Combination of TEM, LM and micro-CT to image insects: the perspective of crop protection strategies

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Background
Invasive insects, in addition to several already established species, are a serious threat to crop production and an important hazard as vectors of pathogens of severe diseases. This is an increasing problem due to trans-continental trade, travel and climate change. Therefore, new strategies for crop protection and new approaches to minimize the adverse effects of pathogen spread are needed. A better understanding of insect functional ultrastructure during animal development and under stress conditions is crucial to facilitate formulation of new innovative solutions. Microscopic analyses enable comprehensive characterization of the structure of insect organs and localization of selected molecules at different scales, and in combination with biochemical analyses that unravel selected aspects at the molecular level, constitute a toolbox for the integration of structure and function and for an in-depth evaluation of the effects of xenobiotics. The first aim of our study was to establish a procedure in which micro-computed tomography, light and transmission electron microscopy will be used in combination to image insect digestive system in larvae and adults, spanning the range from molecular resolution to imaging of the whole organisms. Our second aim was to apply the method to characterize the midgut of two insect species that are important from the perspective of crop protection, spotted wing drosophila (Drosophila suzukii) and Colorado potato beetle (Leptinotarsa decemlineata).

Methods
The insect gut is positioned at the interface between the external and internal environment and it is a likely target of xenobiotics. As the midgut epithelium plays a central role in digestion, nutrient absorption and protection against toxins and pathogens, we have characterized the midgut functional ultrastructure in larvae and adults to get insight into changes during development and to evaluate the alterations of the gut epithelium due to exposure to selected xenobiotics - fungal lectins and protease inhibitors. Micro-CT imaging was performed on whole animals that were chemically fixed. Subsequent processing and segmentation of the micro-CT data was performed using Neoscan80 and Dragonfly software. Sections of the whole larvae or dissected gut samples were imaged by LM and TEM in different regions along the anterior-posterior axis, focused on the characterization of the luminal surface of the epithelium, the distribution of stem/progenitor cells and alterations of the epithelium architecture after exposure to selected xenobiotics.

Results and conclusions
The digestive system in insects consists of foregut, midgut and hindgut. The highly convoluted midgut is the longest part of the alimentary canal and occupies a large part of D. suzukii larva body volume (Figs. 1A, B). The midgut epithelium comprises several cell types (Fig. 1C), enterocytes bear numerous microvilli on the apical surface (Fig. 1D). Stem cells are abundant and appear as clusters of cells in the basal region of the epithelium (Fig. 1E). In the midgut of Colorado potato beetle larvae columnar enterocytes with dense apical microvilli prevail and numerous stem cells reside in clusters in the basal part of the gut epithelium. Stem cells do not form septate junctions with neighbouring cells, while enterocytes display abundant intercellular junctions. Our current work is focused on the determination of the effects of entomotoxic fungal proteins on the digestive system by a combination of imaging methods

and biochemical approaches to identify the target molecules and elucidate their mode of action.

Graphic:

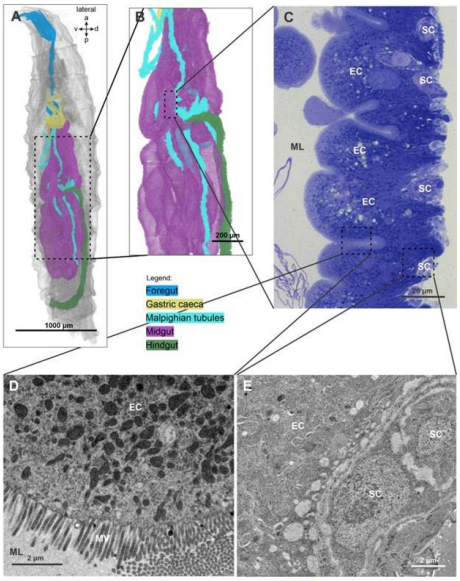

Figure 1: A, B) Micro-CT 3D reconstruction of the digestive system in *D. suzukii larva.* **C)** Histological structure of *D. suzukii* midgut epithelium and peritrophic matrix in the midgut lumen (ML). **D)** Electron micrograph of the apical surface of enterocyte (EC) with microvilli (MV). **E)** Electron micrograph of basally located midgut stem cells (SC).

Keywords:

Midgut, stem cells, insect larvae