RESISTANCE IN APHIDS

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SUMMARY

- The frequency of neonicotinoid resistant *Aphis gossypii* Glover populations has markedy decreased from a high of 94% to a moderate 22% in a single season.
- The decrease in the frequency of neonicotinoid aphids likely relates to a subtle change in the way foliar neonicotinoids are being used against pests other than aphids and where aphids are accidently being selected by that use rather than a reduction in neonicotinoid seed dressing consumption or an increase in seed dressing efficacy (it was shown not to completely control resistant *A. gossypii*).
- Pirimicarb resistant *A. gossypii* remain infrequent and the chemical continues to be a relaible IPM compatible method of aphid control.
- A new quantitative real-time PCR (qPCR) method under development to estimate pirimicarb resistance in *A. gossypii* will potentially increase assay accuracy and decrease test cost.

INTRODUCTION

In Australian cotton systems, the emergence of the aphid vectored disease 'cotton bunchy top' in the 1998-1999 growing season, led to a reduction in aphid tolerance and more targeted insecticide applications for aphid control. During the subsequent growing seasons, resistance levels in *A. gossypii* increased to dimethoate and pirimicarb causing control failures in many Australian cotton growing regions (Herron *et al*. 2001). Currently, aphid control relies on insecticides from several modes of action; however, the major group used for aphid control in Australian cotton is the neonicotiniods primarily as a seed dressing (Maas 2011). Neonicotinoid resistance in *A. gossypii* was first detected in season 2007-2008 from where it quickly increased in both level and abundance particularly causing control issues with the foliar neonicotinoids used against *A. gossypii* such as acetamiprid and clothianidin (Herron and Wilson 2011).

Here I present recent resistance data showing a marked change in neonicotinoid resistance in *A. gossypii* and show the practical consequence of neonicotinoid resistance on *A. gossypii* control plus introduce a new molecular based method to characterise pirimicarb resistance.

METHODS

Aphids were collected from commercial cotton fields or cotton plants in the vicinity of commercial crops. They were sent to the Elizabeth McArthur Agricultural Institute (EMAI) and cultured separately on pesticide-free cotton at 25 ± 4 ^{o}C under natural light (Herron *et al*. 2001). Strain integrity was assured by maintaining aphid populations in purpose built insect proof cages.

Aphid bioassay tests required placing them in a 35 mm Petri dish on an excised cotton plant leaf disc fixed in agar (Herron *et al*. 2001). Briefly, batches of ten apterous adult female aphids per leaf disc were then sprayed with insecticide with the aid of a Potter spray tower. All tests were replicated and included a water-only sprayed control. After spraying, clear plastic film was used to cover the Petri dishes, which were then maintained at 25 ± 0.1 ^{o}C in 16:8 L:D for 24 h after which mortality was assessed.

Pirimicarb resistance was previously detected via bioassay and then an established DNA based method of M^{c}Loon and Herron (2009) but we are concurrently trialling a quantitative real-time PCR (qPCR) method to estimate the resistance allele frequency from pooled DNA.

Conventional PCR detects resistance alleles in individual aphids and typically 20-50 individual aphids are needed to accurately estimate the resistance allele frequency per strain (sampling site). Our new method for estimating the resistance allele frequency is based on Taqman probes for resistant and susceptible alleles in one PCR reaction with DNA extracted from pooled (hundreds) aphids. The resistance allele frequency can be accurately estimated based on the ratio of increased fluorescence intensity between the resistance probe and susceptible probe at each PCR cycle.

The practical consequence of neonicotinoid resistance was tested using a randomised complete block glasshouse trial where neonicotinoid seed dressings were challenged with neonicotinoid resistance aphids. Resistant aphids were challenged against a thiamethoxam seed treatment (Cruiser 350 SF) at consecutive weekly intervals post germination until product failure relative to an untreated control.

RESULTS

Although testing for season 2011-2012 is still incomplete it is obvious there is a significant reduction in neonicotinoid resistance from the previous season high of 94% to 22% (Figure 1). Pirimicarb resistance testing is also still under way

and to date only a single strain from cotton has contained resistant individuals (Figure 2). The DNA based quantitative real time method for pirimicarb allele detection estimated a resistance frequency of 10.5 and 64.4 percent from populations known to contain 10.0 and 65.0 percent pirimicarb resistant alleles respectively (Figure 3). When a thiamethoxam seed treatment was challenged with neonicotinoid resistant aphids they were not completely controlled at any post treatment assessment interval (Figure 4).

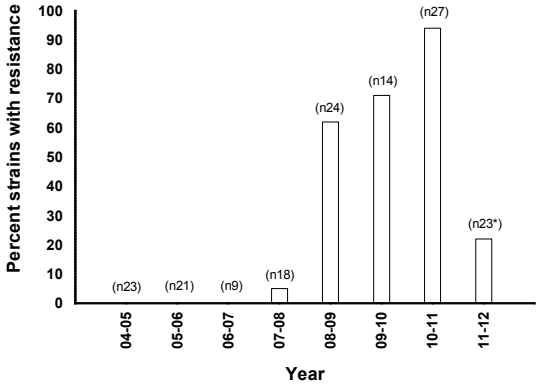

Figure 1. Proportion of *Aphis gossypii* strains showing neonicotinoid resistance (2004-2005 to 2011-2012). *2011-2012 data incomplete at time of writing (23 of 37 strains)

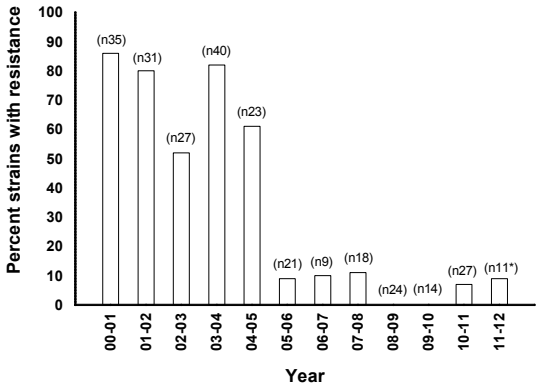

Figure 2. Proportion of *Aphis gossypii* strains showing neonicotinoid resistance (2000-2001 to 2011-2012). *2011-2012 data incomplete at time of writing (11 of 37 strains)

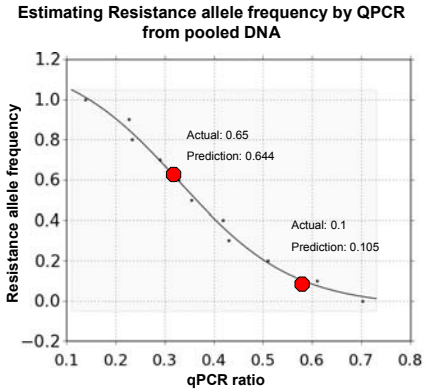

Figure 3. Estimating the pirimicarb resistance allele frequency (orange dots) in two samples of *Aphis gossypii* containing a known 0.10 and 0.65 allele frequency.

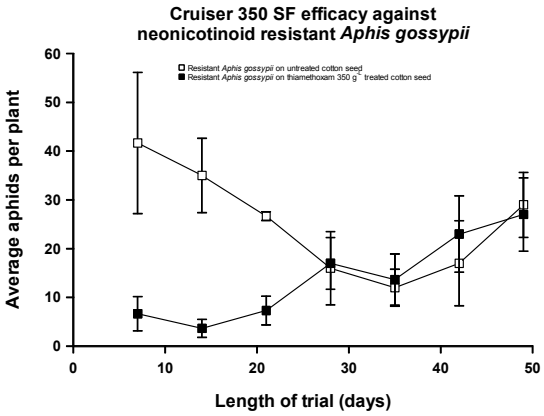

Figure 4. Randomised complete block trial results for neonicotinoid resistant *Aphis gossypii* evaluated against untreated and Cruiser 350 SF treated seed.

DISCUSSION

Resistance monitoring for season 2011-2012 produced some quite unexpected results. The frequency of neonicotinoid resistance in strains tested dropped from a nearly ubiquitous 94% in 2010-2011 to 22% in season 2011-2012. This reduction in the resistance frequency has happened despite the amount of thiamethoxam containing seed dressing used in Australian cotton likely increasing rather than decreasing and a higher rate 'Extreme' product also being available (Rob Eveleigh pers. Com. CSD).

Additionally, seed trial data developed by Kate Marshall at EMAI showed that thiamethoxam treated seed would not fully control neonicotinoid resistant aphids. For that reason I expect neonicotinoid seed treatment to further exacerbate rather than control resistance. None the less, resistance has decreased despite high seed treatment usage likely causing an environment that should be making resistance worse rather than it better.

Clearly something is different and I suspect it is the way foliar neonicotinoid sprays (eg clothianidin or acetamiprid) rather than seed treatments are being used. Resistance issues in *A. gossypii* have often been thought linked to concurrent selection (Herron and Wilson 2011) that simply means aphids being in the wrong place at the wrong time when a neonicotinoid insecticide is sprayed against another pest and aphids are selected. It has been known for some time that *A. gossypii* are often highly pyrethroid resistant (Herron *et al*. 2001) yet those chemicals are not targeting aphids directly.

Foliar neonicotinoid insecticides are used in Australian cotton to control various pests. The green mirid *Creontiades dilutus* (Stål) and green vegetable bug *Nezara viridula* (L.) are targeted for control with neonicotinoids and I consider it likely that neonicotinoid use against pests such as *C. dilutus* and *N. viridula* has subtlety altered and this has coincidently changed the neonicotinoid resistance frequency in *A. gossypii*.

The drop in the neonicotinoid resistance frequency is very good news for aphid control in Australian cotton. Additionally, resistance against the IPM friendly aphicide pirimicarb is also low. The new method being developed by Yizhou Chen at EMAI using qualitative real time PCR technology should greatly increase the precision of future pirimicarb resistance monitoring while simultaneously reducing cost. This is because the new qPCR method can have many aphids included into a single PCR reaction rather than the established method that uses a single PCR reaction per individual aphid.

This is possible because the new method detects resistance alleles rather than resistant individuals so hundreds of aphids can be processed at once for the cost of a single assay and the resistance allele frequency estimated. As we know pirimicarb resistance in *A. gossypii* is nearly always homozygous (Chen, unpublished data) it is straight forward task to relate the proportion of resistant alleles back to an absolute number of resistant *A. gossypii*.

ACKNOWLEDGMENT

Funding for this study is provided by CRDC DAN1203 and related PhD DAN1201. The thiamethoxam seed trial was done by Kate Marshall and forms part of her PhD at UTS. The molecular component is supervised by Dr Yizhou Chen with technical assistance provided by Dr Daniel Bogema. Finally, insect strain maintenance and resistance bioassay is done by Brendan Langfield.

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