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Elucidating Novel Biopesticide Modes of Action in Insects: Physiological, Cellular and Molecular Approaches

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Abstract

This PhD project forms a significant, embedded part of the project, "Novel insecticides and synergists from endemic and exotic flora", funded by the Cotton Research and Development Corporation (CRDC), 2015-2018. I aimed to identify and develop new tools for integrated pest management (IPM) in cotton (*Gossypium hirsutum*). While adoption of transgenic cotton has resulted in reduced synthetic insecticide use against the cotton bollworms *Helicoverpa* spp., secondary pests such as two-spotted spider mite, cotton aphid, green mirid, and silverleaf whitefly continue to be of concern. Thus, there is an urgent need to investigate and develop novel options, such as biopesticides and semiochemicals for insect pest management.

My proof-of-concept study employed two insect cell lines and evaluated three pyrethroid insecticides by combining three *in vitro* methods, absorbance spectrometry, confocal scanning laser microscopy (CSLM) and microelectrode ion flux estimation (MIFE) to assist in elucidating possible mode of action, which could be adopted to evaluate insecticidal activity of complex, unknown, or multi-constituent formulations. I observed that the two cell lines produced distinctly different responses. *Drosophila melanogaster* D.mel-S2 cell line was a useful model to monitor ion flux changes, resulting from insecticides with neural toxicity; however, it was less useful to determine some metabolic pathway indicators of toxic stress. Conversely, the *Spodoptera frugiperda* Sf9 cell line produced acute reactive oxygen species (ROS) in response to insecticide treatments, but was not highly responsive in electrophysiological experiments. I also showed that the natural, multi-constituent botanical extract of pyrethrum elicited different Na^+, Cl^- and Ca^{2+} ion fluxes than its synthetic, single constituent analogues, *α*-cypermethrin and esfenvalerate. These two methods used in combination with absorbance spectrometry measuring cell growth inhibition plus cell mortality assays shed some light on cytotoxic responses in differing model cell lines. The study highlights the importance of utilising multiple cell types and interdisciplinary methods to provide a better insight into mode of insecticidal action. This is especially pertinent to novel biopesticide discovery, as the underlying mechanisms for toxicity in initial screening processes are likely to be unknown.

A laboratory direct application insect bioassay utilising a Potter precision spray tower was employed during the initial foundation project to screen over 400 plant extracts for their efficacy on a selection of cotton insects, after which I identified 20 extracts for further experimentation. I have investigated the insecticidal mode/s of action from a physiological, cellular and genetic standpoint with a focus primarily on three major cotton pests, one model species and two insect cell lines. A combination of insect bioassays, absorbance spectrometry, CSLM, and MIFE have been employed to provide insight into potential target sites for these novel botanical pesticides. Ion channels are the major target sites of action in many insecticides. I found novel physiological responses of two model insect cell lines, D.mel-S2 and Sf9 and a selection of insects to some of the novel botanical extracts using cytotoxicity assay, cell-stress response and ion flux, which provide greater insight for understanding multiple toxicity responses. Moreover, a combination of insect bioassays, RT-qPCR and RNA-sequencing were employed to provide insight into potential molecular targets for these novel botanical pesticides. I identified a large number of differentially expressed genes (DEGs) especially in the categories of membrane transport and oxidative stress that are relevant for the understanding of mode of action (MOA) of these novel extracts on cotton insects. Moreover, I found that, in comparison to the susceptibility of *Aphis gossypii* to the novel botanical extract 68N.M, the DEGs encoding such as chemoreceptors and Ca^{2+} channels may equip *D. melanogaster* with superior capacity to sense and tolerate 68N.M. Many of these key DEGs should be considered for more detailed functional analysis in both *D. melanogaster* and *A. gossypii* to elucidate the gene function in insecticidal MOA in the future.

In summary, by targeting ion transport, ROS production, chemoreceptors and their associated genes in insect cells and insects, there is a great potential to develop reliable laboratory screening methods to identify novel and environmental friendly botanical pesticides for Australian and global agricultural industry in the future.