

NEONICOTINOID RESISTANCE IN COTTON APHID FROM AUSTRALIA

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Summary

We have shown that target site insensitivity in Australian *Aphis gossypii* via the R81T mutation is not the causal mechanism of neonicotinoid resistance despite overseas studies implicating such. Instead we propose metabolic detoxification as the likely causal mechanism for resistance in Australian *A. gossypii* and we are currently trying to validate that hypothesis via transcriptome analysis. It is not clear why the R81T mutation is absent but the difference may relate to limited imidacloprid use in Australian cotton and the progressive nature of the cotton industry itself.

Introduction

In Australia, cotton aphid, *Aphis gossypii* Glover is a destructive pest of cotton and cucurbits and is frequently targeted with chemical sprays for its control. Resistance to the organophosphates, carbamates, pyrethroids and more recently the neonicotinoids has been detected in *A. gossypii* in Australian cotton (Herron et al. 2001; Herron & Wilson 2011; Marshall et al. 2012). Insecticide resistance in *A. gossypii* has two primary routes; target site insensitivity and metabolic detoxification. Target site insensitivity is caused from modification/s in the gene of the target site which prevents binding of the insecticide and renders the chemical ineffective. In metabolic detoxification, enzymes which metabolize the insecticide may be over produced (gene amplification) or up-regulated (gene expression), in each case allowing the insect to metabolize the toxin to a level suitable for survival. Alternatively, enzymes may have a greater affinity for binding to the insecticide, allowing it to be slowly sequestered over time. Detoxification and/or sequestration are not mutually exclusive and often occur together in insects whereby metabolic detoxification is the primary mechanism of resistance.

For the three chemical classes; organophosphates, carbamates and pyrethroids, the mechanisms by which *A. gossypii* confers resistance have been elucidated as either target site insensitivity and/or metabolic detoxification. Against the more recent chemical class, the neonicotinoids, the causal mechanism of resistance has not yet been revealed.

Overseas, imidacloprid (a neonicotinoid) resistance in *A. gossypii* has been linked to target site insensitivity via a modification in the predicted binding site of neonicotinoid insecticides in the nicotinic acetylcholine receptor (nAChR) (Koo et al. 2014; Shi et al. 2012). This mutation, termed R81T results in an arginine (AGA) to threonine (ACA) base substitution at amino acid position 81 in the loop D region of the $\beta 1$ subunit of the nAChR.

To ascertain whether target site insensitivity was responsible for the confirmed resistance in Australian *A. gossypii*, we amplified the mutation site within the loop D region of the $\beta 1$ subunit through PCR and compared the DNA sequence of a thiamethoxam resistant *A. gossypii* strain (Carrington) from Australia against a reference imidacloprid resistant *A. gossypii* strain (GenBank Accession number: JQ627836) from China (Shi et al. 2012). Additionally, the cDNA sequences of a neonicotinoid susceptible strain (F 96) and an additional thiamethoxam resistant strain (Glentown) from Australia were included for sequence analysis.

Methods

1. Bioassay

Insecticide susceptible (strain F 96) and thiamethoxam resistant (strains Carrington and Glentown, both collected off commercial cotton) were bioassayed against the neonicotinoid insecticide thiamethoxam (Actara®). Briefly, aphids in batches of thirty were placed onto an excised cotton leaf discs fixed in agar in

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a petri dish and sprayed using a Potter spray tower with serial dilutions of the insecticide prepared with distilled water (Herron et al. 2001). Each strain was tested against five serial concentrations, selected to achieve $0 < x < 100\%$ mortality. After spraying, each petri dish was covered with cling wrap with tiny perforations to reduce condensation and placed in an incubator at 25°C for 24 hours. After this period aphids were assessed as dead or alive with the aid of a stereo microscope.

2. Data Analysis

Bioassay data was analysed using a stand-alone probit program developed by Barchia (2001), which ensures that variability between replicates is taken into account. Dose response probit regressions were corrected for control mortality (Abbott 1925) and the LC_{50} and $LC_{99.9}$ plus their 95% fiducial-limits were calculated by applying the method of Finney (1971). Resistance factors were calculated by dividing the LC_{50} of the field-collected population by the value of the susceptible strain.

3. PCR Amplification

DNA was extracted from a pooled sample of 200 aphids of strain Carrington and used as a template in a polymerase chain reaction (PCR) protocol using primers (Forward primer: CTGTCCAGAACATGACCGAA and Reverse primer: GTGGTAACTGAGCACCTGT) designed to amplify the mutation site within the loop D region of the $\beta 1$ subunit of the nAChR. The amplified DNA was purified and sequenced by the Australian Genomic Research Facility (AGRF). Using the sequencing software program CodonCode Aligner® the sequencing data of strain Carrington was aligned to the reference imidacloprid resistant *A. gossypii* strain (GenBank accession number: JQ627836) for comparison.

Additionally, cDNA sequences were produced for susceptible strain F 96 and thiamethoxam resistant strains Carrington, and Glentown for further analysis.

Results

Bioassay Results

Strain	LC_{50} (95% FL ^a) (g/L)	Slope± SE ^b	RF ^c (95% CI ^d)
Susceptible	0.00038(0.00031-0.00046)	2.4±0.24	-
Carrington	0.03(0.027-0.039)	2.2±0.19	85.00(65.29-110.66)
Glentown	0.02(0.01-0.03)	1.2±0.20	51.3(30.5-86.2)

^afiducial limits; ^bstandard error; ^cresistance factor; ^dconfidence interval.

TABLE 1. Full log dose probit regression summary of neonicotinoid susceptible strain F 96 and thiamethoxam resistant strains Carrington and Glentown against thiamethoxam

Sequencing Results

Sequence alignment between susceptible strain F 96, thiamethoxam resistant strains Carrington and Glentown and the reference imidacloprid resistant *A. gossypii* strain (Genbank accession number: JQ627836) confirmed that the region amplified were the loop D region of the $\beta 1$ subunit. Comparative sequence analysis identified that all strains sequenced from Australia possessed a nucleotide G at base position 242 in the consensus region of DNA (AGA), whilst the reference imidacloprid resistant *A. gossypii* strain (Genbank accession number: JQ627836) possessed the nucleotide C (ACA), the later resulting in a corresponding codon change at position 81 from arginine to threonine (R81T) (Fig.1).

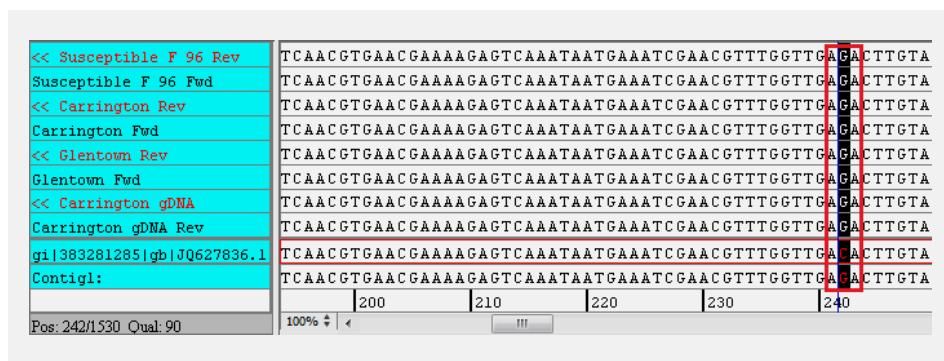


FIGURE 1. Comparative sequence analysis of Aphis gossypii strains susceptible F 96, Carrington (cDNA and gDNA), Glentown and imidacloprid resistant (Genbank accession number JQ627836). (Note: mutation site R81T boxed in red)

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Discussion

Through comparative sequence analysis, our results have illustrated that in Australian *A. gossypii* the causal mechanism of neonicotinoid resistance is not a target site insensitivity via the *R81T* mutation (Fig. 1). Studies overseas which have found the *R81T* mutation in *A. gossypii*, have described it firstly as the putative mechanism for imidacloprid resistance, and secondly as a cross resistance mechanism to other neonicotinoid insecticides (Koo et al. 2014; Shi et al. 2012). Interestingly, our strains display resistance to thiamethoxam (Table 1) but recent bioassay data has shown they are susceptible to imidacloprid (unpubl.data). Although there is confirmed cross resistance between members of the neonicotinoid mode of action group 4A (Shi et al. 2011; Wang et al. 2007) the spectrum of resistance displayed may be dependent on exposure to each chemical. Infrequent use of imidacloprid in Australian cotton may have increased the susceptibility of *A. gossypii* to this chemical. Additionally, in Australia the majority of cotton seed planted is coated with thiamethoxam as a pre germination seed treatment for the control of all early season insect pests. If the *R81T* mutation develops in response to imidacloprid, *A. gossypii* in Australian cotton may not have had enough exposure to develop the *R81T* mutation. Alternatively, we consider in the absence of imidacloprid a metabolic resistance developed via detoxification to the secondary analogs of imidacloprid and in particular to thiamethoxam. Research to validate a metabolic detoxification theory as the primary mechanism of neonicotinoid resistance in Australian *A. gossypii* from cotton is underway.

Conclusion

The mutation responsible for imidacloprid resistance (*R81T*) in *A. gossypii* strains overseas is not present in Australia. Thiamethoxam is used widely in Australian cotton as a pre germination seed treatment whilst imidacloprid use is limited. This could explain the reason

why the causal mechanism of resistance to neonicotinoids in Australia, in particular to thiamethoxam may develop from a different origin.

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