Final Project Summary

Project title	Insights into the defence of honeybees, *Apis mellifera L.*, against insecticides		
Project number	RD-2009-3656	**Final Project Report**	PR573
Start date	01/10/2009	**End date**	30/09/2013
AHDB Cereals & Oilseeds funding	£28,000	**Total cost**	£108,000

What was the challenge/demand for the work?

The first report of colony collapse disorder in in honeybees was in 2006. Currently an indispensable input for global agriculture, pesticides have been widely vocalised in public discussion platforms as a potential contributor to colony collapse disorder. An extensive number of studies found honeybees to be no more sensitive to numerous insecticides than other insect species, but their metabolic capacity may be limited. Since the honeybee genome has been published, it is known that detoxification genes are relatively lower in number in honeybees than in other insects. Other studies have shown that certain genes play an important role in defence mechanisms of the honeybees against specific insecticides (in-hive). As this project demonstrates, honeybees may have intrinsic mechanisms that provide protection against certain insecticide classes. Here, those metabolic enzymes that confer primary defence to different classes of insecticides (mainly neonicotinoid, thiacloprid) in honeybee were studied.

How did the project address this?

Esterases and P450s as phase 1 metabolic enzymes (i.e. act directly upon the intact insecticide) have been reported to metabolise a variety of insecticides such as organophosphates, carbamates, pyrethroids and neonicotinoids.

This project was conducted to determine metabolic enzymes conferring the primary defence to selected insecticides, with a focus on esterases and P450s:

a. Esterases – use the ‘interference assay’ to determine the interactions between esterases and insecticides. Binding of semi-purified esterase from honeybee with insecticides were tested.

b. P450s – identify defence P450(s) by microarray analyses following gene expression induction by sub-lethal concentrations of insecticides. Express candidate genes heterologously and characterise interaction between the P450 and insecticide by functional assays.

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What outputs has the project delivered?

This project has provided additional evidence at the genomic level for the involvement of phase 1 detoxification enzymes in honeybee response after exposure to thiacloprid, through upregulation of detoxification genes.

Purified honeybee esterase failed to bind to tau-fluvalinate, α-cypermethrin, imidacloprid and thiacloprid. Thus, bee esterase does not provide any protection against tested pyrethroids and neonicotinoids.

Pre-treatment of honeybees with a sub-lethal dose of thiacloprid induced protection to the same compound immediately following thiacloprid feeding. Transcriptome profiling, using microarrays, identified a number of genes encoding detoxification enzymes that were overexpressed significantly in insecticide-treated bees compared to untreated controls. These included four candidate P450s, CYP6BE1, CYP305D1, CYP6AS5, CYP315A1 and an esterase CCE8. The four P450s and cytochrome b5 were functionally expressed in *Eschericia coli* and their ability to metabolise thiacloprid examined by LC-MS analysis. There was no obvious metabolism of thiacloprid, thus their role in the metabolism and disposition of thiacloprid is still unclear. CCE8 expression was not achieved using the *E. coli* expression system.

The main findings from this research were published in Insect Molecular Biology, Volume 25, Issue 2, April 2016 (pages 171-180)

Who will benefit from this project and why?

Agrochemical industry, researchers, beekeepers, farming society and the general public.

An increasing global population brings the challenge of maintaining a sustainable agricultural system, with the constraints on land use and environmental protection. One acute problem is that of arthropod pests, chemical control of which remains the foundation of pest control for many growers today and for the foreseeable future. However, pesticides are suspected by many to contribute to the disappearance of honeybees.

It is the one of the main challenges of all parties to protect honeybees and the environment on the one hand, while ensuring the sustainable use of pesticides on the other.

In the scope of this study, identification of the specific honeybee genes that regulate the metabolism of the detoxification enzymes or the alternative target-sites will enable agrochemical R&D to develop

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safer and target-specific new 'bee-friendly' pesticides/synergists to minimise damage to non-target organisms.

These new strategies would aim to design chemicals which have the ability to inhibit pest detoxification activities without damaging the honeybee defence mechanism or possibly even stimulate its detoxification capacity.

If the challenge has not been specifically met, state why and how this could be overcome
This is the first report with honeybees of a sub-lethal dose of an insecticide providing a protective effect to subsequent exposure of the same insecticide. Following these findings, several microarray studies were performed and the candidate detoxification genes from honeybee were identified. Although functional P450 was obtained for all CYP genes expressed, incubation of thiacloprid and imidacloprid with recombinant P450 failed to produce evidence for the metabolism of thiacloprid or imidacloprid as assessed by parent compound depletion. In the future, a more sensitive detection methodology could be used to monitor the appearance of specific P450-mediated insecticidal metabolites using LC-MS/MS. This would require the use of metabolite standards which were not available during the course of this PhD. Additionally, Further investigation of alternative model substrates of recombinant honeybee P450s is required which would facilitate future insecticide screening.

Lead partner	Rothamsted Research
Scientific partners	Liverpool School of Tropical Medicine
Industry partners	Syngenta
Government sponsor	N/A