Resistance testing summary for the 2006-2007 and 2007-2008 cotton seasons: cotton aphid *Aphis gossypii* and two-spotted mite *Tetranychus urticae*

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- Cotton aphid and two-spotted mite were collected from Australian cotton growing regions and tested in the laboratory for insecticide resistance.
- For the third consecutive season molecular testing was used to detect pirimicarb and organophosphate resistance in field collected cotton aphid strains. Bioassay and molecular testing were conducted in parallel. Similar results where obtained by both methods in their characterisation of pirimicarb and organophosphate resistant aphid strains.
- Pirimicarb and organophosphate resistance associated with control failure was detected in one cotton aphid sample from the Macquarie Valley in 2006-2007. In 2007-2008 aphids were scarce in the Macquarie Valley so no samples were collected. However one strain from St George is pirimicarb resistant and one strain from Gwydir has produced resistant isolates (a mix of susceptible and resistant).
- *Prima facie* acetamiprid resistance has been detected in cotton aphid for the first time but additional testing is required for confirmation.
- Propargite, chlorfenapyr (Intrepid®), abamectin and bifenthrin (Talstar®) resistance were detected in two-spotted mite strains but resistance patterns to the specific chemicals were not the same across seasons. High frequency resistance was restricted to bifenthrin with resistance detected in both seasons 2006-2007 and 2007-2008.

Key Words: Aphids, cotton, resistance, spider mites, molecular, bioassay, monitoring

Introduction

With the introduction of transgenic cotton in Australia to control *Helicoverpa* spp., a reduction in chemical insecticide usage has occurred. Subsequently, there has been an increase in the populations of sucking insect pests, such as green mirids, control of which with broad-spectrum insecticides depletes beneficial populations and leads to outbreaks of secondary pests such as two spotted mite (TSM) and cotton aphid. Control of these secondary pests inevitably selects for insecticide resistant strains. Dealing with this issue requires on-going monitoring for resistance in pests to key insecticides if future control problems are to be averted (Herron 2007).

Two-spotted mite is notorious world-wide for developing insecticide resistance including Australia where resistance in cotton continues to evolve, as most recently seen with Talstar® (Herron *et al*. 2001b) and subsequently Intrepid® (Herron *et al*. 2004).

Similarly, cotton aphid is resistant to a range of insecticides in many crops and countries. Of late, high-level resistance to organophosphates (omethoate and dimethoate) and some carbamates (pirimicarb) has developed in cotton aphid (Herron *et al*. 2001a). In Australia the cotton aphid reproduces almost exclusively asexually, essentially they clone themselves. With this method of reproduction very rapid fixing of genotypic changes can occur since there is no influx of alleles from the male of the species. This is particularly evident with insecticide resistance genes and the rapid appearance of resistant strains seen shortly after insecticide usage.

Both TSM and aphids are important pests of cotton. TSM is capable of causing dramatic losses of yield and reductions in fibre quality. Cotton aphid is a vector of cotton vein mosaic virus (Dos Santos, K. B *et al*, 2004), citrus tristeza virus (Yokomi, R. K and DeBorde, R. L, 2005), cotton bunchy top (Reddall, A *et al*, 2004) and promotes bacterial or fungal contaminations via its honey dew excretions which contaminate the lint. Hence, both TSM and cotton aphid would become major problems in the cotton industry if the capacity to control them was limited by insecticide resistance (Herron *et al*. 2001a).

Continued insecticide resistance monitoring, including generation of baseline data for new chemistry, is essential for effective ongoing management of resistance in these pests. Here we present our monitoring data for seasons 2006-2007 and 2007-2008.

Methods

Chemicals tested

Mites and aphids were treated with proprietary commercial insecticide formulations. For aphids these included acetamiprid (Intruder®), thiacloprid (Calypso®), endosulfan (Thiodan®), thiamethoxam (Cruiser®) and pirimicarb (Pirimor®) except diafenthiuron (Pegasus®) for which the UV activated carbodiimide derivative of diafenthiuron, CGA-140408, was tested instead. This was necessary because diafenthiuron is activated by exposure to UV light, which would not normally occur in the laboratory. Note that acetamiprid, thiacloprid and thiamethoxam are all from the same neonicotinoids group. Mite treatments were, bifenthrin (Talstar®), abamectin (Agrimec®), propargite (Comite®), chlorfenapyr (Intrepid®) and diafenthiuron (Pegasus® as CGA140408). With the introduction to Australia of Bollgard II® cotton the use of insecticides to control pests has dramatically reduced. For this reason the organophosphate profenofos (e.g. Curacron®) is no longer available in Australia and is no longer included in our resistance monitoring.

Cotton aphid

Aphids were collected by researchers, CRC Regional Extension Officers, consultants and growers from commercial cotton fields or cotton plants in the vicinity of commercial crops. They were sent to the bioassay laboratory at Camden (Elizabeth McArthur Agricultural Institute) and each field strain cultured separately on pesticide-free cotton (Deltapine 90) at 25 ± 4 ^{o}C under natural light. Strain integrity is assured by maintaining populations in purpose built insect proof cages.

Aphid Bioassay. Aphids were tested by placing them in a 35 mm Petri dish on an excised cotton plant leaf disc fixed in agar (Herron *et al*. 2001a). Briefly, batches of ten adult female aphids per leaf disc were then sprayed with the aid of a Potter spray tower. Each test was 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.

Aphid Molecular Assay. Pirimicarb and organophosphate resistance were detected via established methods (M^{c}Loon and Herron 2006). Briefly, DNA is isolated from a pool of 20 aphids in addition to 10 individual aphids from each of the different field strains. Both the pool of DNA (from the 20 aphids) plus the 10 individual aphid DNA extractions were subject to PCR amplification of the *Ace*1 gene (covering the mutation responsible for resistance) using real time PCR followed by restriction enzyme digests with the enzymes; *Ssp*I (carbamate resistance) and *Pdi*I (organophosphate resistance). Note that the *Ssp*I enzyme detects resistance to pirimicarb, which would normally also give cross resistance to dimethoate and omethoate, while the *Pdi*I enzyme detects another resistance mechanism to organophosphates (profenofos and chlopyrifos-methyl) based on a second mutation within the *Ace*I gene. Agarose gel electrophoresis was performed to visualise the result of the digests. Gel concentrations were 2%, run for 90 minutes at 94V and saved as digital images using the Gel Dock System (Bio Rad).

Two-spotted mite

Strains of TSM were collected from a range of cotton fields in NSW and Qld late in each cotton season and put into culture as above. The bioassay procedure required young adult female mites to be transferred from culture to French bean leaf discs (Herron *et al*. 2004). Briefly, mites and leaf discs were then sprayed with insecticide with the aid of a Potter spray tower as above. Each test was replicated and included a water only sprayed control. After spraying, mites on leaf discs were maintained at 28 ± 0.1 ^{o}C in constant light for 48 h after which mortality is assessed.

Results

Two-spotted spider mite. TSM was collected from the Gwydir and M^{c}Intyre Valleys during 2006-2007 season and Macquarie and Namoi valleys during 2007-2008 (Table 1). Resistance was detected against bifenthrin, chlorfenapyr and propargite during the 2006-2007 season. Resistance to chlorfenapyr was not detected in the following 2007-2008 season however resistance was detected to abamectin (Table 1).

Aphid Bioassay. Cotton aphid strains were collected more widely than TSM with samples isolated from the Gwydir, M^{c}Intyre and Macquarie Valleys in 2006-2007. In this season, pirimicarb resistance was restricted to the Macquarie Valley only (Table 2). In 2007-2008 survivors at the discriminating dose were detected against pirimicarb in one strain and against acetamiprid in two cotton aphid strains (Bin WF and Blan F3) suggesting *Prima Facie* resistance to this insecticide..

Aphid Molecular Assay. The molecular testing for pirimicarb and general organophosphate (OP) resistance of the 2006/2007 aphids identified resistance in a single strain (Wil 21B) in agreement with the strains' bioassay data. However, when the strain was retested months later (using molecular and bioassay) it had lost the resistance profile to both insecticides. The remaining aphid strains all had a susceptible profile for pirimicarb and general OP resistance. Testing of the 2007/2008 aphid strains identified two with a pirimicarb resistant profile (Table 2). One strain, RvlndMo, had a definitive pirimicarb resistance profile. The other strain, Bin WF, had a mixed profile indicative of a strain with low levels of pirimicarb resistance (approximately 5%).

Additional baseline generation for Calypso® showed the minimum effective dose required to kill strain Car 13 was equivalent to the discriminating dose (Figure 1).

Discussion

Despite the overall reduction in sprays associated with Bollgard II®, resistance causing aphid control failure was still an issue in the Macquarie Valley. One strain, Wil 21B, was shown to be highly pirimicarb resistant with associated resultant control failure. The strain was confirmed to have both pirimicarb and chlopyrifos-methyl (Rescue®) resistance via molecular testing and pirimicarb resistance via bioassay. However, when strain Wil 21B was re-tested some four months later the resistance had completely disappeared and was not detected with either bioassay or molecular methods. Reversion of pirimicarb resistance is unlikely however since the mutation giving rise to it is particularly stable. It is more likely a case of mixed aphid cultures present at the collection site or an overall change in the clonal dominance within the strain. The first bioassay result identified 6% of pirimicarb susceptible individuals (either intra or inter strain variants). It is this pool of aphids that has given rise to the next dominant clone, which when tested four months later (in the absence of selection) was pirimicarb susceptible. The 2007/2008 season has produced a single highly pirimicarb resistant strain from St George and a strain from Gwydir showing a low level of resistance. The Gwydir strain was also shown, via bioassay, to have some acetamiprid resistance.

For the first time cotton aphid has survived a discriminating dose of acetamiprid giving a *Prima Facie* detection of resistance. However, additional research is required to confirm the *Prima Facie* acetamiprid resistance that will require survivors from each of the strains being transferred to new cultures and allowed to breed. These new strains will then be subjected to full log-dose probit analysis and their response compared to established baseline data. Only if significantly different will acetamiprid resistance in cotton aphid be confirmed. Any strains showing resistance to acetamiprid will also be evaluated fully against the other neonicotinoids to evaluate the degree of cross resistance. This is important as another neonicotinoid, imidacloprid, is widely used in cotton as a seed treatment (Gaucho) and thiamethoxam is also a seed treatment (Cruiser). This use pattern may be important in selecting for resistance in aphids. If neoncotinoid resistance is confirmed then we may need to reconsider the positioning of the products and use patterns in the insecticide resistance management strategy.

The discriminating dose used for thiacloprid was interpolated from the dose response for cotton aphid strain Susceptible A. The 0.04 g / L chosen was midway between the calculated $LC_{99.9}$ and $LC_{99.99}$ level of response (ie 0.02- 0.054 respectively). However, the additional baseline data showed the minimum effective dose required to kill strain Car 13 was equivalent to the discriminating dose. For that reason the discriminating dose for thiacloprid has been increased for season 2007-2008 to 0.05 g / L to avoid false positive results.

For the third time resistance in cotton aphid has been diagnosed with both molecular and conventional bioassay methods. There is good agreement between the methods and molecular tests will soon be included as part of the routine resistance monitoring.

Propargite, chlorfenapyr and bifenthrin resistance were again detected in two-spotted mite for season 2006/2007 with abamectin resistance detected the following season as well, though chlorfenapyr resistance was not. However this probably reflects the limited number of strains that were collected. It is not encouraging that bifenthrin resistance was detected in both seasons at a discriminating dose mortality of less then 50% despite the rather small sample. Clearly bifenthrin resistance is persisting despite changes to the resistance management strategy and the overall reduction of insecticide use associated with the introduction of Bollgard® II cotton

Resistance to propargite or abamectin tends to be unstable and resistance will continue to be detected however as long as the current strategy is adhered to resistance generally disappears. Abamectin is the 3rd most common insecticide used on Bollgard II and Emamectin is the second most common insecticide used on conventional cotton this probably exposes mites to reasonably consistent selection hence resistance occurs. Propargite resistance is more difficult to understand it's hardly used at all and may be a consequence of cross resistance or from high propargite selection on the field it was collected from.

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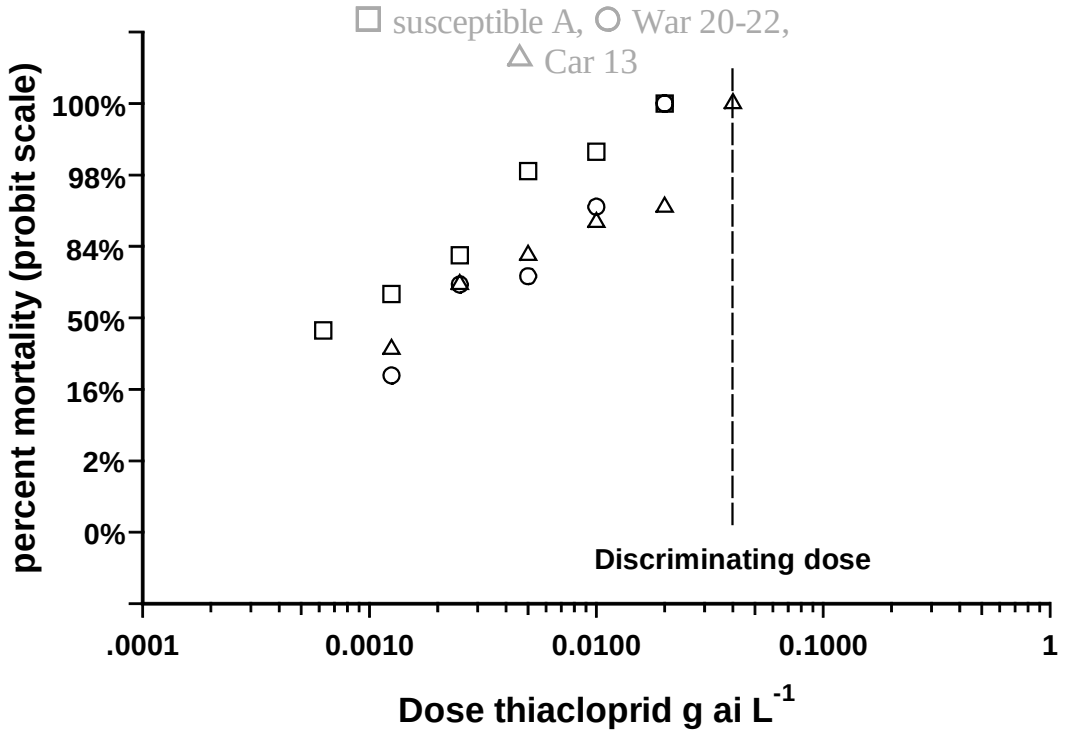

Figure 1. Dose response for Susceptible A, and field strains War 20-22 and Car 13 against Calypso® (thiacloprid) with the 2006/2007 discriminating dose superimposed

Table 1. Percent mortality at the discriminating dose (ie percent susceptible) for various strains of TSM collected during season 2006-2007 and 2007-2008 and evaluated for resistance against Talstar®, Intrepid®, Agrimec®, Comite® and Pegasus® (CGA-140408)

Season	Strain	Area	Chemical				
			Talstar®	Intrepid®	Agrimec®	Comite®	Pegasus® CGA140408
2006-2007	AU	Gwydir	99	100	100	97	100
	NO	Gwydir	45	94	100	100	100
	W	M^{c}Intyre	100	98	100	100	100
2007-2008	WA	Macquarie	46	100	94	99	100
	WI	Namoi	40	100	100	100	100

Table 2. Pirimicarb and Organophosphate (OP) susceptibility using molecular diagnosis and percent mortality at the discriminating dose (ie percent susceptible) using bioassay for various strains of cotton aphid collected during season 2006-2007 and 2007-2008

Season	Strain	Area	Molecular	Test	Bioassay	Chemical					
			*Ssp*I (Pirimicarb)	*Pdi*I (OP)	Pirimor®	[a] Rescue®	Thiodan ®	Intruder®	Pegasus® CGA140408	Calypso®	Cruiser®
2006-2007	Aus Mid 23	Gwydir	S	S	100	100	100	100	100	100	Not tested
	Car 34	M^{c}Intyre	S	S	100	100	100	100	100	100	Not tested
	War 20-22	M^{c}Intyre	S	S	100	100	100	100	100	100	Not tested
	Alch 007	M^{c}Intyre	S	S	100	100	100	100	100	100	Not tested
	Car 13	M^{c}Intyre	S	S	100	100	100	100	100	100	Not tested
	Nor 4	Gwydir	S	S	100	100	100	100	100	100	Not tested
	Byr 55	Macquarie	S	S	100	100	100	100	100	100	Not tested
	Bur 4	Macquarie	S	S	100	100	100	100	100	100	Not tested
	Wil 21B	Macquarie	R & S$^{\Psi}$	R & S$^{\Psi}$	6 & 100$^{\Psi}$	100	100	100	100	100	Not tested
2007-2008	Bel P	St George	S	S	-	-	100	Nf	Not tested	100	100*
	Glen vol	Upper Namoi	S	S	-	-	100	100	Not tested	100	100
	War vol	Upper Namoi	S	S	100*	100*	100	100	Not tested	100	100
	Gos vol	Darling Down	S	S	-	-	100	Nf	Not tested	100	Nf
	Ovr	St George	S	S	-	-	100	100*	Not tested	100	100*
	St G F 134	St George	S	S	-	-	100	100*	Not tested	100	Nf
	Red vol	Gwydir	S	S	-	-	100	100*	Not tested	100	100*

Season	Strain	Area	Molecular	Test	Bioassay	Chemical					
			*Ssp*I (Pirimicarb)	*Pdi*I (OP)	Pirimor®	[a] Rescue®	Thiodan ®	Intruder®	Pegasus® CGA140408	Calypso®	Cruiser®
	Wil F5 vol	Lower Namoi	S	S	-	-	100	100*	Not tested	100	100
	Ros F3 vol	DarlingDown	S	S	-	-	100	Nf	Not tested	100	Nf
	Bin W F	Gwydir	S[#]	S	100	-	100	78	Not tested	100	100
	Blan F3	St George	S	S	-	-	100	96	Not tested	100	100
	Ash vol	St George	S	S	-	-	100	100*	Not tested	100	100*
	M rocks	St George	S	S	-	-	100	100	Not tested	100	100
	Oak C vol	Darling Down	S	S	-	-	100	100*	Not tested	100	100*
	BrkGlenF3	St George	S	S	-	-	100	100	Not tested	100	100*
	Plan Fa F3	St George	S	S	100	-	100	100	Not tested	100	100
	Rvlnd Mo	St George	R	R	7	30	100	100	Not tested	100	100*
	Brk F133-1	St George	S	S	-	-	100	100	Not tested	100	100*

Nf = Not finished
* = Not replicated
[a] = Lorsban® used in 2007-2008
[Ψ] = See discussion
S = Susceptible
R = Resistant
[#] = Low level of resistance (<5%)
- = Not tested unless molecular assay detects resistance (War vol and Plan Fa F3 tested as negative controls)