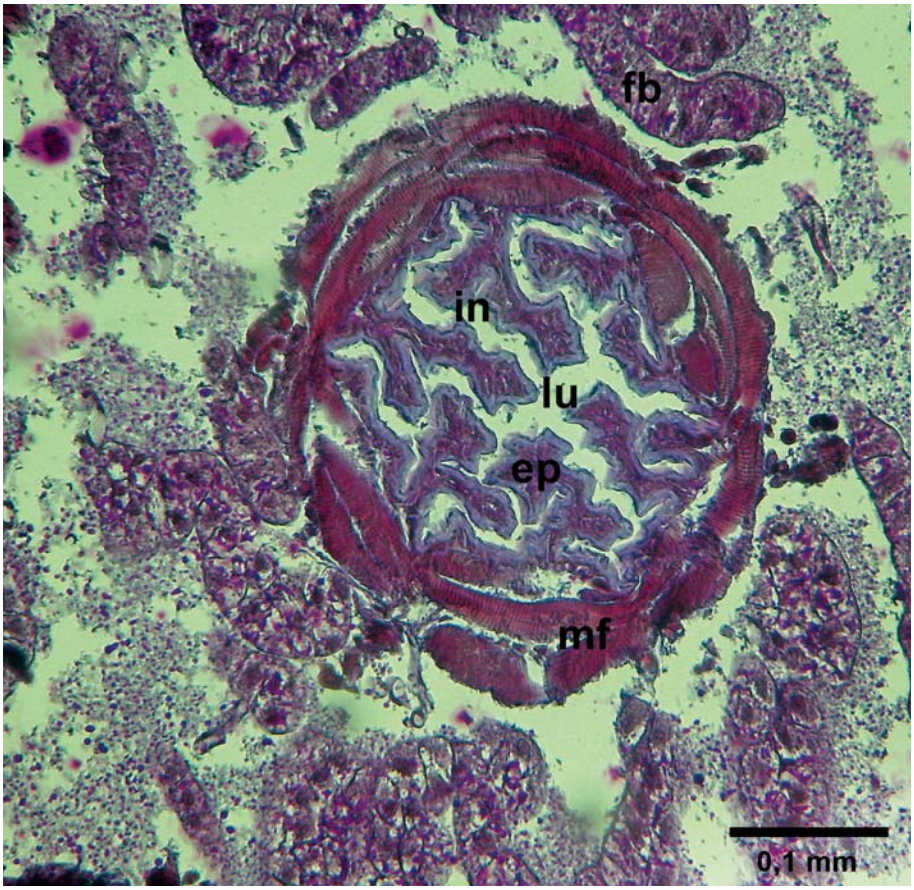

Fig. 5. *Leptinotarsa decemlineata*, rectum: ep – epithelium, fb – fat body, in – intima, lu – lumen, mf – muscle fibers, PAS reaction

DISCUSSION

The present report contributes information on the anatomy and histology of the alimentary canal of the Colorado potato beetle, *Leptinotarsa decemlineata*. Its architecture is similar to that described for other coleopteran insects: *Tribolium, Dendroctonus* or *Dermestes* (A m e e n , R a h m a n , 1973; D í a z et al., 2003; C a l d e i r a et al., 2007). The alimentary canal of insects comprises structurally and embryologically of three distinct regions from the mouth to the anus. The foregut, and hindgut, are of ectodermal origin, lined by a cuticle, shed and renewed at each molt. The midgut, originates from the endoderm and possesses no cuticular intima (C h a p m a n , 1998).

The foregut of *L. decemlineata* opens the alimentary canal from the mouth and extends to the midgut. It is differentiated into the pharynx, esophagus and crop (H a e f t e n et al., 1993) The function of the foregut of the insect is to pass food forward to the midgut. Therefore, the foregut, contains a muscle layer. The cells of the foregut are flattened and undifferentiated since they are not involved in absorption or secretion (C h a p m a n , 1998).

The midgut is made up of cells, usually much thicker than the epidermis of the foregut. Following studies of insects show the importance of the midgut. It is the main site for both digestion of food and absorption of nutrition, and the epithelium is responsible for the production of many digestive enzymes and/or the uptake and transfer of nutrients to the haemolymph (W i g g l e s w o r t h , 1953). The midgut is also characterized as a point of entry of insecticides, viruses and toxins and the release of neurohormones that regulate the activity of various other physiological processes (D o w , 1986; L e h a n e , B i l l i n g s l e y , 1996).

Columnar cells are responsible for enzyme secretion and absorption of the products of digestion (C h a p m a n , 1998). It is suspected that the well-developed microvillar brush border on the apical membrane of the cell has the function of absorbing nutrients from the lumen to the cell. The presence of acid mucopolysaccharides (glycocalix) on the external surface of microvilli of the midgut epithelial cells in *Leptinotarsa* coincides with other reports of these amine sugars in several groups of insects (L a n e et al., 1996). Despite their common occurrence in the midgut, few attempts have been made to establish their possible role. In general, the importance of glycocalix in insects has been inferred from knowledge about its role in mammalian studies, including its participation in ion transport, protection of epithelial cells against damage by abrasion, protease secretion to the lumen, and in buffering extreme pH conditions (L a n e et al., 1996).

The small, undifferentiated cells distributed among cylindrical cells in the midgut make up the cellular nidi and apparently participate in normal epithelial regeneration processes or in replacement of cylindrical cells (C h a p m a n , 1998).

Endocrine cells distributed throughout the epithelium were not detected in our preparations due to difficulty to observe under conventional histological techniques. Nevertheless, they have already been indicated in other works

and have endocrine function (e.g. hormonal control of peristaltic movements and digestion) (M o n t u e n g a , 1989; S e h n a l , Ž i t ň a n , 1996)

The hindgut is made up of the same layers as the foregut, but the epithelial cells are larger and the cuticle is thinner. The general anatomical (hindgut is differentiated into ileum, colon and rectum) and histological structure of the hidgut was the same as already described (S u i c m e z , B i t m i s , 1991) and coincides with that found in other Coleoptera (C h a p m a n , 1998). Cryptonephridial complex isolates the rectum and the distal ends of the Malpighian tubules from the hemolymph (M a r t o j a , B a l l a n - D u f r a n ç a i s , 1984).

This work has been aimed at characterizing of morphological variability in the epithelial cells (columnar, regenerative and endocrine) mainly along the midgut of *L. decemlineata* larvae. The next step will be the effect of the insecticidal proteins on this part of the gut.

Acknowledgments

We thank J. Krejčová for technical assistance in preparation of the manuscript.

REFERENCES

AMEEN, M. – RAHMAN, M. F.: Larval and adult digestive tracts of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Int. J. Insect Morphol. & Embryol., *2*, 1973: 137–152.

BLACKBURN, M. B. – DOMEK, J. M. – GELMAN, D. B. – HU, J. S.: The broadly insecticidal *Phytorhabdus luminescens* toxin complex a (Tca): Activity against the Colorado potato beetle, *Leptinotarsa decemlineata*, and sweet potato whitefly, *Bemisia tabaci*. J. Insect Sci., *5*, 2005: 1–11.

BOLTER, C. J. – JONGSMA, M. A.: Colorado potato beetles (*Leptinotarsa decemlineata*) adapt to proteinase inhibitors induced in potato leaves by methyl jasmonate. J. Insect Physiol., *41*, 1995: 1071–1078.

CALDEIRA, W. – DIAS, A. B. – TERRA, W. R. – RIBEIRO, A. F.: Digestive enzyme compartmentalization and recycling and sites of absorption and secretion along the midgut of *Dermestes* (Coleoptera) larvae. Arch. Insect Biochem. Physiol., *64*, 2007: 1–18.

CHAPMAN, R. F.: The Insects: Structure and Function. 4th ed. Cambridge, Cambridge University Press 1998.

COSTA, S. D. – BARBERCHECK, M. E. – KENNEDY, G. G.: Sublethal acute and chronic exposure of Colorado potato beetle (Coleoptera: Chrysomelidae) to the δ-endotoxin of *Bacillus thuringiensis.* J. Econ. Entomol., *93*, 2000: 680–689.

DÍAZ, E. – ARCINIEGA, O. – SÁNCHEZ, L. – CISNEROS, R. – ZÚÑIGA, G.: Anatomical and histological comparison of the alimentary canal of *Dendroctonus micans*, *D. ponderosae*, *D. pseudotsugae pseudotsugae*, *D. rufipennis*, and *D. terebrans* (Coleoptera: Scolytidae). Ann. Entomol. Soc. Am., *96*, 2003: 144–152.

DOW, J. A. T.: Insect midgut function. Adv. Insect Physiol., *19*, 1986: 187–328.

GRUDEN, K. – ŠTRUKELJ, B. – POPOVIČ, T. – LENARČIČ, B. – BEVEC, T. – BRZIN, J. – KREGAR, I. – HERZOG-VELIKONJA, J. – STIEKEMA, W. J. – BOSCH, D. – JONGSMA, M. A.: The cysteine protease activity of Colorado potato beetle (*Leptinotarsa decemlineata* Say) guts, which is insensitive to potato protease inhibitors, is inhibited by thyroglobulin type-1 domain inhibitors. Insect Biochem. Mol. Biol., *28*, 1998: 549–560.

HAEFTEN, T. V. – SMID, H. M. – SCHOONEVELD, H.: Serotoninergic innervation of the alimentary canal of the Colorado potato beetle, *Leptinotarsa decemlineata*: structural and functional aspects. Cell Tissue Res., *273*, 1993: 475–485.

LANE, N. J. – DALLAI, R. – ASHHURST, D. E.: Structural macromolecules of the cell membranes and the extracellular matrices of the insect midgut. In: LEHANE, M. J. – BILLINGSLEY, P. F. (eds): Biology of the Insect Midgut. London, Chapman & Hall 1996, pp. 115–144.

LEHANE, M. J. – BILLINGSLEY, P. F.: Biology of the Insect Midgut. London, Chapman & Hall 1996.

LOSEVA, O. – IBRAHIM, M. – CANDAS, M. – KOLLER, C. N. – BAUER, L. S. – BULLA, L. A., Jr.: Changes in protease activity and Cry3Aa toxin binding in the Colorado potato beetle: implications for insect resistance to *Bacillus thuringiensis* toxins. Insect Biochem. Mol. Biol., *32*, 2002: 567–577.

MARTOJA, R. – BALLAN-DUFRANÇAIS, CH.: The ultrastructure of the digestive and excretory organs. In: KING, R. C. – AKAI, H. (eds): Insect Ultrastructure. New York, Plenum Press 1984, pp. 199–268.

MONTUENGA, L. M. – BARRENECHEA, M. A. – SESMA, P. – LÓPEZ, J. – VÁZQUEZ, J. J.: Ultrastructure and immunocytochemistry of endocrine cells in the midgut of the desert locust, *Schistocerca gregaria* (Forskal). Cell Tissue Res., *258*, 1989: 577–583.

RASOCHA, V.: Mandelinka bramborová (*Leptinotarsa decemlineata* Say) významný škůdce v porostech brambor (Colorado potato beetle /*Leptinotarsa decemlineata*/ a serious pest in potato stands). Agro, *4*, 2003: 34–36.

SEHNAL, F. – ŽITŇAN, D.: Midgut endocrine cells. In: LEHANE, M. J. – BILLINGSLEY, P. F. (eds): Biology of the Insect Midgut. London, Chapman & Hall 1996, pp. 55–78.

SUICMEZ, M. – BITMIS, K.: Anatomical and histological researches on the digestive system of *Leptinotarsa decemlineata* Say. Turkish J. Biol., *15*, 1991: 158–165.

VACEK, Z.: Histologie a histologická technika, učebnice pro zdravotnické školy (Histology and histological technology, textbook for medical schools). Praha, Avicenum 1988.

VISAL, S. – TAYLOR, M. A. J. – MICHAUD, D.: The proregion of papaya proteinase IV inhibits Colorado potato beetle digestive cysteine proteinases. FEBS Letters, *434*, 1998: 401–405.

WIGGLESWORTH, V. B.: The Principles of Insect Physiology. London, Butler & Tanner Ltd. 1953.

Received for publication on May 25, 2010
Accepted for publication on June 21, 2010

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Anatomie a histologie trávicího traktu larev mandelinky bramborové.

Scientia Agric. Bohem., *41*, 2010: 134–139.

Tato práce se zabývá studiem anatomie a histologie trávicího traktu mandelinky bramborové, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). Trávicí trubice je rozdělena na tři hlavní části: přední střevo (stomodeum), střední střevo (mesodeum) a zadní střevo (proctodeum). Nejkratší částí trávicí trubice je přední střevo. Přední střevo tvoří silná kutikula (intima), tenký jednovrstevný epitel, bazální membrána a svalová vrstva. Nejdelší částí trávicí trubice je střední střevo. Horní okraj epitelu středního střeva tvoří mikrovilli, charakteristické pro tuto oblast střeva. Základem epitelu středního střeva jsou tři různé typy buněk: trávicí, endokrinní a regenerační (též nazývané nidi). Epitel je obklopen vrstvou svaloviny. Zadní střevo tvoří stejné vrstvy jako přední střevo s tím rozdílem, že buňky epitelu zadního střeva jsou větší a kutikula je tenčí. Ve všech částech střeva je epitel tvořen jednou vrstvou buněk. Začátek šesti malpigických trubic se nachází na rozhraní středního a zadního střeva a samostatně ústí do konečníku.

Leptinotarsa decemlineata; přední střevo; střední střevo; zadní střevo

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