## The Maldi-Imaging Technique Applied to the Investigation of the Effects of Insecticides on Bees

<u>Roat, T.C<sup>1</sup></u>, Catae, A.F.<sup>1</sup>, Pratavieira, M.<sup>1</sup>, Da Silva Menegasso, A.R.<sup>1</sup>, Palma, M.S.<sup>1</sup>, Malaspina, O<sup>1</sup>.

1. Centro de Estudos de Insetos Sociais, Instituto de Biociências, Universidade Estadual Paulista (UNESP), Rio Claro, SP, Brazil.

Introduction: The growth of agricultural areas increased the use of insecticides to control pests. However, non-target insects can also be affected by this use, such as Apis mellifera that are considered important pollinators. Among most widely used insecticide classes in agricultural crops there are the neonicotinoids, such as thiamethoxam that is neurotoxic. Objectives: Considering the possible effects of these products on bees, this study aimed to examine the alterations in the spatial distribution of neuroligin and amyloid protein precursor like CG7727-PA in the brains of A. mellifera exposed to this pesticide through the use of MALDI-Imaging. Material and Methods: For this, forager bees were exposed for 8 days to a diet containing a sublethal concentration LC<sub>50/100</sub> of thiamethoxam (0.0227 ng thiamethoxam/ µL diet). Individuals were collected 1 and 8 days after the beginning of food supply and their brains were sectioned by criostate. The chemical printer ChIP-1000 (Shimadzu) was used for trypsin and matrix deposition, and the MALDI (Mass Spectrometric Imaging) MS spectra were acquired by MALDI ToF-ToF (Shimadzu). The spectra were converted into images by MSiReader v0.05 software, creating density maps for proteins. Results and Discussion: Results showed that thiamethoxam caused an increase of the relative expression of neuroligins, that are membrane proteins (type I) that connect neurons pre and postsynaptic in the synapses mediating the signaling through synapses. Thus, the over-expression of this protein in the exposed groups indicates a disorder in the nervous system, probably signaling an increase in the chemical synapses. Likewise, thiamethoxam increased the relative expression of amyloid protein precursor like CG7727-PA, suggesting a neuronal degeneration. Amyloids are ordained protein aggregates usually associated with neurodegenerative diseases. **Conclusion:** Thus, thiamethoxam can affect the synaptic patterns, causing damage to the nervous system. The MALDI-imaging technique is an innovative and efficient tool to investigate the possible alteration in brains of africanized honeybees exposed to pesticide.

Keywords: insecticide, neonicotinoid, sublethal dose, spectrometry Acknowledgments: FAPESP (2012/13370-8 and 2013/07251-9)