Characterisation of neonicotinoid resistance in the cotton aphid, *Aphis gossypii* from Australian cotton

A thesis submitted in (partial) fulfilment of the requirements for the award of the degree

Doctor of Philosophy

from

University of Technology Sydney

By

Kate L. Langfield (née Marshall)
Bachelor of Animal and Veterinary Bioscience (Hons)

School of Life Sciences

APRIL 2017

Certificate of Original Authorship

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as part of the collaborative doctoral degree and/or fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Signature of Student:

Date:

Refereed Journal Publications

This thesis includes a chapter that has been published as the following journal article:

Chapter 3:

Marshall, **K.L.,** Collins, D., Wilson, L.J. & Herron, G.A. 2014. Efficacy of two thiamethoxam pre-germination seed treatments and a phorate side-dressing against neonicotinoid and pirimicarb resistant cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae). Austral Entomology. **54**(4): 351-357

Conference Proceedings

Marshall, K.L., Wilson, L.J. & Herron, G.A. 2012. Do the neonicotinoid seed treatments Cruiser® and Cruiser Extreme® control resistant aphid? p. 102. In: *16th Australian Cotton Conference.* Cotton Research and Development Corporation, Broadbeach, Australia, August 14th - 16th.

Marshall, K.L., Herron, G.A. & Chen, Y. 2014. Neonicotinoid Resistance in Cotton Aphid from Australia. In: *17th Australian Cotton Conference*. Cotton Research and Development Corporation, Broadbeach, Australia, 5th to 7th August.

Marshall, K.L. 2014. Characterisation of Neonicotinoid Resistance in the Cotton Aphid. Presented at the: *17th Australian Cotton Conference*. Broadbeach, Australia, 5th to 7th August.

Acknowledgements

First and foremost, I would like to acknowledge Dr. Grant Herron, my primary supervisor at the Elizabeth Macarthur Agricultural Institute. I deeply appreciate all of the guidance, encouragement, and knowledge you bestowed upon me during my graduate studies at the Elizabeth Macarthur Agricultural Institute. For all of your help and direction I will always be indebted. To Drs. Yizhou Chen and Thomas Walsh, thank you both for your ongoing support and valuable contributions, and lastly, thank you to Professor Steven Djordjevic for accepting me as your graduate student at the University of Technology, Sydney.

Without the financial support of the Cotton Research and Development Corporation which offered me a scholarship for graduate studies, this work would not have been possible. Special thanks go to Tracey Leven and Susan Maas for their unwavering support and dedication throughout the course of this project. My appreciation is also extended to the staff of the Elizabeth Macarthur Agricultural Institute, especially those who I have worked alongside in the Insecticide Resistance Unit.

Thank you to my family and friends, especially my sister Jane, brother Luke and best friend Romy, for helping me survive all of the stress, especially from this last year and for the continued encouragement as I neared the end.

To my parents, I would like to acknowledge the tremendous support you have given me throughout my life. Together, you are the inspiration behind what led me to take on this endeavour. Most of all, thank you for both being a constant example of what '*energy well directed*' can achieve: success!

Finally, I would like to thank my husband Brendan for providing me with endless love and encouragement throughout the duration of this doctorate. Put simply, I have never met anyone who believes in me more. I feel incredibly lucky to have met you and look forward to spending the rest of my life with you.

Contents

List of Tables

List of Figures

List of Abbreviations and Acronyms

ABC: Adenosine triphosphate-binding cassette

Ace: Acetylcholinesterase gene

AChE: Acetylcholinesterase (the target of organophosphate and carbamate insecticides)

ACh: Acetylcholine

ATP: Adenosine triphosphate

CBT: Cotton Bunchy Top

CE: Carboxylesterase

CLR: Cotton Leaf Roll

COG: Cluster of orthologous groups

cys-LGIC: cys-loop Ligand gated ion channel

CYP: Family of P450 genes

DEF: *S,S,S*-tributyl phosphorotrithioate (a synergist)

ddNTPs: di-deoxynucleotidetriphosphates

DDT: Dichlorodiphenyltrichloroethane

DEG: Differentially expressed genes

EMAI: Elizabeth Macarthur Agricultural Institute

EMS: Ethyl methanesulfonate

EST: Esterase

FPKM: Fragments aligned per thousand bases per million reads

GABA: gamma-aminobutyric acid (the target receptor of organochlorines and Phenylpyrazoles (Fiproles) insecticides)

GST: Glutathione-S-transferase

GO: Gene Ontology

IPM: Integrated Pest Management

IRAC: Insecticide Resistance Action Committee

IRMS: Insecticide Resistance Management Strategy

***kdr*; super-*kdr*:** knock down resistance (knock-down resistance traits)

KEGG: Kyoto Encyclopedia of Genes and Genomes

LC_{50}: The dose required to kill half the tested population

LC_{99}: The dose required to kill 99% of the tested population

LBD: Ligand binding domain

nAChR: Nicotinic acetylcholine receptor (the target of neonicotinoid insecticides)

NADPH: Nicotinamide Adenine Dinucleotide Phosphate

NHC: Nitromethylene heterocyclic compounds

NGS: Next generation sequencing

NR: Non-redundant

NSW: New South Wales

***o-Ace*:** orthologous gene to *Drosophila* gene *Ace*

***p-Ace*:** paralogous gene to *Drosophila* gene *Ace*

PBO: Piperonyl butoxide (a detoxification enzyme inhibitor)

PCR: Polymerase chain reaction

P450: Cytochrome P450-dependent monooxygenase

QLD: Queensland

qRT-PCR: Quantitative real-time polymerase chain reaction

***Rdl*:** Resistance to dieldrin gene

RR: Resistance ratio

RFLP: Restriction fragment length polymorphism

RNA-Seq: RNA-Sequencing

SNP: Single nucleotide polymorphism

UGT: UDP-glucuronosyltransferase

USA: United States of America

USD: United States dollar

VGSC: *para*-type voltage gated sodium channel (the target of pyrethroid insecticides, pyrethrins and DDT)

WHO: World Health Organisation

Abstract

The cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is a highly polyphagous pest that inflicts serious damage to a broad range of agricultural, horticultural and greenhouse crops. In Australia, *A. gossypii* is a significant pest of cotton and is difficult to control with insecticides because of its high propensity to develop resistance. Neonicotinoids are among the most effective insecticides used to control *A. gossypii* but the recent detection of resistance threatens their longevity. Consequently, I aimed to restore neonicotinoid efficacy against *A. gossypii* through elucidation of underlying resistance mechanism(s).

Bioassay was used to measure thiamethoxam (neonicotinoid) response in three field strains collected from commercial cotton. Resistance ratios between 49- and 85-fold were produced and resistance was correlated with potential field control failures via a glasshouse efficacy trial. Results showed that resistant *A. gossypii* could complete their development on cotton grown from thiamethoxam-treated seed. A second trial investigated the use of phorate (an organophosphate) as an alternative pre-germination treatment to thiamethoxam. Phorate effectively controls neonicotinoid resistant *A. gossypii* but cross resistance between phorate and the carbamate insecticide pirimicarb must be carefully considered as part of any sustainable management strategy.

PCR-Sequencing was employed to identify if mutation R81T known to confer resistance to neonicotinoid compounds was present in Australian *A. gossypii*. Comparative sequence analysis between susceptible and resistant strains confirmed the absence of mutation R81T. Potential biochemical mechanisms of thiamethoxam resistance in *A. gossypii* were then studied using synergist bioassays. The use of the synergist piperonyl butoxide in tandem with thiamethoxam completely or partially suppressed resistance. This suggests that resistance is at least in part, mediated by overexpression of detoxification enzymes that could subsequently be targeted to achieve improved field control of resistant aphids.

High-throughput sequencing of the *A. gossypii* transcriptome found differences in gene expression associated with thiamethoxam resistance. Two transcripts involved in the

detoxification of xenobiotics (putatively annotated as cytochrome P450 gene *6K1*-like) were found differentially expressed between resistant and susceptible strains. Transcript expression was further validated by qRT-PCR and showed a similar tendency in up-regulation of expression. As such I identified this gene as the strongest candidate for thiamethoxam resistant *A. gossypii.*

This study has generated a comprehensive transcriptome resource for *A. gossypii* that has characterised the expression of numerous important transcripts encoding proteins involved in insecticide resistance. Consequently, my study will contribute to future research relating to molecular characterization of insecticide resistance mechanisms in *A. gossypii* and other insect pests.