FIVE YEARS OF RESISTANCE MONITORING FOR SILVERLEAF WHITEFLY IN COTTON

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SUMMARY

- No changes to the current Insecticide Resistance Management Strategy for SLW.
- Resistance to Admiral® has been recorded in the horticultural production areas of Ayr and Bowen highlighting the need to comply with the IRMS.
- No resistance has been detected for Movento® or Pegasus® which make them important options for managing resistance.
- Low levels of resistance to bifenthrin is an additional reason to avoid the use of this broad spectrum, disruptive insecticide.

BODY OF PAPER

Introduction

Since its identification in Australia in 1994, Silverleaf whitefly, *Bemisia tabaci* (SLW) has continued to broaden its geographic range, progressively spreading further south and now being well established in the Gwydir and Namoi regions of NSW. Its infestation of each new cotton valley has been typically characterised by high field numbers during the initial “outbreak” season followed by reduced but persistent pest levels in the following seasons. This boom bust outbreak pattern is thought to be due in part to the delayed response of important natural enemies such as *Eretmocerus hayati* which later become more prevalent and exert area wide suppression of resident SLW populations. Initial outbreaks in each region (Central Highlands, Balonne, Western Downs), have created the impetus for people to give additional consideration to early and mid-season insecticide choices to minimise natural enemy disruption potential. Experience from northern growers suggest that early season use of broad spectrum insecticides will often encourage a later SLW problem and the use of products such as dimethoate early season for mirids has been generally abandoned.

The damage potential of SLW is not the direct result of plant feeding but rather the excretion of honeydew. As whitefly feed on the sap, the high concentration of carbohydrates relative to nitrates means that much of the excess sugars are excreted as waste. This liquid is deposited onto the leaves and cotton lint where it forms a glossy sheen. The presence of honeydew promotes the growth of sooty mould which can reduce leaf processes such as photosynthesis, as well as causing downgrades to lint quality due to colour and stickiness. It is the effect on lint quality that is most problematic.

Compared with aphids which also secrete honeydew, silverleaf whitefly honeydew has unique characteristics that make it particularly problematic. The sugar, trehalulose, produced and excreted by the whitefly in the honeydew has a very low melting point (Hequet and Abidi, 2002). During spinning and milling, buildings may reach 40ºC or more, add to this the temperatures created during friction while spinning, and trehalulose transforms from a solid to a liquid. In its liquid phase, the sugars stick to machinery and foul equipment, increasing the frequency of cleaning and thus running costs.

Management of SLW is complicated by the insects rapidly evolving resistance to insecticides. Overseas, widespread resistance exists to all known older forms of chemistry including carbamates, organophosphates and pyrethroids. It is believed that SLW entered Australia in 1994 already possessing resistance to these insecticides (Gunning et al., 1995).

Five years ago, DAFF entomology Toowoomba commenced resistance monitoring for SLW. The cotton industry had concerns that this pest could develop resistance to the few insecticides registered for its control including pyriproxifen (Admiral®), the ‘cornerstone’ in effective SLW management.

Overseas experience indicated high levels of resistance to Admiral® could occur in cotton farming systems with field failures being recorded in Israel, although all strong cases of Admiral® resistance was later identified as Q biotype (Horowitz et al., 2005). In Arizona, there has also been an observed decline in susceptibility in bioassays after 10 years of use (Ma et al., 2010). Resistant laboratory strains showed no fitness disadvantages indicating that resistance had the potential to develop quickly (Crowder et al., 2008).

The ramifications for the development of resistance were high for the Australian cotton industry at the time this project commenced. At that time, there were only limited products registered for SLW management, with the primary control option being Admiral®. Pegasus® (diafenthiuron) is also relied on for the control of lower density infestations. The only other product registered at the time, bifenthrin (eg Talstar®), had only limited efficacy and was disruptive to beneficial insects.

Resistance monitoring forms the basis of the cotton Insecticide Resistance Management Strategy (IRMS) which is updated each year based on the current resistance profile of the pest. The content of this report includes the results of resistance monitoring conducted between 2007 and 2012 in cotton in Australia, an analyses of changes in resistance over this time period and opportunities for future research that will best equip the industry to manage resistance into the future..

Methodology

A ‘susceptible’ strain of SLW was obtained from CSIRO in 2007 where it had been in laboratory culture since 1995. The susceptible strain is not a true susceptible strain as it has had prior exposure to or risk of exposure to insecticides. It is believed that SLW entered Australia already possessing resistance to insecticides (Gunning et al., 1995). Further, the susceptible strain has been exposed to gene flow from the addition of wild type SLW of unknown origin prior to 2005 and was exposed to Malathion once prior to 2007.

Field collections of whitefly were taken from cotton in Queensland and northern New South Wales between 2007 and 2012. Whitefly were collected either as fourth instar nymphs on leaves or as adults collected using a modified vacuum. Whitefly were identified as *Bemisia tabaci* based on morphological characteristics and a sample was kept in alcohol should molecular confirmation need to be performed.

Bioassays were conducted on the offspring of field collected material with the aim that all bioassays were conducted within four generations in culture so as to best represent the genetics of the field collected strains. Serial dilutions of each of the four registered chemicals; Admiral® (pyriproxyfen), Talstar® (bifenthrin), Pegasus® (diafenthiuron) and Movento® (spirotetramat) were performed using leaf dip bioassays on the relevant development stage of the whitefly. Bioassays were conducted on eggs for Admiral®, first instar nymphs for Movento® and adults for Talstar® and Pegasus® and assessed for mortality at each of the different rates of insecticide.

In 2011-12 an Admiral® discriminating dose was developed as a first screen. Colonies that had high mortality in these bioassays compared to the susceptible strain were then put through a more rigorous dose response assay in the subsequent generation.

Full dose response assays were analysed using Probit 5 for Windows (Gillespie, 1995) and correcting for control mortality using Abbott’s formula. Resistance Factors (RF) were calculated by dividing the lethal concentration (LC) for 50 percent and 90 percent mortality of field strains by the corresponding LC value for the susceptible strain. Discriminating doses were subject to Henderson-Tilton's Corrected Mortality formula. Strain responses were considered significantly different if the fiducial limits did not overlap.

To confirm the presence of resistance, colonies exhibiting elevated resistance factors were pressured with a sublethal dose determined as the rate that killed 70% of the population. A full dose response assay was performed on the offspring of the pressured generation and pressuring was attempted twice to induce a shift in resistance factors. Significant increases in resistance factors compared to the parent strain confirmed resistance.

Results and Discussion

Pyriproxyfen (Admiral®)

As a cautionary note, the baseline data obtained for Admiral® is not true baseline data. The whitefly had already potentially been exposed to Admiral® from as early as 2001 prior to resistance monitoring commencing in 2007-08.

Since testing commenced in 2007-08, elevated resistance factors have been detected for Admiral® in field collections from Ayr, Bowen, St George (QLD) and Warburn (NSW) (see appendix). An Ayr colony that was pressured with a sublethal dose showed increases in resistance factors confirming the presence of resistance. These results corroborate the work of other researchers in Australia and overseas that SLW has the capacity to develop resistance to Admiral® and shows that resistance to this insecticide has been selected for in Australia.

The insecticide resistance management strategy (IRMS) for cotton is more restrictive than for horticulture. Since Admiral® was made available under permit in cotton in 2001, there has been a maximum of one spray per season. In contrast in horticulture, there is a maximum of two applications per crop and several crops per year. This translates into multiple selection events for Admiral® each year in horticulture.

The differences in Admiral® usage patterns between cotton and horticulture would suggest that horticulture regions may have a greater risk of elevated resistance in SLW than in cotton dominated regions. This has been observed in the very high resistance factors recorded in Bowen and the Burdekin as well as growers and consultants' personal observations of reduced field efficacy. The resistance to Admiral® in the Burdekin is due to horticulture usage as no Admiral® has been used to treat cotton grown in the Burdekin (Grundy pers. comm 2012). Despite the restrictions placed on Admiral®, elevated resistance factors have been detected in St George.

Elevated resistance factors for St George have been recorded each season since resistance monitoring commenced. These collections have all been made pre-spray and there has not been any increase in resistance factors between seasons. Because resistance factors have remained both relatively low and stable, there has not been sufficient cause to change the IRMS for this region. However, resistance monitoring will continue each season and growers should remain cognisant of the need to comply with the current IRMS that advocates a maximum of one application per season to conserve the efficacy of this product.

It is uncertain why St George would have elevated resistance factors compared to other cotton dominated regions. Admiral® usage is heavily restricted in cotton and while there is some melon production, there is also some melon production in Emerald which remains susceptible to Admiral®. St George has also had fewer years of exposure to this product with the first whitefly outbreaks in St George occurring in 2003-04, two years after Emerald. Future work includes pressuring colonies from St George to establish whether elevated resistance factors are due to natural variability or resistance.

Resistance factors for Emerald, Theodore, the Darling Downs, Moree and Narrabri remain the same as the susceptible strain. This provides reason to maintain the current IRMS of a maximum of one spray per season.

Admiral® remains the cornerstone of effective management of high density infestations of cotton in Australia. In the event of resistance developing as has occurred in the Burdekin in horticulture, the current IRMS would need to be changed. But with usage for Admiral® currently restricted to a maximum of one spray per season the options for managing further resistance to this product are limited. In Israel, a 'pyriproxyfen free year' strategy has been adopted whereby every couple of seasons this product is rested (Horowitz et al., 1999).

Future opportunities for research into Admiral® resistance in SLW include investigating the 'refuge strategy' to determine how different rates of mixing between susceptible and resistant individuals affects the speed of resistance build up in a population (Carriere et al., 2012).

Diafenthiuron (Pegasus®)

Results for Pegasus® (diafenthiuron) show some elevated resistance factors of 2-8 (see Appendix). Attempts to pressure a colony with higher resistance factors this season was unsuccessful after two attempts. This indicates that this colony did not have resistance present and suggests that the observed differences in resistance factors are natural variability. The resistance factors are similar across all regions and have been observed since resistance testing commenced which is indicative of natural variability.

Pegasus® has not been used widely elsewhere in the world due to phytotoxicity in some crops. The limited information available for Pegasus® comes almost exclusively from its use in Israel where it is registered in cotton and since the introduction of Q biotype, has been the cornerstone in effective management of whitefly. In Israel, monitoring has detected resistance factors of 1 – 8, but this has not changed significantly over time.

Bifenthrin (Talstar®)

Results for Talstar® (bifenthrin) have shown elevated resistance factors each season however it is inconclusive at this stage whether this is due to resistance or natural variability in tolerance to this product. To establish whether the elevated resistance factors are resistance or natural variability, colonies will be exposed to high rates of insecticide in the laboratory. If resistance factors are significantly higher in the offspring then the parents this will indicate that the population has resistance present.

Talstar® registration occurred well before DAFF Entomology commenced resistance testing, so no true baseline data could be established. However, growers who have used multiple consecutive sprays of bifenthrin observed a drop in efficacy with the third spray having almost complete field failure (pers. comm.). Insecticides should be rotated with other products with a different mode of action so as to reduce the risk of field failure due to resistance.

A limitation of conventional resistance monitoring is that resistance will not be detected at very low frequencies or may not be detected in heterozygote individuals. Future opportunities in research include testing molecular markers for resistance. Currently, molecular markers have been detected for pyrethroid resistance (Chung et al.). This marker has not been tested against bifenthrin resistant SLW. Having a molecular marker for resistance would complement the existing monitoring program by providing further information on the genetics of resistance.

Spirotetramat (Movento®)

Movento® (spirotetramat) is the newest SLW product to be registered for use in cotton. Movento® has a unique mode of action making it a useful fit as part of the insecticide resistance management strategy. At this stage Movento® has not been used widely for whitefly control but it is being used in aphid management so there is indirect exposure to this product.

All bioassays remain susceptible at this stage with no differences in resistance factors compared to the susceptible strain and steep slopes in full dose response assays indicating the populations are made up of homozygous susceptible individuals.

CONCLUSION

Five years since resistance monitoring commenced, SLW resistance status remains relatively stable and in some ways is in a better state than when monitoring commenced in 2007 due to the introduction of a fourth insecticide, Movento®.

Silverleaf whitefly remains susceptible to Movento® and Pegasus®, providing useful options from a resistance management strategy perspective. Resistance is present to Admiral® in three horticultural regions and elevated resistance factors have also been recorded in St George, although this has not been confirmed as resistance or natural variability at this stage. Talstar® may have low levels of resistance present in cotton regions however, while registered for SLW control, it is not recommended due to disruption to beneficial insects and poor efficacy due to no translaminar or systemic activity. The registration of Movento® has increased the number of available insecticides for SLW control and this has reduced the resistance risk by reducing selection pressure on the other registered products.

Opportunities for future research include validating literature on potential management strategies for delaying the onset of resistance including the 'refuge strategy' and the 'Admiral® free year' strategy. Other areas of interest include assessing molecular markers for resistance to complement the existing monitoring program.

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APPENDIX

Table 1. Pyriproxyfen resistance factors from 2007-2012

	Cotton Season				
Region	2007-08	2008-09	2009-10	2010-11	2011-12
Burdekin	Resistant (22)	Resistant (108)	Susceptible	Resistant (48)	
		Resistant (125)	Elevated RF (3)	Resistant (20)	
			Susceptible		
Bowen				Resistant (41)	Resistant (13)
					Resistant (19)
					Resistant (15)
					Elevated RF (8)
Emerald	Susceptible	Susceptible			Susceptible
Theodore/Biloela	Susceptible	Susceptible	Elevated RF (2)		Susceptible
St George	Susceptible		Elevated RF (4)	Elevated RF (3)	Susceptible
	Resistant (17)			Susceptible	Susceptible
				Elevated RF (3)	Susceptible
				Susceptible	
				Elevated RF (3)	
Darling Downs	Susceptible	Susceptible	Susceptible		
			Susceptible		
Moree		Susceptible		Susceptible	Susceptible
				Susceptible	Susceptible
					Susceptible
					Susceptible
Narrabri		Susceptible		Susceptible	
		Susceptible		Susceptible	
		Susceptible		Susceptible	
		Susceptible			
Gatton					Susceptible

* The resistance factor (RF) is the rate that kills 90% of a field strain divided by the rate that kills 90% of the susceptible strain

Table 2. Bifenthrin resistance factors from 2007-2012

	Cotton Season				
Region	2007-08	2008-09	2009-10	2010-11	2011-12
Burdekin	Elevated RF(6)			Resistant (RF382)	
Bowen				Resistant (RF>1000)	Resistant (2265)
				Resistant (RF>1000)	Resistant (>2000)
					Resistant (>2000)
					Resistant (2399)
Emerald	Susceptible	Susceptible	Elevated RF (4)		Elevated RF (2)
			Elevated RF (3)		Elevated RF (4)
Theodore/Biloela		Elevated RF (3)	Elevated RF (11)		Elevated RF (2)
		Susceptible			
St George		Susceptible	Elevated RF (3)	Elevated RF (10)	Susceptible
		Susceptible		Elevated RF (11)	Elevated RF (4)
		Susceptible		Elevated RF (4)	Elevated RF (7)
				Elevated RF (2)	
Darling Downs	Susceptible	Elevated RF (2)	Elevated RF (2)		
	Elevated RF (13)		Elevated RF (8)		
			Elevated RF (2)		
Moree		Susceptible		Elevated RF (6)	Elevated RF (3)
				Elevated RF (7)	Elevated RF (6)
					Elevated RF (4)
					Susceptible
Narrabri		Elevated RF (2)		Elevated RF (10)	
		Elevated RF (2)		Elevated RF (6)	
				Elevated RF (5)	

* The resistance factor (RF) is the rate that kills 90% of a field strain divided by the rate that kills 90% of the susceptible strain

Table 3. Diafenthiuron resistance factors from 2007-2012

	Cotton Season				
Region	2007-08	2008-09	2009-10	2010-11	2011-12
Burdekin	Susceptible				
Bowen				Elevated RF (2)	
Emerald	Susceptible	Susceptible	Elevated RF(2)		Elevated RF (2)
			Susceptible		
			Elevated RF (2)		
Theodore/Biloela	Susceptible	Susceptible	Elevated RF (3)		Susceptible
		Susceptible			
St George	Susceptible	Susceptible	Elevated RF (8)	Susceptible	Elevated RF (2)
	Elevated RF(5)	Susceptible		Elevated RF (4)	Elevated RF (2)
		Susceptible		Elevated RF (3)	Elevated RF (4)
				Elevated RF (5)	
Darling Downs	Susceptible	Susceptible	Elevated RF (2)		
			Elevated RF (5)		
			Elevated RF (8)		
Moree		Elevated RF (2)	Susceptible	Elevated RF (4)	Susceptible
				Elevated RF (3)	Elevated RF (2)
					Elevated RF (2)
					Susceptible
Narrabri		Susceptible		Elevated RF (2)	Susceptible
		Susceptible		Elevated RF (2)	Susceptible
		Susceptible		Elevated RF (6)	Elevated RF (2)
		Susceptible			

* The resistance factor (RF) is the rate that kills 90% of a field strain divided by the rate that kills 90% of the susceptible strain

Table 4. Spirotetramat resistance factors from 2007-2012

	Cotton Season				
Region	2007-08	2008-09	2009-10	2010-11	2011-12
Burdekin		Susceptible			
Bowen				Susceptible	Susceptible
					Susceptible
					Susceptible
					Susceptible
Emerald			Susceptible		Susceptible
			Susceptible		Susceptible
Theodore/Biloela		Susceptible	Susceptible		Susceptible
St George			Susceptible		Susceptible
					Susceptible
					Susceptible
Darling Downs			Susceptible	Susceptible	
Moree		Susceptible		Susceptible	Susceptible
				Susceptible	Susceptible
					Susceptible
					Susceptible
Narrabri				Susceptible	Susceptible
				Susceptible	Susceptible
				Susceptible	Susceptible

* The resistance factor (RF) is the rate that kills 90% of a field strain divided by the rate that kills 90% of the susceptible strain

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