



Australian Government
**Cotton Research and
Development Corporation**

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FINAL REPORT

**"Insecticide resistance management in cotton aphid (*Aphis gossypii*) and
cotton mite (*Tetranychus urticae*)"**

DAN 163c

1 July 2002 to 30 June 2005

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**NSW DEPARTMENT OF
PRIMARY INDUSTRIES**

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Annual, Progress and Final
Reports

Part 1 - Summary Details

REPORTS

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Project Title: Insecticide resistance management in cotton aphid (*Aphis gossypii*) and cotton mite (*Tetranychus urticae*)

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Key highlights –

Aphids

1. Thiodan®, Confidor®, Actara®, Intruder® and Pegasus® resistance was not detected. These chemical can be used with confidence against cotton aphid.
2. It was initially thought by the product supplier that Rescue® may have been a 'silver bullet' for cotton aphid control. This was not the case and Rescue® resistance was consistently detected in 30-40% of strains tested.
3. Pirimor® resistance was more abundant than Rescue® resistance with a low of 52% resistant strains detected in 2002-2003 to a maximum of 77% resistant strains in 2003-2004. Pirimor® resistance continued at 61% of strains in 2004-2005 despite additional use restrictions introduced in season 2003-2004. It is likely that the continuing Pirimor® resistance was caused by a known cross-resistance to Folimat® or Rogor® and their use against other pests, possibly mirids, with coincident selection of concurrent aphids. The 2004-2005 management strategy for aphids included a warning that aphid resistance should be considered when applying Folimat® or Rogor® for mirid control.
4. Aphids were additionally collected from hosts other than cotton and monitoring detected resistant populations in farm backyards and on weeds. These rogue overwintering aphid populations have the potential to provide a nucleus of future control problems and so should be eliminated where practical. The resistance management strategy was modified to include that information with a recommendation that overwintering aphids specifically be targeted for non chemical control.
5. Cross-resistance biochemical studies demonstrated that the Temik® target site resistance was different to Pirimor® target site resistance. Consequently Temik® could be considered a unique rotation group. For the 2004-2005 cotton season the aphid rotation groupings were Temik®, organophosphates and carbamates ort han Temik®, the neonicotinoids, Pegasus®, Thiodan® and Fullfill®. This was included into the aphid resistance management strategy.

Mites

6. No resistance was detected to Agrimec® or Pegasus®.
7. Incipient Comite® resistance was detected in 2003-2004 but not in the following season. This suggests that the resistance management strategy for that product is working well and gives confidence in the resistance management strategy for two-spotted mite
8. Talstar® resistance was common during the study making the product at best unreliable for two-spotted mite control.
9. Intrepid® resistance was common and continued to be detected despite a modification to the resistance management strategy in season 2003-2004 that further restricted product use from two to one spray per season. Encouragingly resistance frequencies in each strain were generally less than in seasons 2003-2004. It will be interesting to see if the trend continues with Intrepid® resistance frequencies per strain continuing to drop. I don't consider this indicates a flaw with the management strategy because Intrepid® failed in cotton after a single application yet in cotton it survived for some 5 seasons before Intrepid® resistance was detected in two-spotted mite.
10. Curacron® resistance in two-spotted mite was ubiquitous and often at high frequencies. Resistance was such that Curacron could no longer be considered a useful control for two-spotted mite

1. Outline the background to the project.

Up until the introduction of transgenic cotton the cotton bollworm, *Helicoverpa armigera* (Hübner) has been the major pest of the Australian cotton industry. However following the introduction of the single gene Ingard® transgenic cotton sucking insect pests such as aphids, mites and mirids have become more troublesome, so requiring increased targeted insecticide control. Of late, high-level organophosphate and carbamate resistance has developed in cotton aphid (Herron *et al.* 2001). Two-spotted mite *Tetranychus urticae* Koch has a proven ability to develop resistance if targeted with and recently developed resistance to chlorfenapyr (Intrepid®)(Herron *et al.* 2004a). Other sporadic but troublesome sucking pests include green peach aphid *Myzus persicae* (Sulzer), bean spider mite *Tetranychus ludeni* Zacher, thrips (including western flower thrips) and green mirids (Forrester and Wilson 1988).

Cotton aphid, *Aphis gossypii* Glover

The pest status of aphids is often related to the contamination of the cotton lint with sugary 'honey-dew'. However, earlier outbreaks in the crop growth cycle can significantly reduce yield and recently cotton aphid was confirmed as a vector for 'Cotton Bunchy Top' syndrome, a virus type disease of cotton linked to aphids. These changes in the system mean that the need for effective tools to control aphids and resistance management for those chemical tools is critical to the cotton industry.

Cotton aphid reproduces asexually that allows very rapid changes in resistance level to develop. Management of aphids is further complicated because there is no dilution of resistance by outcrossing to susceptibles, as is used to manage *Bt* resistance in *Helicoverpa* spp. Therefore, aphids can very quickly become a major problem when chemical control fails due to resistance (Herron 2001).

Effective management of cotton aphid will be best achieved by pursuing an integrated approach, including monitoring, cross-resistance studies resistance, mechanism elucidation and evaluation of new chemistry and the effect on beneficials. Without this study Australia's reputation as a producer of clean cotton could be dramatically affected.

Two-spotted mite, *T. urticae*

Two-spotted mite is notorious world-wide for developing resistance with Australian researchers publishing many first citations (eg Herron *et al.* 1993, Herron and Rophail 1998).

As each new compound has become available we have pro-actively established baseline resistance levels and cross-resistance profiles and initiated routine resistance monitoring. Unfortunately, resistance continues to evolve as seen most recently to bifenthrin (Herron *et al.* 2001a) and then chlorfenapyr (Herron *et al.* 2004). This is occurring largely due to use of these compounds against other pests, rather than mites themselves, and is disturbing, as it reduces the number of chemicals available for two-spotted mite control.

Management of mites is complicated because most chemicals are also used against other pests such as aphids or *Helicoverpa* spp. and this has contributed to resistance development to organophosphates, bifenthrin and chlorfenapyr. Effective management of two-spotted mite will be best achieved by pursuing an integrated approach that includes resistance management, based on a sound understanding of the mites resistance and cross-resistance

spectra. Continued resistance monitoring, and the timely inclusion of new chemistry, is essential for effective ongoing management of this pest species.

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2. List the project objectives and the extent to which these have been achieved.

The project aimed to achieve effective resistance management of cotton aphid and two-spotted mite

- Effective resistance management of cotton aphid and two-spotted mite was achieved through regular updating of the aphid and mite resistance management strategy in line with contemporary resistance monitoring outputs. For instance the 2002-2003 management strategy included warnings not to follow a seed or in furrow insecticide treatment with the first foliar spray from the same chemical group. The subsequent 2003-2004 strategy had Pirimor® and Folimat® use against aphids further restricted by strategic windowing and Intrepid® use against two-spotted mite (including *Helicoverpa* spp sprays) reduced to one application per season. The 2004-2005 strategy had aldicarb removed to its own rotation group giving an additional alternation group for aphid management plus a warning that Folimat®/Rogor® sprays against mirids could select Pirimor® resistance in concurrent aphids.

3. Detail the methodology and justify the methodology used.

Aphids

Aphids were collected by researchers, CRC Industry Development Officers, consultants and growers from cotton fields, weeds and domestic back yards. They were then sent to the bioassay laboratory at Camden (EMAI) and each field strain cultured separately on pesticide-free cotton (Deltapine 90) at $25 \pm 4^{\circ}\text{C}$ under natural light. Strain integrity was assured by maintaining populations in purpose built insect proof cages. When established in culture, the 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.* 2001). Batches of ten adult female aphids per leaf disc were then be sprayed with the aid of a Potter spray tower producing an aqueous insecticide deposit of

1.6 ± 0.07 mg cm⁻² with a 2 mL insecticide aliquot. Each test was replicated and included a water only sprayed control. After spraying, the clear plastic film covered Petri dishes were maintained at 25 ± 0.1 °C in 16:8 Light : Day (L:D) for 24 h after which mortality was assessed.

Mites

Strains of two-spotted mite were randomly collected 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 (Edge and James 1982). Mites and leaf disc were then sprayed with insecticide with the aid of a Potter spray tower as above. Each test was replicated and includes a water only sprayed control. After spraying, mites on leaf discs were maintained at 28 ± 0.1 °C in constant light for 48 h after which mortality is assessed.

Analysis

Data were analysed using a Probit program written in GENSTAT 5 statistical software (Barchia, 2001). LC₅₀ and LC₉₉ values plus their 95% fiducial limits were calculated using the probit method outlined in Finney (1971) and included control mortality correction (Abbott 1925). Resistance factors (RF) (RF₅₀ and RF₉₉) plus their associated 95% confidence intervals (CI) were calculated as outlined in Robertson and Preisler (1992). Discriminating-dose tests were corrected for control mortality (Abbott 1925).

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4. Detail and discuss the results including the statistical analysis of results.

4.1 Aphids

Cotton aphid resistance testing: season 2002-2003

For season 2002-2003 resistance was not detected against Thiodan® (endosulfan), Confidor® (imidacloprid) or Pegasus® (diafenthiuron)(Table 4.1.1) In contrast Pirimor® (pirimicarb) and Rescue® (chlorpyrifos methyl) resistance was relatively common. Pyrethroid resistance was often detected in cotton aphid although the product is not registered for that use. This clearly illustrates the problem of non-target selection with coincident populations.

For season 2002-2003 strains from Goondiwindi were particularly interesting as only three out of nine strains tested showed Pirimor® resistance and none were resistant to Rescue®.

This is in stark contrast to strains from other regions with 100% resistance to both chemicals being common. It is interesting these strains are from a region committed to IPM and currently trialing season long soft management.

The second significant outcome is results from strains collected from backyards or weeds. It is now clear these backyard resistance refuges may be providing a nucleus for future control problems in nearby cotton.

Finally, in contrast to most eastern Australian results, Northern Territory strains showed no Pirimor® resistance but one strain did show a low frequency resistance to Rescue®.

Table 4.1.1. Percent mortality at the discriminating dose (ie percent susceptible) for various strains of cotton aphid collected during season 2002-2003 and evaluated for resistance against Pirimor® (Pir), Rescue® (Res), Pegasus® (CGA-140408) (Peg), Confidor® (Con), Talstar® (Tal) and Thiodan® (Thio).

Strain	Region	Chemical					
		Pir	Res	Peg	Con	Tal	Thio
NT 1	N Territory	100	100	100	100	100	100
NT 2	N Territory	100	100	100	100	100	100
NT 3	N Territory	100	94	100	100	100	100
Farm Garden 1	Namoi	100	100	100	100	100	100
Farm Garden 2	Namoi	99	81	100	100	92	100
Farm Garden 3	Namoi	28	100	100	100	100	100
Em 1	Emerald	100	100	100	100	40	100
Em 2	Emerald	2	100	100	100	100	100
Em 3	Emerald	45	80	100	100	100	100
ACRI regrowth	Namoi	100	83	100	100	100	100
ACRI 1	Namoi	29	95	100	100	100	100
ACRI 2	Namoi	20	33	100	100	100	100
Bladder Ketmia	Toowoomba	16	68	100	100	100	100
G 1	Goondiwindi	96	100	100	100	100	100
G 2	Goondiwindi	100	100	100	100	95	100
G 3	Goondiwindi	100	100	100	100	100	100
G 4	Goondiwindi	100	100	100	100	100	100
G 5	Goondiwindi	100	100	100	100	100	100
G 6	Goondiwindi	100	100	100	100	100	100
G 7	Goondiwindi	90	100	100	100	100	100
G 8	Goondiwindi	100	100	100	100	100	100
G 9	Goondiwindi	86	100	100	100	88	100
N 1	Namoi	11	38	100	100	100	100
N 2	Namoi	100	77	100	100	42	100
N 3	Namoi	36	18	100	100	100	100
N 4	Namoi	30	34	100	100	100	100
D 1	Dalby	89	100	100	100	100	100
Overall proportion with resistance		52	41	0	0	18	0

Cotton aphid resistance testing: season 2003-2004

For season 2003-2004 the resistance management strategy for Folimat® (omethoate) and Pirimor® (pirimicarb) was changed to reduce Pirimor® selection against cotton aphid. The aim was to reduce Pirimor® resistance frequencies and restore confidence in this useful IPM friendly compound. Despite the change to the strategy Pirimor resistance has remained high and approximately equal to frequencies found in 2002-2003 season (Table 4.1.2). Despite our best efforts resistance levels have remained static and we suspect this may be attributable to coincident product use against other pests, especially Folimat or Rogor® (dimethoate). It is clear from the data that coincident product use is a problem, for instance, Talstar® (bifenthrin) is not registered for aphid control yet some 30 percent of populations show some level of Talstar® resistance. Folimat® and Rogor® are popular chemicals that unfortunately confer cross-resistance to Pirimor® in aphids but are also used to control mirids. It is notable that the 2003-2004 cotton season required many cotton growers to spray for mirid control and those sprays were undoubtedly Folimat® or Rogor®. It seems reasonable to suspect that mirid control is now adversely affecting the management of cotton aphid.

For the 2003-2004 season strains were collected so that Pirimor® resistance could be tabulated relative to collection site ie from domestic yards, weeds, cotton or crops other than cotton (Table 4.1.3). It is notable that domestic back yards and weeds have a very high proportion of resistant populations. This is important because cotton aphid can develop a winged form that can disperse if food quality drops or aphids become too crowded. Consequently resistant aphids can move from farm gardens, weeds or domestic backyards to cotton fields. If food quality is good the non-winged form is found which has limited mobility explaining why aphids occur as 'hotspots', then after a time can be suddenly found widely as the winged forms spread over the field.

Table 4.1.2. Percent mortality at the discriminating dose (ie percent susceptible) for various strains of cotton aphid collected during season 2003-2004 and evaluated for resistance against Pirimor® (Pir), Rescue® (Res), Pegasus® (CGA-140408) (Peg), Confidor® (Con), Talstar® (Tal) and Thiodan® (Thio).

Strain	Region	Where	Chemical					
			Pir	Res	Peg	Con	Tal	Thio
Adam St Narrabri	Namoi	Dom yard	8	74	100	100	100	100
Tinkelara	Emerald	Cot crop	98	100	100	100	100	100
Parker Farm	Emerald	Cot crop	98	100	100	100	100	100
Greenbah Garden	Namoi	Dom yard Nr cotton	16	23	100	100	100	100
ACRI F18 sample 1873	Namoi	Weed	96	100	100	100	100	100
ACRI F18 sample 1874	Namoi	Volunteer Cotton	98	100	100	100	100	100
ACRI F18 sample 1878	Namoi	Volunteer Cotton	97	100	100	100	100	100
ACRI F18 sample 1884	Namoi	Ratoon Cotton	93	100	100	100	100	100
Waterways	Emerald	Cot crop	34	79	100	100	100	100
5 ways	Emerald	Cot crop	88	100	100	100	100	100
Brearley Downs	Emerald	Cot crop	43	100	100	100	100	100
Jabiwarra	Emerald	Cot crop	80	74	100	100	96	100
Orvieto	Emerald	Cot crop	74	100	100	100	100	100
Shananda	Emerald	Cot crop	10	100	100	100	100	100

Strain	Region	Where	Chemical					
			Pir	Res	Peg	Con	Tal	Thio
Deneliza	Emerald	Cot crop	59	100	100	100	96	100
ACRI 1989	Namoi	Sunflower	41	81	100	100	100	100
ACRI 1935	Namoi	Sunflower Crop	65	100	100	100	100	100
ACRI 1936	Namoi	Weed Nr cotton	36	73	100	100	100	100
ACRI 1938	Namoi	Mung bean crop	Did	Not	Establis h			
ACRI 1939	Namoi	Weed Nr cotton	19	62	100	100	100	100
Field 10	Pittswort h Qld	Cot crop	100	97	100	100	90	100
UTCN - ACRI	Namoi	Cot crop	24	100	100	100	100	100
AET 7 - ACRI	Namoi	Cot crop	47	88	100	100	100	100
Warendi	Goondi	Cot crop	100	100	100	100	100	100
Morella	Goondi	Cot crop	100	100	100	100	100	100
Mullala	Goondi	Cot crop	68	100	100	100	72	100
Alcheringi	Goondi	Cot crop	100	100	100	100	100	100
Mimosa Field 55	Gwydir	Cot crop	100	100	100	100	48	100
Warilea Field 2	Namoi	Cot crop	100	100	100	100	86	100
Listowell Field 3	Gwydir	Cot crop	Did Not Establish					
Pindara Field 5	Namoi	Cot crop	100	100	100	100	76	100
Oakey Point southern river block	Namoi	Cot crop	Did Not Establish					
Lochelgrin Field 13	Namoi	Cot crop	31	100	100	100	100	100
Havana Field 12	Namoi	Cot crop	100	100	100	100	99	100
Riverway 1933	Namoi	Cot crop	78	77	100	100	100	100
ACRI 1945	Namoi	Weed	72	60	100	100	100	100
ACRI 1948	Namoi	Weed	Did Not Establish					
Greenbah 1960	Namoi	Dom yard Nr cotton	Did Not Establish					
Greenbah 1962	Namoi	Dom yard Nr cotton	Did Not Establish					
Greenbah 1963	Namoi	Dom yard Nr cotton	Did Not Establish					
Greenbah 1965	Namoi	Dom yard Nr cotton	Did Not Establish					
Rosilda	Dalby	Cot crop	58	77	100	100	82	100
Boshammer	Dalby	Cot crop	Did Not Establish					
Sunnyside bridge 1977	Namoi	Cot crop	Did Not Establish					
Narrabri RSL	Namoi	Dom yard	85	91	100	100	99	100
Riverway 2016	Namoi	Cot crop	67	100	100	100	92	100
Riverway 2017	Namoi	Weed Nr cot	48	83	100	100	100	100

Strain	Region	Where	Chemical					
			Pir	Res	Peg	Con	Tal	Thio
Riverway 2024	Namoi	Weed Nr cot	70	100	100	100	96	100
Sunnyside farm 2035	Namoi	Cot crop	67	99	100	100	100	100
Carsons Block 2064	Namoi	Weed Nr Cot	Did Not Establish					
Collins Bridge 2065	Namoi	Weed not Nr cot	21	100	100	100	100	100
Greenbah 2082	Namoi	Dom yard Nr cot	Did Not Establish					
Overall proportion with resistance			77	35	0	0	30	0

Where: Dom = domestic, Nr = near, Cot = cotton

Table 4.1.3 Percent of populations (strains) showing Pirimor® (pirimicarb) resistance collected from domestic yards, weeds, cotton or other than a cotton crop

Source	Number populations collected	Number populations with resistance	Proportion resistant
Domestic yards	3	3	100
Weeds	9	9	100
Cotton	26	18	69
Crop other than cotton	2	2	100

Cotton aphid resistance testing: season 2004-2005

Pirimor® resistance was detected in 61% of cotton aphid populations (Table 4.1.4). As Pirimor® resistance is extremely high level, control failure would likely result. Unfortunately Pirimor® is known to cause cross resistance to Folimat® and Rogor® so those products would also be compromised by resistance. Pirimor resistance remains despite a change to the management strategy in 2003-2004 when Pirimor® and Folimat® use was restricted by the implementation of chemical use windows. It is possible that control of mirids with Folimat® or Rogor® may be selecting concurrent aphids so producing Pirimor®, Folimat® and Rogor® resistance in aphids.

Table 4.1.4. Percent mortality at the discriminating dose (ie percent susceptible) for various strains of cotton aphid collected during season 2004-2005 and evaluated for resistance against Pirimor® (Pir), Pegasus® (CGA-140408)(Peg), Thiodan® (Thio), Actara® (Act) and Intruder® (Int)

Strain	Region	Chemical				
		Pir	Peg	Thio	Act	Int
Norw	Gwydir	92	100	100	100	100
Lamer	Gwydir	87	100	100	100	100
F2#1 AC	Namoi	did	not	establish	into	culture
Mapl F1	Goondi	100	100	100	100	100
Carring	Goondi	100	100	100	100	100

Pall F4	Goondi	100	100	100	100	100
McD K3	St George	95	100	100	100	100
Alc F007	Goondi	did	not	establish	into	culture
Mor Win	Goondi	did	not	establish	into	culture
Car F2-7	Goondi	did	not	establish	into	culture
Mor E4	Goondi	did	not	establish	into	culture
War18-22	Goondi	did	not	establish	into	culture
Tuck	Goondi	did	not	establish	into	culture
Car20-25	Goondi	99	100	100	100	100
Caffery	Dalby	100	100	100	100	100
Lat F15	Bourke	100	100	100		
Lat F17	Bourke	96	100	100	100	
Lat F18	Bourke	100	100	100	100	100
Lat LM3	Bourke	90	100	100	100	100
Bee F16	Gwydir	did	not	establish	into	culture
Tara	Namoi	95	100	100	100	100
Hava F4	Namoi	91	100	100	100	100
Beech	Namoi	did	not	establish	into	culture
Purl F42	Gwydir	91	100	100	100	100
Milo	Gwydir	did	not	establish	into	culture
Glen F6B	Namoi	100	100	100	100	100
Mira F2	Namoi	100	100	100	100	100
Veth F5	Gwydir	93	100	100	100	100
LFL F1 B	Hillston	97	100	100	100	100
LFL30/2015	Hillston	90	100	100	100	100
LFLG16-26	Hillston	100	100	100	100	100
LFLY12-15	Hillston	did	not	establish	Into	culture
LFL B lat	Hillston	92	100	100	100	100
LFL dr lat	Hillston	93	100	100	100	100
Overall with resistance	proportion	61	0	0	0	0

4.2 Mites

Two-spotted mite resistance testing: season 2002-2003

Agrimec®, Comite®, Talstar® and CGA-140408 (the UV activated carbodiimide derivative of Pegasus®) resistance was not detected in two-spotted mite (Table 4.2.1). Intrepid® resistance was detected in 4 of the 6 strains tested (Figure 4.2.1) with a maximum of 8.7-fold resistance in strain G at LC50 level. Curacron® resistance was detected at a maximum level of 541-fold in strain W (Figure 4.2.2), however, strain HA had 100% mortality at the discriminating dose and so is susceptible.

Intrepid® resistance was first detected in season 2001-2002 with season 2002-2003 producing an alarming increase in level and abundance of Intrepid® resistance (Figure 4.2.1). In response the mite management strategy for Intrepid® was modified for season 2003-2004 requiring a reduction in total Intrepid sprays to one per season for either *Helicoverpa* spp or two-spotted mite. Intrepid® resistant two-spotted mite from Japan were found to be incompletely dominant and monogenic (Uesugi et al. 2002). If resistant Australian two-spotted are a similar single locus then resistance would be expected to evolve faster with increased insecticide use (Tabashnik 1990) and so the halving of the Intrepid® selection pressure would be expected to extend the useful life of the product.

During the 1999-2000 cotton season, Talstar® resistance was detected in 9 of the ten strains. During 2000-2001 Talstar® resistance was detected in 5 out of 6 strains tested. Similarly in season 2001-2002 resistance was detected in 7 of the 8 strains. In this 2002-2003 season resistance was detected in 4 of the 6 strains suggesting that the abundance of Talstar® resistance is static. Consequently, Talstar® would be unreliable for two-spotted mite control

For season 2001-2002, Curacron® resistance peaked at an all time high of 1718x in strain Warriana Field 4. This season resistance dropped to a maximum of 541x in strain W and interestingly strain HA from Hillston was susceptible but high level vigour tolerant. It would be more than a decade since Curacron® susceptible TSM have been found in cotton.

Table 4.2.1. Testing results for the 2002/2003 cotton season- six strains of two-spotted mite against a 0.02% discriminating dose (DD) of Comite®, 0.0001% DD of Agrimec®, 0.02 % DD of Talstar® and 0.002% DD of Pegasus® (CGA-140408).

Strain	Mortality at DD- Agrimec	Mortality at DD-Talstar	Mortality at DD-Pegasus	Mortality at DD- Comite
G	100	48	100	100
W	100	55	100	100
Y	100	72	100	100
RA	100	100	100	100
TO	100	86	100	100
HA	100	100	100	100

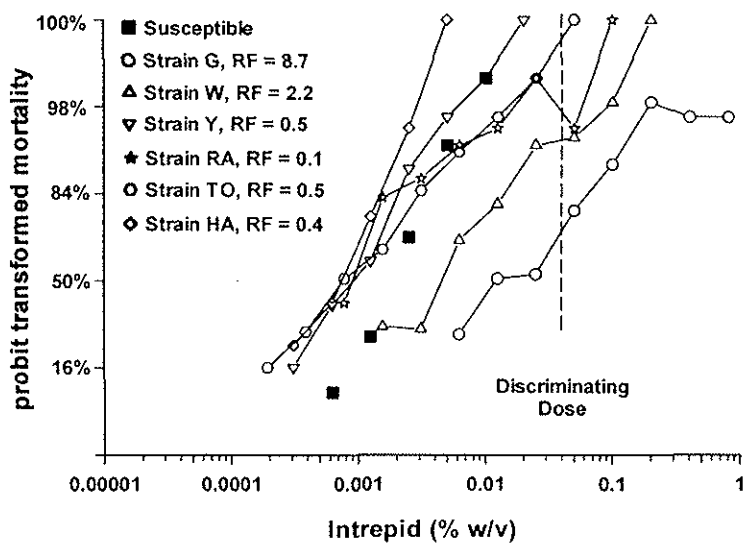


Figure 4.2.1. Dose-response for Intrepid® against six strains of two-spotted mite collected during the 2002/2003-cotton season

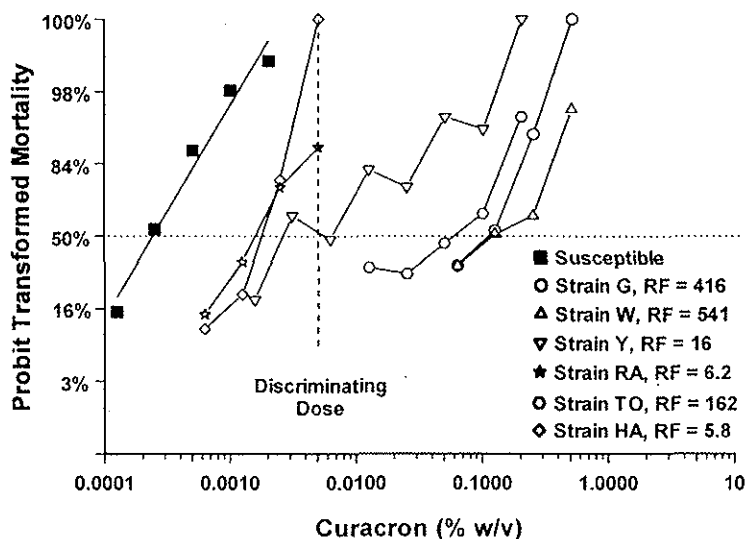


Figure 4.2.2. Dose-response for Curacron® against six strains of two-spotted mite collected during the 2002/2003-cotton season

References

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Uesugi R., Goka K. and Osakabe M.H. 2002. Genetic basis of resistance to chlorfenapyr and etoxazole in the two-spotted spider mite (Acari: Tetranychidae). *Journal of Economic Entomology* **95**: 1267-1274

Two-spotted mite resistance testing: season 2003-2004

Agrimec® and Pegasus® resistance were not detected (Table 4.2.2). In contrast, Curacron® resistance was detected in every population tested (Table 4.2.2). Intrepid® resistance was detected in three strains (Table 4.2.2) peaking at 4.0 fold at the LC₅₀ level in strain RA (Figure 4.2.3). Comite® resistance was detected in one strain only (Table 4.2.2) and at 2.7 fold at the LC₅₀ level (Figure 4.2.4).

Season 2002-2003 produced an alarming trend of increasing level and abundance of Intrepid® resistance. In response the mite management strategy for Intrepid was modified with a reduction in total Intrepid® sprays. Despite the change to the management strategy, Intrepid® resistance was again detected in 3 of the 7 strains tested during season 2003-2004.

In the 2002-2003 season resistance was detected in 4 of the 6 strains and in season 2003-2004 in 5 of the 7 strains tested. This suggests that the abundance of Talstar® resistance remains relatively static and would continue to be unreliable for mite control.

Table 4.2.2. Testing results for the 2003/2004 cotton season: eight strains of two-spotted mite against a 0.0001% discriminating dose (DD) of Agrimec® (Agr), 0.02 % DD of Talstar® (Tal), 0.04% DD of Intrepid® (Int), 0.002% DD of Pegasus® (CGA-140408), 0.02% DD of Comite® (Com) and 0.005% DD of Curacron® (Cur).

Strain	Chemical					
	Agr	Tal	Int	Peg	Com	Cur
CH	100	97	100	100	100	11
G1	100	100	100	100	100	48
G	100	81	96	100	100	36
LO	100	49	100	100	97	4
H	100	70	100	100	100	8
P	100	67	79	100	100	20
RA	100	100	73	100	100	67

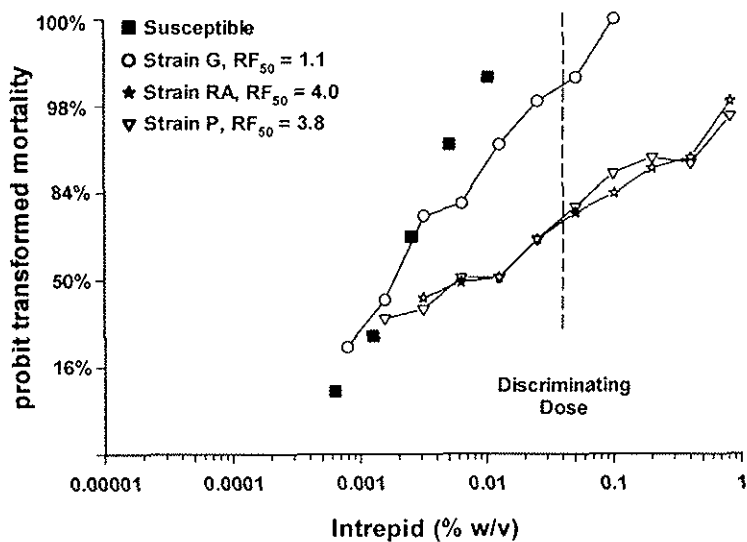


Figure 4.2.3. Dose-response for Intrepid® against a laboratory reference and three suspect resistant strains of two-spotted mite collected during the 2003-2004-cotton season

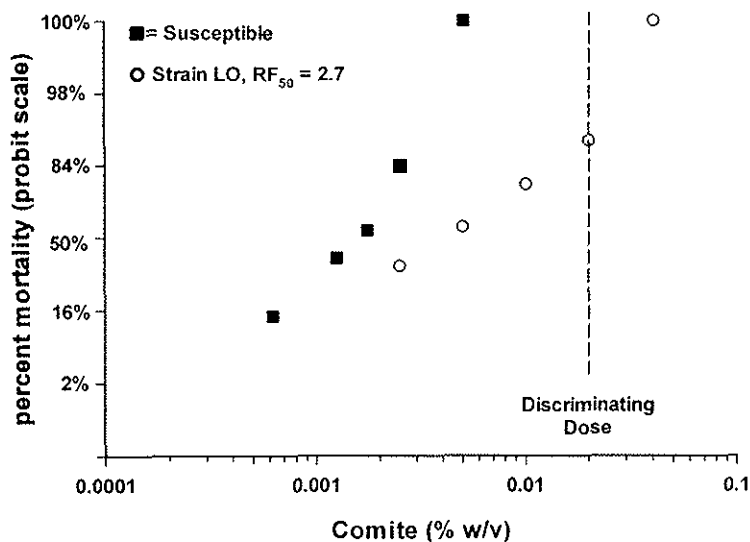


Figure 4.2.4. Dose-response for Comite® against a laboratory reference and single suspect resistant strain of two-spotted mite collected during the 2003-2004 cotton season

Two-spotted mite resistance testing: season 2004-2005

Strains were collected from the Namoi, Gwydir and Griffith areas (Table 4.2.3). Agrimec®, Comite® and Pegasus® resistance was not detected, but Intrepid® resistance was detected in 3 of the 7 strains and Talstar® resistance in 4 of the 7 stains. Curacron resistance was ubiquitous. A baseline response was established using a reference susceptible strain against Paramite® (Figure 4.2.5).

Season 2004-2005 saw the new ovicide Paramite® (extoxazole) registered for use against mites in cotton. However, the generation of baseline to produce a discriminating dose proved particularly time consuming and elusive. After much experimentation the cause was found to be two fold. Firstly, the formulated product was extremely thick and the minute quantities required for bioassay would not pipette reproducibly. This was solved by weighing rather than pipetting product. Next there was a problem with the bioassay not reaching end point. After much testing the bioassay was found not be reaching endpoint at the conventional 8 day withholding period. The withholding period has now been extended to 10 days. With these two modifications baseline data was generated against our standard laboratory susceptible strain. Data can be extrapolated with precision to the LC99.9 level and a discriminating concentration for resistance monitoring estimated. A discriminating dose for resistance monitoring would be approximately 0.0001% ai Paramite®.

Intrepid® resistance was again detected in 3 out of 7 strains during 2004-2005 but encouragingly resistance frequencies in each strain were generally less than in season 2003-2004. It will be interesting to see if the trend continues in 2005-2006 with Intrepid® resistance frequencies per strain continuing to drop.

Again Talstar® resistance was detected in 4 out of 7 strains suggesting Talstar® resistance remains relatively static and so continuing to make the product unreliable for mite control.

Table 4.2.3. Percent mortality at the discriminating dose (i.e. percent susceptible) for various strains of two-spotted mite collected during cotton season 2004-2005 and evaluated for resistance against Talstar® (Tal), Intrepid® (Int), Agrimec® (Agr), Comite® (Com), Pegasus® (Peg) and Curacron® (Cur)

Strain	Area	Chemical					
		Tal	Int	Agr	Com	Peg	Cur
AN	Namoi	29	96	100	100	100	12
G	Namoi	31	84	100	100	100	19
TG	Griffith	100	100	100	100	100	43
GL	Gwydir	100	100	100	100	100	10
NH	Namoi	44	90	100	100	100	17
RA	Griffith	100	100	100	100	100	79
PU	Namoi	50	100	100	100	100	10

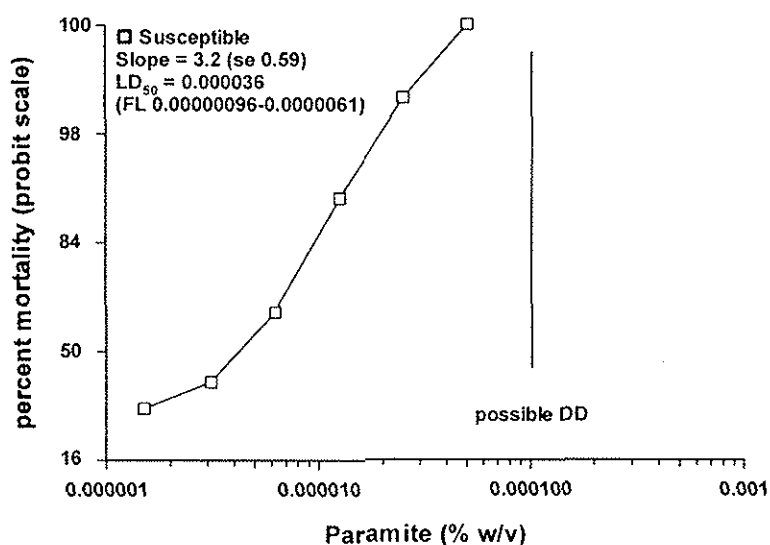


Figure 4.2.5. Dose-response for Paramite® against a laboratory reference strain of two-spotted mite (se = standard error, LD = lethal dose and FL = fiducial limit)

4.3 New chemistry

AMPARO®: A NEW COTTON SEED-DRESSING MIXTURE OF IMIDACLOPRID AND THIODICARB

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Summary

The new cotton-seed dressing Amparo® is a mixture of two chemical groups (chloronicotinyl and carbamate) that must be accurately positioned within the Australian cotton Integrated Resistance Management Strategy (IRMS). Against cotton aphid, *Aphis gossypii* Glover, the carbamate component of the mixture did not significantly reduce aphid numbers. In contrast, the chloronicotinyl component was significantly better than the control and gave statistically equivalent efficacy at all rates tested. Consequently, the current chloronicotinyl grouping of Amparo within the cotton IRMS is valid.

Key words: mixtures, seed-dressing, resistance management, *Aphis gossypii*, cotton aphid

Introduction

The Integrated Resistance Management Strategy (IRMS) for Australian cotton was originally designed to manage resistance in *Helicoverpa* spp. but increasingly considers a range of insecticide resistant secondary pests (Johnson and Farrell 2003). *Aphis gossypii* Glover is one such pest that has recently risen in status due to increasing resistance and associated control failures (Herron *et al.* 2001). The aphid management strategy in cotton is based on the grouping of like chemicals including foliar sprays and seed treatments with chemical alternation between groups (Rossiter *et al.* 2003).

For the 2003-2004 cotton season, Bayer CropScience Pty. Ltd. will introduce a new seed treatment for aphid control that is a combination of the chloronicotinyl, imidacloprid and the carbamate, thiodicarb. Other seed dressings and in-furrow insecticides currently in use in Australian cotton include the carbamate aldicarb (Temik® 150 G), the organophosphate phorate (Thimet® 200 G), and the chloronicotinyls imidacloprid (Gaucho® 600 FS) and thiamethoxam (Cruiser® 350 FS)(Johnson and Farrell 2003). Due to cross-resistance in *A. gossypii* between the organophosphate and carbamate groups pesticides belonging to these classes are considered as one group in the IRMS (Rossiter *et al.* 2003).

To accurately position Amparo within the management strategy we aimed to evaluate its individual components for efficacy against *A. gossypii*.

Materials and methods

Aphids

The susceptible strain was collected from an unsprayed Sydney backyard. Its response to a range of chemicals has been previously described (Herron *et al.* 2000).

Chemicals

Bayer CropScience Pty. Ltd. supplied cottonseed variety Sicala V3i previously treated with formulated imidacloprid or thiodicarb at various rates (Table 1).

Table 1. Seed treatments

Treatment	Active ingredient
1	fungicide (QAP ¹) only control
2	imidacloprid 600 g L ⁻¹ Flowable Concentrate for Seed Treatment (FS) 580 mL 100 kg ⁻¹ + thiodicarb 500 g L ⁻¹ FS 250 mL 100 kg ⁻¹
3	imidacloprid 600 g L ⁻¹ FS 580 mL 100 kg ⁻¹ + thiodicarb 500 g L ⁻¹ FS 500 mL 100 kg ⁻¹
4	imidacloprid 600 g L ⁻¹ FS 580 mL 100 kg ⁻¹
5	imidacloprid 600 g L ⁻¹ FS 875 mL 100 kg ⁻¹
6	thiodicarb 500 g L ⁻¹ FS 500 mL 100kg ⁻¹

¹every treatment included Quintozene, Apron and Peridiam (QAP) fungicides.

Seed germination

On the 7th August 2003 five treated seeds were evenly planted into plastic pots (15 cm diameter) filled with Yates Green Earth premium potting mix. Pots contained only one treatment and each treatment was replicated three times. Pots were placed into individual saucers and watered from above and below with 100 mL. The pots were transferred to a plant germination room maintained at 28 ± 2 °C where they were watered with a further 100 mL on Day 3 and Day 6 and left for a total of 7 d until dicotyledons had emerged.

On the 14th August pots were transferred into one of three aphid proof cages in an insectary (maintained at 25 ± 4 °C). Each cage contained all treatments in a randomised complete block design with pot position randomly assigned.

Each plant was challenged with two adult insecticide susceptible *A. gossypii* aptera at weekly intervals until 29-30th September 2003. Four days post challenge the plants were examined and those with leaves with 0, <10 or >10 aphids noted. During the interval plants were watered (150-200 mL) twice a week at the base of the plant only. After the final challenge the

leaves were removed from each plant and number of aphids counted with the aid of a stereomicroscope.

Statistical analysis

Final aphid numbers per plant were analysed using a generalised linear mixed model with errors assumed to follow a Poisson distribution with a logarithmic link function as follows:

$$\text{Ln}(\text{count}) = \text{offset} + \text{treatment} + \textit{block} + \textit{plot}$$

where “offset” is natural logarithmic of Control mean and the “*italicised*” terms are included in the model as random effects. Parameters were estimated using the residual maximum likelihood (REML) technique through ASREML statistical software (Gilmour *et al.* 1999). Predicted values of ratio of treatment means over Control mean and percent efficacy were calculated.

Results

Treatment effects (ie % efficacy) were highly significant ($P < 0.001$) with all imidacloprid treatments having similar efficacy that was significantly different from the thiodicarb only treatment (Table 2). Interestingly, thiodicarb (treatment 6) seemed to have an inhibitory effect on aphid numbers during the first week of the trial (Table 3).

Table 2. Mean calculated as a ratio to the control count

Treatment	Ln(mean)	SE	Mean	%Efficacy
2	-7.0788	2.3568	0.0008	99.92a
3	-4.1231	0.681	0.0162	98.38a
4	-5.4845	1.0435	0.0042	99.58a
5	-5.8647	1.2147	0.0028	99.72a
6	-0.0782	0.4862	0.9248	7.52b

Note: Significant differences were determined using Z-value at $P < 0.001$ on Ln(mean)

Discussion

The seed treatments Gaucho® 600 FS and Amparo® both contain the active ingredient imidacloprid but with varying amounts of active ingredient. The Gaucho® 600 formulation has a higher application rate of 525 g ai (875 mL product) while Amparo® is applied at 348 g ai (580 mL product) 100 kg⁻¹ seed.

However, in the current study both rates provided statistically equivalent control at trial's end. In contrast, the carbamate component of the Amparo® mixture did not significantly reduce aphid numbers. Consequently, in terms of the cotton IRMS, Amparo® should be included in a chloronicotinyl grouping with Cruiser® 350 FS and Gaucho® 600 FS.

Interestingly, our results suggest that the thiodicarb component of the Amparo® mixture when applied at 250 g ai (500 mL product 100 kg⁻¹ seed) had a short-lived inhibitory effect on aphid numbers. The phenomenon may be worthy of further study to ascertain if the effect is statistically significant.

Acknowledgements

Ms Brie Collier provided technical assistance. Dr Idris Barchia is thanked for the statistical analysis

Table 3. The number of plants in each treatment with leaves infested with 0, <10 or >10 aphids when assessed at six different challenge dates

Date counted	No. of plants (max. 15) with leaves containing	Treatment					
		1	2	3	4	5	6
18 August	0 aphids	2	11	14	14	14	9
	<10 aphids	10	0	0	0	0	2
	>10 aphids	0	0	0	0	0	0
25 August	0 aphids	1	10	13	14	14	3
	<10 aphids	11	1	1	0	0	8
	>10 aphids	0	0	0	0	0	0
1 September	0 aphids	0	10	13	12	14	0
	<10 aphids	1	1	1	2	0	11
	>10 aphids	11	0	0	0	0	0
8 September	0 aphids	0	3	12	12	12	0
	<10 aphids	0	8	2	2	2	3
	>10 aphids	12	0	0	0	0	8
16 September	0 aphids	0	9	9	10	11	0
	<10 aphids	0	2	5	4	3	0
	>10 aphids	12	0	0	0	0	11
22 September	0 aphids	0	9	5	8	10	0
	<10 aphids	0	2	8	4	4	0
	>10 aphids	12	0	0	2	0	11
29-30 September	Total aphids	6864	6	160	38	26	6479

see table 1 for key to treatments

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Insecticide Resistance Where Are We Now? Dec 2003. Cotton Information Sheet TRC
43 12/03. Australian Cotton Cooperative Research Centre, Narrabri. pp. 4.

TESTING INSECTICIDE SUSCEPTIBLE TWO-SPOTTED MITE AGAINST BAYER EXPERIMENTAL PRODUCTS DC-026 and BSN- 2060

Aim

- To evaluate the relative efficacy of two experimental acaricides against laboratory reference susceptible two-spotted mite.

Method

Strain Tested

The susceptible strain was collected from an unsprayed Sydney backyard in 1987 and its response to several chemicals has been previously published (Herron *et al.* 1998).

Chemicals Tested

Formulated product was supplied by Bayer Crop Science and included DC-026 240 g / L SC, BSN-2060 240 g / L SC and the spray adjuvant Pulse.

Bioassay

Edge and James (1982) have described the bioassay procedure used in detail. Briefly, the method requires young adult female mites to be transferred from culture to French bean leaf discs. With the aid of a Potter spray tower mites and leaf disc were sprayed with insecticide and 0.1% pulse or insecticide and water. Each test included a pulse only and/or water only sprayed controls.

Analysis

Where appropriate data were corrected for control mortality (Abbott 1925).

Results and Discussion

Table 1. Dose response for various aqueous concentrations of DC-026 and BSN-2060

Concentration	DC-026 240 g / L SC			BSN-2060 240 g / L SC		
	Number	Dead	%M	Number	Dead	%M
0.1%W/V	27	18	65	24	10	39
0.01%W/V	26	14	52	28	13	44
0.001%W/V	25	19	75	27	16	57
0.0001%W/V	26	0	0	25	3	8
Water only	27	1	4	24	1	4

Table 2. Dose response for various aqueous concentrations of DC-026 and BSN-2060 and 0.1% Pulse

Concentration	DC-026 240 g / L SC			BSN-2060 240 g / L SC		
	Number	Dead	%M	Number	Dead	%M
0.1%W/V	25	25	100	23	23	100
0.01%W/V	25	25	100	21	21	100
0.001%W/V	26	26	100	26	26	100
0.0001%W/V	28	28	100	24	24	100
0.1% Pulse only	26	18	69	21	16	76
Water only	23	1	4.3	28	1	3.6

When sprayed against susceptible two-spotted mite DC-026 and BSN-2060 both gave poor control with increasing concentration. The addition of 0.1% Pulse adjuvant produced 100% mortality at all concentrations tested, however, data show that Pulse alone was very toxic. In fact, Pulse was more toxic than either DC-026 or BSN-2060.

DC-026 and BSN-2060 do not appear to be particularly good acaricides and are probably not worth developing further.

Acknowledgments

Mrs Jeanette Rophail, Senior Technical Officer Scientific, provided technical assistance.

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Edge, V.E. and James, D.G. (1982) Detection of cyhexatin resistance in two-spotted mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) in Australia. *Journal of the Australian Entomological Society*, **21**: 198.

Herron G.A., Edge V.E., Wilson L.J. and Rophail J. 1998. Organophosphate resistance in spider mites (Acari: Tetranychidae) from cotton in Australia. *Experimental and Applied Acarology* **22**: 17-30.

TESTING OF VARIOUS WITH HOLDING PERIODS WITH BAYER EXPERIMENTAL PRODUCTS DC-026 AND BSN-2060 AGAINST INSECTICIDE SUSCEPTIBLE TWO-SPOTTED MITE

Aim

- To evaluate the relative efficacy of two experimental acaricides at various withholding periods against laboratory reference susceptible two-spotted mite.

Method

Strain Tested

The susceptible strain was collected from an unsprayed Sydney backyard in 1987 and its response to several chemicals has been previously published (Herron *et al.* 1998).

Chemicals Tested

Formulated product was supplied by Bayer Crop Science and included DC-026 240 g / L SC and BSN-2060 240 g / L SC.

Bioassay

Edge and James (1982) have described the bioassay procedure used in detail. Briefly, the method requires young adult female mites to be transferred from culture to French bean leaf discs. With the aid of a Potter spray tower mites and leaf disc were sprayed with insecticide or water only control. Numbers of alive and dead mites were counted pre treatment and then at 48, 72 and 96 h after treatment

Results and Discussion

Table 1. Dose response for various aqueous concentrations of DC-026 and BSN-2060 at various withholding periods

Concentration sprayed	BSN-2060 240 g / L SC				DC-026 240 g / L SC			
	N	@48h	@72h	@96h	N	@48h	@72h	@96h
0.1%W/V	25	11	20	21	22	14	18	19
0.01%W/V	21	7	12	15	24	11	17	19
0.001%W/V	26	9	15	21	27	12	13	14
0.0001%W/V	24	2	2	9	23	2	2	3
Water only	21	1	2	3	24	0	1	1

Where: N = pre treatment total number of mites, @48h = number of mites dead at 48 hours after treatment, @72h = number of mites dead at 72 hours after treatment, and @96h = number of mites dead at 96 hours after treatment.

When sprayed against susceptible two-spotted mite DC-026 and BSN-2060 both gave poor control with increasing concentration. However, as withholding period was extended so efficacy increased and the bioassay may not have reached a stable end point mortality within the 96 hour test period.

Acknowledgments

Mrs Jeanette Rophail, Senior Technical Officer Scientific, provided technical assistance.

References

Edge, V.E. and James, D.G. 1982. Detection of cyhexatin resistance in two-spotted mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) in Australia. *Journal of the Australian Entomological Society*, **21**: 198.

Herron G.A., Edge V.E., Wilson L.J. and Rophail J. 1998. Organophosphate resistance in spider mites (Acari: Tetranychidae) from cotton in Australia. *Experimental and Applied Acarology* **22**: 17-30.

5. Provide a conclusion as to research outcomes compared with objectives. What are the “take home messages”?

Aims:

- Monitor resistance levels in mites and aphids.
- Establish baseline for new acaricides and aphicides.
- Cross-resistance profiles for current and experimental chemicals.
- To elucidate mechanisms of resistance in cotton aphid.
- Use all the above information to develop and/or refine resistance management strategies for mites and aphids in cotton

Take home messages:

Aphids

- Thiodan®, Confidor®, Actara®, Intruder® and Pegasus® resistance was not detected.
- Rescue® is not the magic silver bullet for aphid control. Rescue® resistance was consistently detected in 30-40% of strains.
- Pirimor® resistance was more abundant than Rescue® resistance with a low of 52% resistant strains detected in 2002-2003 to a maximum of 77% resistant strains in 2003-2004. Pirimor® resistance continued at 61% of strains in 2004-2005 despite additional use restrictions introduced in season 2003-2004. It is likely that the continuing Pirimor® resistance was caused by a known cross-resistance to Folimat® or Rogor® and their use against other pests, possibly mirids, with coincident selection of concurrent aphids. The 2004-2005 management strategy for aphids included a warning to that effect and aphid resistance should be considered when applying Folimat® or Rogor® for mirid control.
- Aphids were additionally collected from hosts other than cotton and monitoring detected resistant populations in farm backyards and on weeds. These rogue overwintering aphid populations have the potential to provide a nucleus of future control problems and so should be eliminated where practical.
- Biochemical studies have allowed aldicarb to be considered a unique rotation group. For the 2004-2005 cotton season the aphid rotation groupings were Temik®, organophosphates and carbamates, neonicotinoids, Pegasus®, Thiodan® and Fullfil®.

Mites

- No resistance was detected to Agrimec® or Pegasus®.
- Incipient Comite® resistance was detected in 2003-2004 but not in the following season. This suggests that the resistance management strategy for this product is working well and gives confidence to the strategy as a whole
- Talstar® resistance was common during the study making the product at best unreliable for two-spotted mite control.
- Intrepid® resistance was common and continued to be detected despite a modification to the resistance management strategy that further restricted product use from two to one spray per season. However, I don't consider this indicates a flaw with the management strategy because Intrepid® failed in horticulture after a single application. In contrast in cotton it survived for some 5 seasons before Intrepid® resistance was detected in two-spotted mite
- Curacron® resistance in two-spotted mite was ubiquitous and often at high frequencies. Resistance was such that Curacron could no longer be considered a useful control for two-spotted mite

6. Detail how your research has addressed the Corporation's three Outputs - Economic, Environmental and Social?

Economic: Aphids and mites have the potential to cause substantial loss to yield and to lint quality. The capacity to effectively manage aphids and mites is critical to the profitability and competitiveness of cotton production. Effective proactive insecticide resistance monitoring will contribute significantly to maintaining the capacity for effective insecticidal control.

Environmental and / or social: Field control failures caused by resistance invariably lead to an increase in pesticide use as growers struggle for field control. Such sprays may well be inappropriate with potential for adverse community impact. The ability to effectively detect and consequently manage resistance in sucking pests will prevent the unnecessary application of ineffective controls, thereby potentially reducing the risk of any adverse community impact.

7. Provide a summary of the project ensuring the following areas are addressed:

a) technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.)

The resistance management strategy for aphids and mites has been technically improved by including use limits for Intrepid® use against two-spotted mite and strategic use of Pirimor®, Folimat® and Rogor® against cotton aphid.

b) other information developed from research (eg discoveries in methodology, equipment design, etc.)

Aphid strains with confirmed resistance were forwarded to colleagues (DAN164c) for further biochemical analysis. Chemical assays showed Temik® target site resistance was different from Pirimor® or OP target site resistance allowing the creation of a Temik® only rotation group.

c) are changes to the Intellectual Property register required?

Nil

8. Detail a plan for the activities or other steps that may be taken:

(a) to further develop or to exploit the project technology.

The target site resistances identified above will be examined with molecular methods. The aim to see if resistance is as described in overseas studies and ascertain the viability of a rapid diagnostic molecular based technique to detect Pirimor® resistance.

(b) for the future presentation and dissemination of the project outcomes.

Cotton resistance extension tour and cotton conference

(c) for future research.

- Aphid resistance monitoring to continue and include new chemicals such as thiamethoxam
- Mite resistance monitoring to continue and may include new chemistries such as etoxazole
- Methods investigated for mirid bioassay and culture (Linked to M. Khan at QDPI)
- Existing collaborative links to continue
- See above for molecular methods. Also French researchers are looking at cotton aphid using micro satellites. They consider there to be one major clone on cotton worldwide that causes resistance. As this study has generated strains with known resistance status from cotton and weeds it may be useful to subject the local strains to micro satellite analysis. A simple project for a French /Australian collaboration could be instigated explores specific questions on clone variability. We could tap into this for more specific questions / answers related to resistance.

9. List the publications arising from the research project and/or a publication plan. (NB: Where possible, please provide a copy of any publication/s)

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10. Have you developed any online resources and what is the website address?

No

11. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. Where possible include a statement of the costs and potential benefits to the Australian cotton industry or the Australian community.

Key Points

1. Resistance monitoring showed a high proportion of aphid populations showing Pirimor resistance despite a change to the management strategy. I consider it likely this relates to high omethoate or dimethoate use against other pests, possibly mirids, with coincident selection of concurrent aphids.
2. Aphids were also collected from hosts other than cotton where monitoring detected resistant populations in farm backyards and on weeds. These rogue overwintering aphid populations have the potential to provide a nucleus of future control problems and so should be eliminated where practical.
3. Biochemical studies using post bioassay aphids sourced from DAN163c have allowed aldicarb to be considered its own rotation group. For the 2004-2005 cotton season the aphid rotation groupings were Temik®, organophosphates and carbamates, neonicotinoids, Pegasus®, Thiodan® and Fulfil®.

IRACGroup	Chemical	Sold as
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	Aldicarb	Temik
1A	Carbamate	Foliar: Pirimor, Aphidex
1B	Organophosphates	At planting or side dress: Thimet Foliar: Dimethoate, omethoate, chlorpyrifos, chlorpyrifos-methyl, parathion-methyl profenofos
4A	Neonicotinoids	Seed treatments: Gaucho, Amparo, Cruiser Foliar: Confidor, Intruder, Actara
12B	Diafenthiuron	Pegasus
2A	Endosulfan	Thiodan <i>etc</i>
9A	Pymetrozine	Fulfil

4. Intrepid® resistance in two-spotted mite increased quickly in both level and proportion of resistant strains detected in season 2002-2003. In response, the 2003-2004 resistance management strategy for Intrepid® use in cotton was altered and recommended a further restriction of use from two to one spray per season.

Part 4 – Final Report Executive Summary

Provide a one page Summary of your research that is not commercial in confidence, and that can be published on the World Wide Web. Explain the main outcomes of the research and provide contact details for more information. It is important that the Executive Summary highlights concisely the key outputs from the project and, when they are adopted, what this will mean to the cotton industry.

Up until the introduction of transgenic cotton the cotton bollworm has been the major pest of the Australian cotton industry. However following the introduction of the single gene Ingard® transgenic cotton sucking insect pests such as aphids, mites and mirids have become more troublesome, so requiring increased targeted insecticide control. Targeted control has caused resistance to develop with resultant control problems.

Cotton aphid and two-spotted mite were collected from Australian cotton growing regions and tested in the laboratory for resistance. The two-spotted spider mite *Tetranychus urticae* was tested over three years for resistance against a range of chemicals registered in cotton for its control. Encouragingly, Agrimec® and Pegasus® resistance was not detected at all during the study. Comite® resistance was detected in season 2003–2004 but was absent in the subsequent 2004-2005 season. We consider the 2004-2005 result for Comite® together with that of Agrimec® support the effectiveness of the resistance management strategy. However, during season 2001-2002 Intrepid® survivors were detected for the first time in two-spotted mite. Season 2002-2003 produced an alarming trend of increasing level and abundance of Intrepid® resistance. In response the mite management strategy for Intrepid was modified from season 2003-2004 with a reduction in total Intrepid sprays to one per season for either *Helicoverpa* spp or two-spotted mite control. Unfortunately during season 2004-2005 resistance was again detected in 3 out of 7 strains tested but encouragingly resistance frequencies in each strain were generally less than in seasons 2003-2004. The modified

strategy restricting Intrepid® use will be maintained so it will be interesting to see if the trend continues with Intrepid® resistance frequencies per strain continuing to drop.

The cotton aphid *Aphis gossypii* was also screened for resistance against several chemicals used for its control in cotton. Earlier screening included Pirimor®, Rescue®, Pegasus®, Confidor®, Endosulfan and Talstar®. Pegasus® and Confidor® resistance was not detected and endosulfan resistance rarely detected. Pirimor® resistance was common as was resistance to Rescue®. Interestingly, Talstar® resistance was detected in some 10-20% of strains although the product is not registered for that use. The result highlights the issue of accidental selection with concurrent pest species. Pirimor® resistance was detected in 61% of cotton aphid populations and as Pirimor® is known to cause cross resistance to Folimat® / Rogor® those products would also be compromised by resistance. Pirimor® resistance remained despite a change to the management strategy in 2003-2004 when Pirimor® and Folimat® use was restricted by the implementation of chemical use windows. It is possible that control of mirids with Folimat® or Rogor® may be selecting concurrent aphids so producing Pirimor®, Folimat® and Rogor® resistance in aphids. The resistance strategy for aphids in cotton has now been amended to include reference to mirids

Collaborative cross-resistance studies with Drs Gunning and Cottage caused the carbamate Temik® to be split from Pirimor® for cotton aphid control (see also DAN164c). This has given growers access to an extra rotation group for the purposes of resistance management.

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Australian Government
**Cotton Research and
Development Corporation**

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FINAL REPORT

"Insecticide resistance management in cotton aphid (*Aphis gossypii*) and
cotton mite (*Tetranychus urticae*)"

DAN 163c

1 July 2002 to 30 June 2005

Dr G.A. Herron, Department of Primary Industries,
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**NSW DEPARTMENT OF
PRIMARY INDUSTRIES**

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Australian Government
**Cotton Research and
Development Corporation**

Annual, Progress and Final Reports

Part 1 - Summary Details

REPORTS

Please use your TAB key to complete Parts 1 & 2.

CRDC Project Number: DAN 163c
Annual Report: Due 30-September
Progress Report: Due 31-January
Final Report: Due 30-September
(or within 3 months of completion of project)

Project Title: Insecticide resistance management in cotton aphid (*Aphis gossypii*) and cotton mite (*Tetranychus urticae*)

Project Commencement Date: 1 July 2002 **Project Completion Date:** 30 June 2005
Research Program: 3 Crop Protection

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Signature of Research Provider Representative: _____

Key highlights –

Aphids

1. No resistance was detected in aphids to Thiodan® (endosulfan), Confidor® (imidacloprid), Actara® (thiamethoxam), Intruder® (acetamiprid) or Pegasus® (difenthiuron).
2. It was initially thought by the product supplier that Rescue® (chlorpyrifos methyl) may be effective against cotton aphid due to anecdotal evidence of efficacy against suspect resistant strains. Unfortunately, resistance to Rescue® was detected in 30-40% of strains tested.
3. Pirimor® (pirimicarb) resistance ranged from a low of 52% of strains tested showing resistance in 2002-2003 to a maximum of 77% of strains showing resistance in 2003-2004. Pirimor® resistance remained high, with 61% of strains tested in 2004-2005 showing resistance despite the addition of further use restrictions in 2003-2004. Pirimor® resistance is probably being maintained by cross resistance with Folimat® (omethoate) or Rogor® (dimethoate). These are used against other pests, especially mirids, and hence select for resistance in aphid populations present at the same time. The 2004-2005 management strategy for aphids included a warning that aphid resistance should be considered when applying Folimat® or Rogor® for mirid control.
4. Aphids were additionally collected from hosts other than cotton and monitoring detected resistant populations in farm backyards and on weeds. These rogue overwintering aphid populations have the potential to provide a nucleus of future control problems and so should be eliminated where practical. The resistance management strategy was modified to include that information with a recommendation that overwintering aphids specifically be targeted for non-chemical control.
5. Biochemical studies of cross-resistance demonstrated that the Temik® (aldicarb) target site resistance was different to Pirimor® target site resistance. Consequently, Temik® could be considered a unique rotation group. For the 2004-2005 cotton season the aphid rotation groupings were Temik®, organophosphates and carbamates other than Temik®, the neonicotinoids, Pegasus®, Thiodan® and Fullfill® (pymetrozine). This was included into the aphid resistance management strategy.

Mites

1. No resistance was detected to Agrimec® (abamectin) or Pegasus®.
2. Incipient Comite® (propargite) resistance was detected in 2003-2004 but not in the following season. This suggests that the resistance management strategy for that product is working well and gives confidence in the resistance management strategy for two-spotted mite.
3. Talstar® (bifenthrin) resistance was common during the study making the product at best unreliable for two-spotted mite control.
4. Intrepid® (chlorfenapyr) resistance was common and continued to be detected despite a modification to the resistance management strategy in season 2003-2004 that further restricted product use from two to one spray per season. Encouragingly resistance frequencies in each strain were generally less than in seasons 2003-2004. It will be interesting to see if the trend continues with Intrepid® resistance frequencies per strain continuing to drop. I don't consider this indicates a flaw with the management strategy because Intrepid® failed in horticulture after a single application yet in cotton it survived for some 5 seasons before Intrepid® resistance was detected in two-spotted mite.
5. Curacron® (profenofos) resistance in two-spotted mite was ubiquitous and often at high frequencies. Resistance was such that Curacron could no longer be considered a useful control for two-spotted mite.

1. Outline the background to the project.

Up until the introduction of transgenic cotton the cotton bollworm, *Helicoverpa armigera* (Hübner) has been the major pest of the Australian cotton industry. However, following the introduction of the single gene Ingard® transgenic cotton, sucking insect pests such as aphids, mites and particularly mirids have become more troublesome, so requiring increased targeted insecticide control. Of late, high-level organophosphate and carbamate resistance has developed in cotton aphid (Herron *et al.* 2001). Two-spotted mite *Tetranychus urticae* Koch has a proven ability to develop resistance if targeted with pesticides and has recently developed resistance to chlorfenapyr (Intrepid®)(Herron *et al.* 2004a). Other sporadic but troublesome sucking pests include green peach aphid *Myzus persicae* (Sulzer), bean spider mite *Tetranychus luedeni* Zacher, thrips (including western flower thrips) and green mirids (Forrester and Wilson 1988).

Cotton aphid, *Aphis gossypii* Glover

The pest status of aphids is often related to the contamination of the cotton lint with sugary 'honey-dew'. However, earlier outbreaks in the crop growth cycle can significantly reduce yield and recently cotton aphid was confirmed as a vector for 'Cotton Bunchy Top' syndrome, a virus type disease of cotton linked to aphids. These changes in the system mean that there is a critical need in the cotton industry for effective tools to control aphids and for development of effective resistance management strategies.

Cotton aphid reproduces asexually, so elimination of susceptible 'clone' and selection for survival of insecticide resistant 'clones' can result in rapid changes in resistance within populations. Management of aphids is further complicated because there is no dilution of resistance by outcrossing to susceptibles, as is used to manage *Bt* resistance in *Helicoverpa* spp. Therefore, aphids can very quickly become a major problem when chemical control fails due to resistance (Herron 2001).

Effective management of cotton aphid will be best achieved by pursuing an integrated approach, including sampling, management of alternative hosts, resistance monitoring, rational pest thresholds, cross-resistance studies, resistance mechanism elucidation and evaluation of new chemistry and effect on beneficials. Without this research, Australia's reputation as a producer of clean cotton could be dramatically affected.

Two-spotted mite, *T. urticae*

Two-spotted mite is notorious world-wide for developing resistance and Australian researchers have published many first citations of new resistances (eg Herron *et al.* 1993, Herron and Rophail 1998).

As each new compound has become available in cotton we have pro-actively established baseline resistance levels and cross-resistance profiles and initiated routine resistance monitoring. Unfortunately, resistance continues to evolve as seen most recently to bifenthrin (Herron *et al.* 2001a) and then chlorfenapyr (Herron *et al.* 2004). Management of mites is complicated because most chemicals are also used against other pests, such as aphids or *Helicoverpa* spp. and this has contributed to resistance development to organophosphates, bifenthrin and chlorfenapyr. Effective management of two-spotted mite will be best achieved by pursuing an integrated approach that includes resistance management, based on a sound understanding of the mites resistance and cross-resistance spectra. Continued resistance

monitoring, and the timely inclusion of new chemistry, is essential for effective ongoing management of this pest species.

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2. List the project objectives and the extent to which these have been achieved.

The project aimed to achieve effective resistance management of cotton aphid and two-spotted mite. Effective resistance management of cotton aphid and two-spotted mite was achieved through;

- Yearly monitoring of resistance levels to pesticides in aphid and mite populations collected across the cotton regions. This provides precise information on the resistance status of pests and geographic variability in resistance and form the backbone for resistance management.
- Development of appropriate scientifically valid resistance monitoring techniques for new compounds and development of baseline data against which to measure future changes.
- Regular updating of the aphid and mite resistance management strategy, via contributions to the TIMS committee, in line with resistance monitoring results. For instance the 2002-2003 management strategy included warnings not to follow a seed or in furrow insecticide treatment with the first foliar spray from the same chemical group. The subsequent 2003-2004 strategy had Pirimor® and Folimat® use against aphids further restricted by strategic windowing and Intrepid® use against two-spotted mite (including *Helicoverpa* spp sprays) reduced to one application per season. The 2004-2005 strategy had aldicarb moved to its own rotation group giving an addition alternation group for aphid management plus a warning that Folimat®/Rogor® sprays against mirids could select Pirimor® resistance in concurrent aphids.

3. Detail the methodology and justify the methodology used.

Aphids

Aphids were collected by researchers, CRC Industry Development Officers, consultants and growers from cotton fields, weeds and domestic back yards. They were then sent to the bioassay laboratory at Camden (EMAI) and each field strain cultured separately on pesticide-free cotton (Deltapine 90) at $25 \pm 4^\circ\text{C}$ under natural light. Strain integrity was assured by maintaining populations in purpose built insect proof cages. When established in culture, the 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.* 2001). Batches of ten adult female aphids per leaf disc were then sprayed with the aid of a Potter spray tower which produced an aqueous insecticide deposit of $1.6 \pm 0.07 \text{ mg cm}^{-2}$ with a 2 mL insecticide aliquot. Each test was replicated and included a water only sprayed control. After spraying, the clear plastic film covered Petri dishes were maintained at $25 \pm 0.1^\circ\text{C}$ in 16:8 Light : Day (L:D) for 24 h after which mortality was assessed.

Mites

Strains of two-spotted mite were randomly collected 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 (Edge and James 1982). 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 $28 \pm 0.1^\circ\text{C}$ in constant light for 48 h after which mortality is assessed.

Analysis

Data were analysed using a Probit program written in GENSTAT 5 statistical software (Barchia, 2001). LC_{50} and LC_{99} values plus their 95% fiducial limits were calculated using the probit method outlined in Finney (1971) and included control mortality correction (Abbott 1925). Resistance factors (RF) (RF_{50} and RF_{99}) plus their associated 95% confidence intervals (CI) were calculated as outlined in Robertson and Preisler (1992). Discriminating-dose tests were corrected for control mortality (Abbott 1925).

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4. Detail and discuss the results including the statistical analysis of results.

4.1 Aphids

Cotton aphid resistance testing: season 2002-2003

For season 2002-2003 resistance was not detected against Thiodan® (endosulfan), Confidor® (imidacloprid) or Pegasus® (diafenthiuron)(Table 4.1.1). In contrast Pirimor® (pirimicarb) and Rescue® (chlorpyrifos methyl) resistance was relatively common. Pyrethroid resistance was often detected in cotton aphid although no pyrethroids are registered their control. This clearly illustrates the problem of non-target selection with insecticides applied against a target, in this case *Helicoverpa* spp., coincident populations of other pests, in this case aphids.

In the 2002-2003 season, strains from the McIntyre region (Goondiwindi) were particularly interesting as only three out of nine strains tested showed Pirimor® resistance and none were resistant to Rescue®. This was in stark contrast to strains from other regions where 100% resistance to both chemicals was common. It is interesting that the McIntyre region, with the low proportion of resistant strains, has a history of high commitment to IPM and is currently trialling season long 'soft' pest management.

The second significant outcome is results showing resistance in strains collected from backyards or weeds. This work was done in this and other years in collaboration with a CRDC funded CSIRO project CSP145C 'Improving understanding of the ecology and management of cotton aphid'. Aphids in backyard and farm gardens showed resistance profiles very similar to those of aphids collected from cotton crops. This suggests that backyards and farm gardens may provide a refuge where resistant aphids from cotton can survive winter, especially in drought years when alternative hoast may be scarce, and form a nucleus for future control problems in nearby cotton.

Finally, in contrast to most eastern Australian results, Northern Territory (Katherine) strains collected from cotton showed no Pirimor® resistance but one strain did show a low frequency resistance to Rescue®.

Table 4.1.1. Percent mortality at the discriminating dose (ie percent susceptible) for various strains of cotton aphid collected during season 2002-2003 and evaluated for resistance against Pirimor® (Pir), Rescue® (Res), Pegasus® (CGA-140408) (Peg), Confidor® (Con), Talstar® (Tal) and Thiodan® (Thio).

Strain	Region	Chemical					
		Pir	Res	Peg	Con	Tal	Thio
NT 1	N Territory	100	100	100	100	100	100
NT 2	N Territory	100	100	100	100	100	100
NT 3	N Territory	100	94	100	100	100	100
Farm Garden 1	Namoi	100	100	100	100	100	100
Farm Garden 2	Namoi	99	81	100	100	92	100
Farm Garden 3	Namoi	28	100	100	100	100	100
Em 1	Emerald	100	100	100	100	40	100
Em 2	Emerald	2	100	100	100	100	100
Em 3	Emerald	45	80	100	100	100	100
ACRI regrowth	Namoi	100	83	100	100	100	100

ACRI 1	Namoi	29	95	100	100	100	100
ACRI 2	Namoi	20	33	100	100	100	100
Bladder Ketmia	Toowoomba	16	68	100	100	100	100
G 1	Goondiwindi	96	100	100	100	100	100
G 2	Goondiwindi	100	100	100	100	95	100
G 3	Goondiwindi	100	100	100	100	100	100
G 4	Goondiwindi	100	100	100	100	100	100
G 5	Goondiwindi	100	100	100	100	100	100
G 6	Goondiwindi	100	100	100	100	100	100
G 7	Goondiwindi	90	100	100	100	100	100
G 8	Goondiwindi	100	100	100	100	100	100
G 9	Goondiwindi	86	100	100	100	88	100
N 1	Namoi	11	38	100	100	100	100
N 2	Namoi	100	77	100	100	42	100
N 3	Namoi	36	18	100	100	100	100
N 4	Namoi	30	34	100	100	100	100
D 1	Dalby	89	100	100	100	100	100
Overall proportion with resistance		52	41	0	0	18	0

Cotton aphid resistance testing: season 2003-2004

In 2003-2004, the resistance management strategy for Folimat® (omethoate) and Pirimor® (pirimicarb) was changed to reduce Pirimor® selection against cotton aphid. The aim was to reduce Pirimor® resistance frequencies and restore confidence in this useful IPM friendly compound. Despite the change to the strategy, Pirimor® resistance has remained high and approximately equal to frequencies found in the 2002-2003 season (Table 4.1.2). Despite our best efforts, resistance levels have remained static and we suspect this may be attributable to coincident product use against other pests, especially Folimat or Rogor® (dimethoate). It is clear from the data that coincident product use is a problem, for instance, Talstar® (bifenthrin) is not registered for aphid control yet some 30 percent of populations show some level of Talstar® resistance. Folimat® and Rogor® are popular chemicals that unfortunately confer cross-resistance to Pirimor® in aphids but are also used to control mirids. It is notable that the 2003-2004 cotton season required many cotton growers to spray for mirid control and a proportion of those sprays were undoubtedly Folimat® or Rogor®, due to their low cost. It seems reasonable to suspect that mirid control is now adversely affecting the management of cotton aphid.

In the 2003-2004 season strains were collected so that Pirimor® resistance could be tabulated relative to collection site i.e. from domestic yards, weeds, cotton or crops other than cotton (Table 4.1.3). It is notable that domestic back yards and weeds have a very high proportion of resistant populations. This is important because cotton aphid can develop a winged form that can disperse if food quality drops or aphids become too crowded. Consequently resistant aphids can move from farm gardens, weeds or domestic backyards to cotton fields. If food quality is good the non-winged form is found which has limited mobility explaining why aphids occur as 'hotspots', then after a time can be suddenly found widely as the winged forms spread over the field.

Table 4.1.2. Percent mortality at the discriminating dose (ie percent susceptible) for various strains of cotton aphid collected during season 2003-2004 and evaluated for resistance against Pirimor® (Pir), Rescue® (Res), Pegasus® (CGA-140408) (Peg), Confidor® (Con), Talstar® (Tal) and Thiodan® (Thio).

Strain	Region	Where	Chemical					
			Pir	Res	Peg	Con	Tal	Thio
Adam St Narrabri	Namoi	Dom yard	8	74	100	100	100	100
Tinkelara	Emerald	Cot crop	98	100	100	100	100	100
Parker Farm	Emerald	Cot crop	98	100	100	100	100	100
Greenbah Garden	Namoi	Dom yard Nr cotton	16	23	100	100	100	100
ACRI F18 sample 1873	Namoi	Weed	96	100	100	100	100	100
ACRI F18 sample 1874	Namoi	Volunteer Cotton	98	100	100	100	100	100
ACRI F18 sample 1878	Namoi	Volunteer Cotton	97	100	100	100	100	100
ACRI F18 sample 1884	Namoi	Ratoon Cotton	93	100	100	100	100	100
Waterways 5 ways	Emerald	Cot crop	34	79	100	100	100	100
Brearley Downs	Emerald	Cot crop	88	100	100	100	100	100
Jabiwarra	Emerald	Cot crop	43	100	100	100	100	100
Orvieto	Emerald	Cot crop	80	74	100	100	96	100
Shananda	Emerald	Cot crop	74	100	100	100	100	100
Deneliza	Emerald	Cot crop	10	100	100	100	100	100
ACRI 1989	Namoi	Sunflower Crop	59	100	100	100	96	100
ACRI 1935	Namoi	Sunflower Crop	41	81	100	100	100	100
ACRI 1936	Namoi	Weed Nr cotton	65	100	100	100	100	100
ACRI 1938	Namoi	Mung bean crop	36	73	100	100	100	100
ACRI 1939	Namoi	Weed Nr cotton	Did Not Establish					
Field 10	Pittsworth h Qld	Cot crop	19	62	100	100	100	100
UTCN - ACRI	Namoi	Cot crop	100	97	100	100	90	100
AET 7 - ACRI	Namoi	Cot crop	24	100	100	100	100	100
Warenda	Goondi	Cot crop	47	88	100	100	100	100
Morella	Goondi	Cot crop	100	100	100	100	100	100
Mullala	Goondi	Cot crop	100	100	100	100	100	100
Alcheringi	Goondi	Cot crop	68	100	100	100	72	100
Mimosa Field 55	Gwydir	Cot crop	100	100	100	100	100	100
Warilea Field 2	Namoi	Cot crop	100	100	100	100	48	100
Listowell Field 3	Gwydir	Cot crop	Did Not Establish					
Pindara Field 5	Namoi	Cot crop	100	100	100	100	86	100
			100	100	100	100	76	100

Strain	Region	Where	Chemical					
			Pir	Res	Peg	Con	Tal	Thio
Oakey Point southern river block	Namoi	Cot crop	Did Not Establish					
Lochelgrin Field 13	Namoi	Cot crop	31	100	100	100	100	100
Havana Field 12	Namoi	Cot crop	100	100	100	100	99	100
Riverway 1933	Namoi	Cot crop	78	77	100	100	100	100
ACRI 1945	Namoi	Weed	72	60	100	100	100	100
ACRI 1948	Namoi	Weed	Did Not Establish					
Greenbah 1960	Namoi	Dom yard Nr cotton	Did Not Establish					
Greenbah 1962	Namoi	Dom yard Nr cotton	Did Not Establish					
Greenbah 1963	Namoi	Dom yard Nr cotton	Did Not Establish					
Greenbah 1965	Namoi	Dom yard Nr cotton	Did Not Establish					
Rosilda	Dalby	Cot crop	58	77	100	100	82	100
Boshammer	Dalby	Cot crop	Did Not Establish					
Sunnyside bridge1977	Namoi	Cot crop	Did Not Establish					
Narrabri RSL	Namoi	Dom yard	85	91	100	100	99	100
Riverway 2016	Namoi	Cot crop	67	100	100	100	92	100
Riverway 2017	Namoi	Weed Nr cot	48	83	100	100	100	100
Riverway 2024	Namoi	Weed Nr cot	70	100	100	100	96	100
Sunnyside farm 2035	Namoi	Cot crop	67	99	100	100	100	100
Carsons Block 2064	Namoi	Weed Nr Cot	Did Not Establish					
Collins Bridge 2065	Namoi	Weed not Nr cot	21	100	100	100	100	100
Greenbah 2082	Namoi	Dom yard Nr cot	Did Not Establish					
Overall proportion with resistance			77	35	0	0	30	0

Where: Dom = domestic, Nr = near, Cot = cotton

Table 4.1.3 Percent of populations (strains) showing Pirimor® (pirimicarb) resistance collected from domestic yards, weeds, cotton or other than a cotton crop

Source	Number populations collected	Number populations with resistance	Proportion resistant
Domestic yards	3	3	100
Weeds	9	9	100
Cotton	26	18	69
Crop other than cotton	2	2	100

Cotton aphid resistance testing: season 2004-2005

Pirimor® resistance was detected in 61% of cotton aphid populations (Table 4.1.4). As Pirimor® resistance is extremely high level, control failure would likely result. Pirimor resistance remains despite a change to the management strategy in 2003-2004 when Pirimor® and Folimat® use was restricted by the implementation of chemical use windows. It is possible that control of mirids with Folimat® or Rogor® may be selecting concurrent aphids so producing Pirimor®, Folimat® and Rogor® resistance in aphids, as stated earlier.

Table 4.1.4. Percent mortality at the discriminating dose (ie percent susceptible) for various strains of cotton aphid collected during season 2004-2005 and evaluated for resistance against Pirimor® (Pir), Pegasus® (CGA-140408)(Peg), Thiodan® (Thio), Actara® (Act) and Intruder® (Int)

Strain	Region	Chemical				
		Pir	Peg	Thio	Act	Int
Norw	Gwydir	92	100	100	100	100
Lamer	Gwydir	87	100	100	100	100
F2#1 AC	Namoi	did	not	establish	into	culture
Mapl F1	Goondi	100	100	100	100	100
Carring	Goondi	100	100	100	100	100
Pall F4	Goondi	100	100	100	100	100
McD K3	St George	95	100	100	100	100
Alc F007	Goondi	did	not	establish	into	culture
Mor Win	Goondi	did	not	establish	into	culture
Car F2-7	Goondi	did	not	establish	into	culture
Mor E4	Goondi	did	not	establish	into	culture
War18-22	Goondi	did	not	establish	into	culture
Tuck	Goondi	did	not	establish	into	culture
Car20-25	Goondi	99	100	100	100	100
Caffery	Dalby	100	100	100	100	100
Lat F15	Bourke	100	100	100		
Lat F17	Bourke	96	100	100	100	
Lat F18	Bourke	100	100	100	100	100
Lat LM3	Bourke	90	100	100	100	100
Bee F16	Gwydir	did	not	establish	into	culture
Tara	Namoi	95	100	100	100	100
Hava F4	Namoi	91	100	100	100	100
Beech	Namoi	did	not	establish	into	culture
Purl F42	Gwydir	91	100	100	100	100
Milo	Gwydir	did	not	establish	into	culture
Glen F6B	Namoi	100	100	100	100	100

Mira F2	Namoi	100	100	100	100	100
Veth F5	Gwydir	93	100	100	100	100
LFL F1 B	Hillston	97	100	100	100	100
LFL30/2015	Hillston	90	100	100	100	100
LFLG16-26	Hillston	100	100	100	100	100
LFLY12-15	Hillston	did	not	establish	Into	culture
LFL B lat	Hillston	92	100	100	100	100
LFL dr lat	Hillston	93	100	100	100	100
Overall proportion with resistance		61	0	0	0	0

4.2 Mites

Two-spotted mite resistance testing: season 2002-2003

Agrimec®, Comite®, Talstar® and CGA-140408 (the UV activated carbodiimide derivative of Pegasus®) resistance was not detected in two-spotted mite (Table 4.2.1). Intrepid® resistance was detected in 4 of the 6 strains tested (Figure 4.2.1) with a maximum of 8.7-fold resistance in strain G at LC50 level. Curacron® resistance was detected at a maximum level of 541-fold in strain W (Figure 4.2.2), however, strain HA had 100% mortality at the discriminating dose and so is susceptible.

Intrepid® resistance was first detected in season 2001-2002 with season 2002-2003 producing an alarming increase in level and abundance of Intrepid® resistance (Figure 4.2.1). In response the mite management strategy for Intrepid® was modified for season 2003-2004 requiring a reduction in total Intrepid sprays to one per season for either *Helicoverpa* spp or two-spotted mite. Intrepid® resistant two-spotted mite from Japan were found to be incompletely dominant and monogenic (Uesugi et al. 2002). If resistant Australian two-spotted are a similar single locus then resistance would be expected to evolve faster with increased insecticide use (Tabashnik 1990) and so the halving of the Intrepid® selection pressure would be expected to extend the useful life of the product.

During the 1999-2000 cotton season, Talstar® resistance was detected in 9 of the ten strains. During 2000-2001 Talstar® resistance was detected in 5 out of 6 strains tested. Similarly in season 2001-2002 resistance was detected in 7 of the 8 strains. In this 2002-2003 season resistance was detected in 4 of the 6 strains suggesting that the abundance of Talstar® resistance is static. Consequently, Talstar® would be unreliable for two-spotted mite control

For season 2001-2002, Curacron® resistance peaked at an all time high of 1718x in strain Warriana Field 4. This season resistance dropped to a maximum of 541x in strain W and interestingly strain HA from Hillston was susceptible but high level vigour tolerant. It would be more than a decade since Curacron® susceptible TSM have been found in cotton.

Table 4.2.1. Testing results for the 2002/2003 cotton season- six strains of two-spotted mite against a 0.02% discriminating dose (DD) of Comite®, 0.0001% DD of Agrimec®, 0.02 % DD of Talstar® and 0.002% DD of Pegasus® (CGA-140408).

Strain	Mortality at DD- Agrimec	Mortality at DD-Talstar	Mortality at DD-Pegasus	Mortality at DD- Comite
G	100	48	100	100
W	100	55	100	100
Y	100	72	100	100
RA	100	100	100	100
TO	100	86	100	100
HA	100	100	100	100

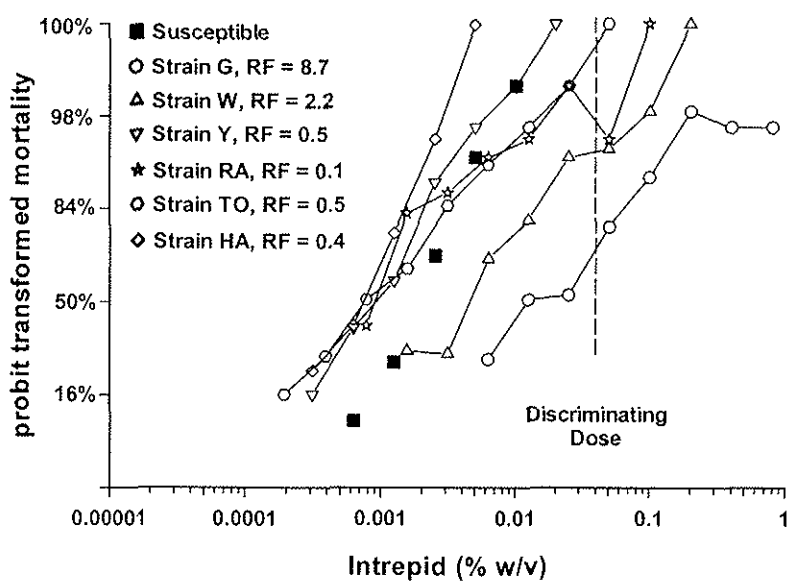


Figure 4.2.1. Dose-response for Intrepid® against six strains of two-spotted mite collected during the 2002/2003-cotton season

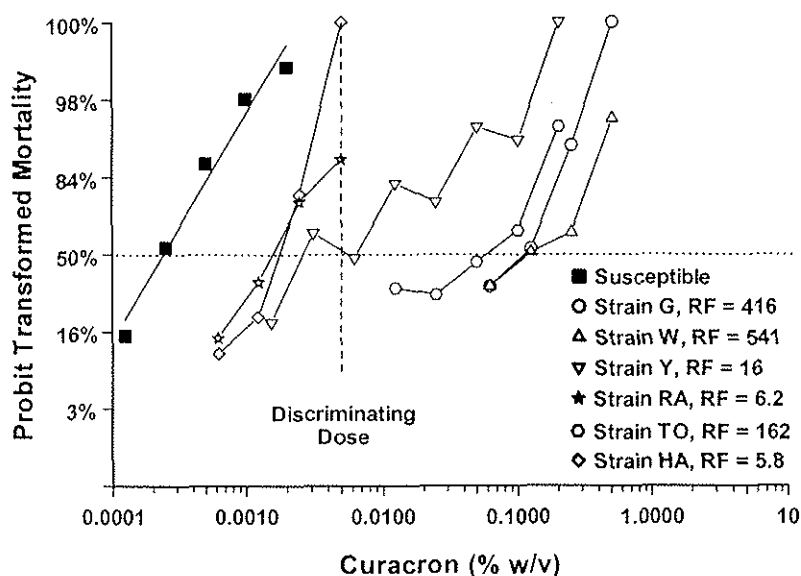


Figure 4.2.2. Dose-response for Curacron® against six strains of two-spotted mite collected during the 2002/2003-cotton season

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Two-spotted mite resistance testing: season 2003-2004

Agrimec® and Pegasus® resistance were not detected (Table 4.2.2). In contrast, Curacron® resistance was detected in every population tested (Table 4.2.2). Intrepid® resistance was detected in three strains (Table 4.2.2) peaking at 4.0 fold at the LC₅₀ level in strain RA (Figure 4.2.3). Comite® resistance was detected in one strain only (Table 4.2.2) and at 2.7 fold at the LC₅₀ level (Figure 4.2.4).

Season 2002-2003 produced an alarming trend of increasing level and abundance of Intrepid® resistance. In response the mite management strategy for Intrepid was modified with a reduction in total Intrepid® sprays. Despite the change to the management strategy, Intrepid® resistance was again detected in 3 of the 7 strains tested during season 2003-2004.

In the 2002-2003 season resistance was detected in 4 of the 6 strains and in season 2003-2004 in 5 of the 7 strains tested. This suggests that the abundance of Talstar® resistance remains relatively static and would continue to be unreliable for mite control.

Table 4.2.2. Testing results for the 2003/2004 cotton season: eight strains of two-spotted mite against a 0.0001% discriminating dose (DD) of Agrimec® (Agr), 0.02 % DD of Talstar® (Tal), 0.04% DD of Intrepid® (Int), 0.002% DD of Pegasus® (CGA-140408), 0.02% DD of Comite® (Com) and 0.005% DD of Curacron® (Cur).

Strain	Chemical					
	Agr	Tal	Int	Peg	Com	Cur
CH	100	97	100	100	100	11
G1	100	100	100	100	100	48
G	100	81	96	100	100	36
LO	100	49	100	100	97	4
H	100	70	100	100	100	8
P	100	67	79	100	100	20
RA	100	100	73	100	100	67

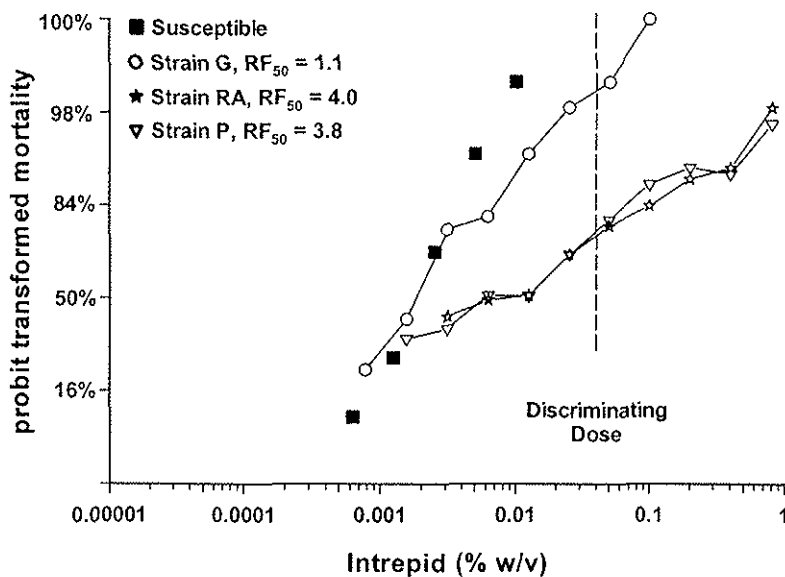


Figure 4.2.3. Dose-response for Intrepid® against a laboratory reference and three suspect resistant strains of two-spotted mite collected during the 2003-2004-cotton season

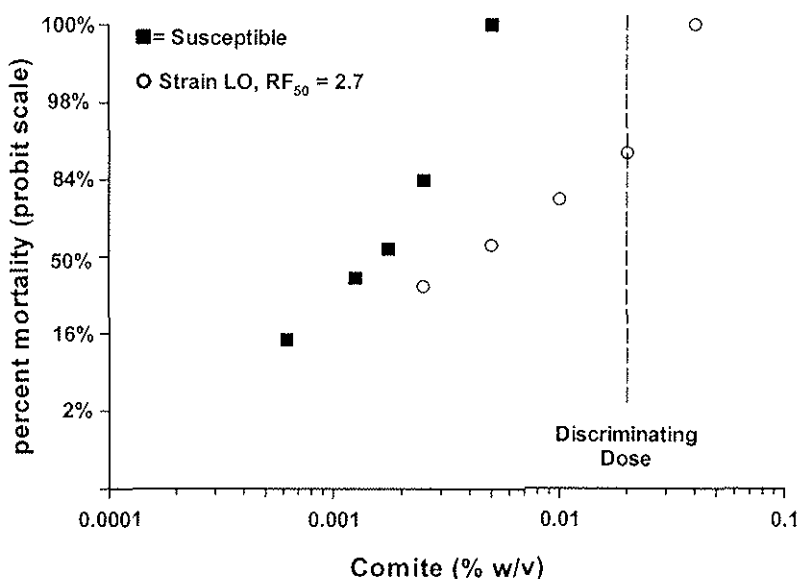


Figure 4.2.4. Dose-response for Comite® against a laboratory reference and single suspect resistant strain of two-spotted mite collected during the 2003-2004 cotton season

Two-spotted mite resistance testing: season 2004-2005

Strains were collected from the Namoi, Gwydir and Griffith areas (Table 4.2.3). Agrimec®, Comite® and Pegasus® resistance was not detected, but Intrepid® resistance was detected in 3 of the 7 strains and Talstar® resistance in 4 of the 7 strains. Curacron resistance was ubiquitous. A baseline response was established using a reference susceptible strain against Paramite®, discussed below (Figure 4.2.5).

Season 2004-2005 saw the new ovicide Paramite® (extoxazole) registered for use against mites in cotton. However, the generation of baseline to produce a discriminating dose proved particularly time consuming and elusive. After much experimentation the cause of difficulty was found to be two fold. Firstly, the formulated product was extremely thick and the minute quantities required for bioassay would not pipette reproducibly. This was solved by weighing rather than pipetting product. Next there was a problem with the bioassay not reaching end point. After much testing the bioassay was found not be reaching endpoint at the conventional 8 day withholding period. The withholding period has now been extended to 10 days. With these two modifications baseline data was generated against our standard laboratory susceptible strain. Data can be extrapolated with precision to the LC99.9 level and a discriminating concentration for resistance monitoring estimated. A discriminating dose for resistance monitoring would be approximately 0.0001% ai Paramite®.

Intrepid® resistance was again detected in 3 out of 7 strains during 2004-2005 but encouragingly resistance frequencies in each strain were generally less than in season 2003-2004. It will be interesting to see if the trend continues in 2005-2006 with Intrepid® resistance frequencies per strain continuing to drop. This is possible given the limited use of this product due to high uptake of Bollgard II® cotton.

Again Talstar® resistance was detected in 4 out of 7 strains suggesting Talstar® resistance remains relatively static and so continuing to make the product unreliable for mite control.

Table 4.2.3. Percent mortality at the discriminating dose (i.e. percent susceptible) for various strains of two-spotted mite collected during cotton season 2004-2005 and evaluated for resistance against Talstar® (Tal), Intrepid® (Int), Agrimec® (Agr), Comite® (Com), Pegasus® (Peg) and Curacron® (Cur)

Strain	Area	Chemical					
		Tal	Int	Agr	Com	Peg	Cur
AN	Namoi	29	96	100	100	100	12
G	Namoi	31	84	100	100	100	19
TG	Griffith	100	100	100	100	100	43
GL	Gwydir	100	100	100	100	100	10
NH	Namoi	44	90	100	100	100	17
RA	Griffith	100	100	100	100	100	79
PU	Namoi	50	100	100	100	100	10

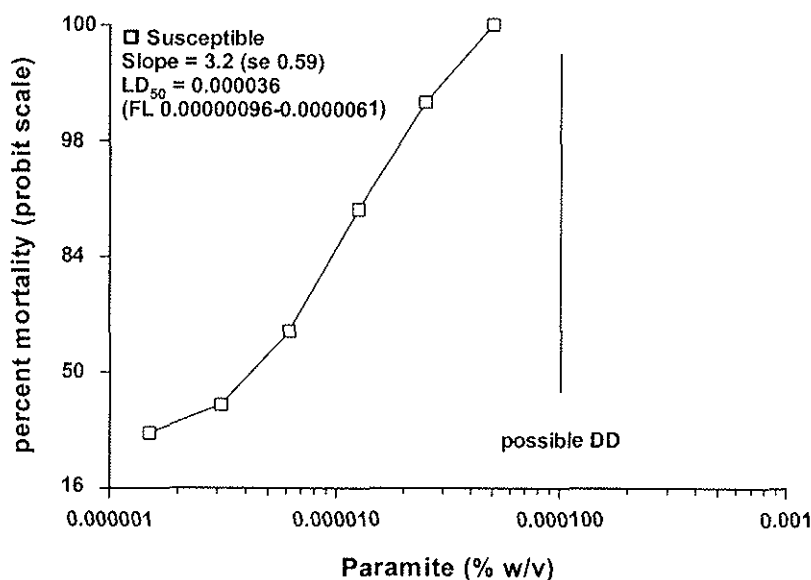


Figure 4.2.5. Dose-response for Paramite® against a laboratory reference strain of two-spotted mite (se = standard error, LD = lethal dose and FL = fiducial limit)

4.3 New chemistry

AMPARO®: A NEW COTTON SEED-DRESSING MIXTURE OF IMIDACLOPRID AND THIODICARB

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Summary

The new cotton-seed dressing Amparo® is a mixture of two chemical groups (chloronicotinyl and carbamate) that must be accurately positioned within the Australian cotton Integrated

Resistance Management Strategy (IRMS). Against cotton aphid, *Aphis gossypii* Glover, the carbamate component of the mixture did not significantly reduce aphid numbers. In contrast, the chloronicotinyl component was significantly better than the control and gave statistically equivalent efficacy at all rates tested. Consequently, the current chloronicotinyl grouping of Amparo within the cotton IRMS is valid.

Key words: mixtures, seed-dressing, resistance management, *Aphis gossypii*, cotton aphid

Introduction

The Integrated Resistance Management Strategy (IRMS) for Australian cotton was originally designed to manage resistance in *Helicoverpa* spp. but increasingly considers a range of insecticide resistant secondary pests (Johnson and Farrell 2003). *Aphis gossypii* Glover is one such pest that has recently risen in status due to increasing resistance and associated control failures (Herron *et al.* 2001). The aphid management strategy in cotton is based on the grouping of like chemicals including foliar sprays and seed treatments with chemical alternation between groups (Rossiter *et al.* 2003).

For the 2003-2004 cotton season, Bayer CropScience Pty. Ltd. will introduce a new seed treatment for aphid control that is a combination of the chloronicotinyl, imidacloprid and the carbamate, thiodicarb. Other seed dressings and in-the-seed-furrow insecticides currently in use in Australian cotton include the carbamate aldicarb (Temik® 150 G), the organophosphate phorate (Thimet® 200 G), and the chloronicotinyls imidacloprid (Gaucho® 600 FS) and thiamethoxam (Cruiser® 350 FS)(Johnson and Farrell 2003). Due to cross-resistance in *A. gossypii* between the organophosphate and carbamate groups pesticides belonging to these classes are considered as one group in the IRMS (Rossiter *et al.* 2003).

To accurately position Amparo within the management strategy we aimed to evaluate its individual components for efficacy against *A. gossypii*.

Materials and methods

Aphids

The susceptible strain was collected from an unsprayed Sydney backyard. Its response to a range of chemicals has been previously described (Herron *et al.* 2000).

Chemicals

Bayer CropScience Pty. Ltd. supplied cottonseed variety Sicala V3i previously treated with formulated imidacloprid or thiodicarb at various rates (Table 1).

Table 1. Seed treatments

Treatment	Active ingredient
1	fungicide (QAP ¹) only control
2	imidacloprid 600 g L ⁻¹ Flowable Concentrate for Seed Treatment (FS) 580 mL 100 kg ⁻¹ + thiodicarb 500 g L ⁻¹ FS 250 mL 100 kg ⁻¹
3	imidacloprid 600 g L ⁻¹ FS 580 mL 100 kg ⁻¹ + thiodicarb 500 g L ⁻¹ FS 500 mL 100 kg ⁻¹
4	imidacloprid 600 g L ⁻¹ FS 580 mL 100 kg ⁻¹
5	imidacloprid 600 g L ⁻¹ FS 875 mL 100 kg ⁻¹
6	thiodicarb 500 g L ⁻¹ FS 500 mL 100kg ⁻¹

¹every treatment included Quintozene, Apron and Peridiam (QAP) fungicides.

Seed germination

On the 7th August 2003 five treated seeds were evenly planted into plastic pots (15 cm diameter) filled with Yates Green Earth premium potting mix. Pots contained only one treatment and each treatment was replicated three times. Pots were placed into individual saucers and watered from above and below with 100 mL. The pots were transferred to a plant germination room maintained at 28 ± 2 °C where they were watered with a further 100 mL on Day 3 and Day 6 and left for a total of 7 d until dicotyledons had emerged.

On the 14th August pots were transferred into one of three aphid proof cages in an insectary (maintained at 25 ± 4 °C). Each cage contained all treatments in a randomised complete block design with pot position randomly assigned.

Each plant was challenged with two adult insecticide susceptible *A. gossypii* aptera at weekly intervals until 29-30th September 2003. Four days post challenge the plants were examined and those with leaves with 0, <10 or >10 aphids noted. During the interval plants were watered (150-200 mL) twice a week at the base of the plant only. After the final challenge the leaves were removed from each plant and number of aphids counted with the aid of a stereomicroscope.

Statistical analysis

Final aphid numbers per plant were analysed using a generalised linear mixed model with errors assumed to follow a Poisson distribution with a logarithmic link function as follows:

$$\text{Ln}(\text{count}) = \text{offset} + \text{treatment} + \textit{block} + \textit{plot}$$

where “offset” is natural logarithmic of Control mean and the “*italicised*” terms are included in the model as random effects. Parameters were estimated using the residual maximum likelihood (REML) technique through ASREML statistical software (Gilmour *et al.* 1999). Predicted values of ratio of treatment means over Control mean and percent efficacy were calculated.

Results

Treatment effects (ie % efficacy) were highly significant ($P < 0.001$) with all imidacloprid treatments having similar efficacy that was significantly different from the thiodicarb only treatment (Table 2). Interestingly, thiodicarb (treatment 6) seemed to have an inhibitory effect on aphid numbers during the first week of the trial (Table 3).

Table 2. Mean calculated as a ratio to the control count

Treatment	Ln(mean)	SE	Mean	%Efficacy
2	-7.0788	2.3568	0.0008	99.92a
3	-4.1231	0.681	0.0162	98.38a
4	-5.4845	1.0435	0.0042	99.58a
5	-5.8647	1.2147	0.0028	99.72a
6	-0.0782	0.4862	0.9248	7.52b

Note: Significant differences were determined using Z-value at $P < 0.001$ on Ln(mean)

Discussion

The seed treatments Gaucho[®] 600 FS and Amparo[®] both contain the active ingredient imidacloprid but with varying amounts of active ingredient. The Gaucho[®] 600 formulation

has a higher application rate of 525 g ai (875 mL product) while Amparo[®] is applied at 348 g ai (580 mL product) 100 kg⁻¹ seed.

However, in the current study both rates provided statistically equivalent control at trial's end. In contrast, the carbamate component of the Amparo[®] mixture did not significantly reduce aphid numbers. Consequently, in terms of the cotton IRMS, Amparo[®] should be included in a chloronicotinyl grouping with Cruiser[®] 350 FS and Gaucho[®] 600 FS.

Interestingly, our results suggest that the thiodicarb component of the Amparo[®] mixture when applied at 250 g ai (500 mL product 100 kg⁻¹ seed) had a short-lived inhibitory effect on aphid numbers. The phenomenon may be worthy of further study to ascertain if the effect is statistically significant.

Acknowledgements

Ms Brie Collier provided technical assistance. Dr Idris Barchia is thanked for the statistical analysis

Table 3. The number of plants in each treatment with leaves infested with 0, <10 or >10 aphids when assessed at six different challenge dates

Date counted	No. of plants (max. 15) with leaves containing	Treatment					
		1	2	3	4	5	6
18 August	0 aphids	2	11	14	14	14	9
	<10 aphids	10	0	0	0	0	2
	>10 aphids	0	0	0	0	0	0
25 August	0 aphids	1	10	13	14	14	3
	<10 aphids	11	1	1	0	0	8
	>10 aphids	0	0	0	0	0	0
1 September	0 aphids	0	10	13	12	14	0
	<10 aphids	1	1	1	2	0	11
	>10 aphids	11	0	0	0	0	0
8 September	0 aphids	0	3	12	12	12	0
	<10 aphids	0	8	2	2	2	3
	>10 aphids	12	0	0	0	0	8
16 September	0 aphids	0	9	9	10	11	0
	<10 aphids	0	2	5	4	3	0
	>10 aphids	12	0	0	0	0	11
22 September	0 aphids	0	9	5	8	10	0
	<10 aphids	0	2	8	4	4	0
	>10 aphids	12	0	0	2	0	11
29-30 September	Total aphids	6864	6	160	38	26	6479

see table 1 for key to treatments

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TESTING INSECTICIDE SUSCEPTIBLE TWO-SPOTTED MITE AGAINST BAYER EXPERIMENTAL PRODUCTS DC-026 and BSN- 2060

Aim

- To evaluate the relative efficacy of two experimental acaricides against laboratory reference susceptible two-spotted mite.

Method

Strain Tested

The susceptible strain was collected from an unsprayed Sydney backyard in 1987 and its response to several chemicals has been previously published (Herron *et al.* 1998).

Chemicals Tested

Formulated product was supplied by Bayer Crop Science and included DC-026 240 g / L SC, BSN-2060 240 g / L SC and the spray adjuvant Pulse.

Bioassay

Edge and James (1982) have described the bioassay procedure used in detail. Briefly, the method requires young adult female mites to be transferred from culture to French bean leaf discs. With the aid of a Potter spray tower mites and leaf disc were sprayed with insecticide and 0.1% pulse or insecticide and water. Each test included a pulse only and/or water only sprayed controls.

Analysis

Where appropriate data were corrected for control mortality (Abbott 1925).

Results and Discussion

Table 1. Dose response for various aqueous concentrations of DC-026 and BSN-2060

Concentration	DC-026 240 g / L SC			BSN-2060 240 g / L SC		
	Number	Dead	%M	Number	Dead	%M
0.1%W/V	27	18	65	24	10	39
0.01%W/V	26	14	52	28	13	44
0.001%W/V	25	19	75	27	16	57
0.0001%W/V	26	0	0	25	3	8
Water only	27	1	4	24	1	4

Table 2. Dose response for various aqueous concentrations of DC-026 and BSN-2060 and 0.1% Pulse

Concentration	DC-026 240 g / L SC			BSN-2060 240 g / L SC		
	Number	Dead	%M	Number	Dead	%M
0.1%W/V	25	25	100	23	23	100
0.01%W/V	25	25	100	21	21	100
0.001%W/V	26	26	100	26	26	100
0.0001%W/V	28	28	100	24	24	100
0.1% Pulse only	26	18	69	21	16	76
Water only	23	1	4.3	28	1	3.6

When sprayed against susceptible two-spotted mite DC-026 and BSN-2060 both gave poor control with increasing concentration. The addition of 0.1% Pulse adjuvant produced 100% mortality at all concentrations tested, however, data show that Pulse alone was very toxic. In fact, Pulse was more toxic than either DC-026 or BSN-2060.

DC-026 and BSN-2060 do not appear to be particularly good acaricides and are probably not worth developing further.

Acknowledgments

Mrs Jeanette Rophail, Senior Technical Officer Scientific, provided technical assistance.

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Herron G.A., Edge V.E., Wilson L.J. and Rophail J. 1998. Organophosphate resistance in spider mites (Acari: Tetranychidae) from cotton in Australia. *Experimental and Applied Acarology* **22**: 17-30.

TESTING OF VARIOUS WITH HOLDING PERIODS WITH BAYER EXPERIMENTAL PRODUCTS DC-026 AND BSN-2060 AGAINST INSECTICIDE SUSCEPTIBLE TWO-SPOTTED MITE

Aim

- To evaluate the relative efficacy of two experimental acaricides at various withholding periods against laboratory reference susceptible two-spotted mite.

Method

Strain Tested

The susceptible strain was collected from an unsprayed Sydney backyard in 1987 and its response to several chemicals has been previously published (Herron *et al.* 1998).

Chemicals Tested

Formulated product was supplied by Bayer Crop Science and included DC-026 240 g / L SC and BSN-2060 240 g / L SC.

Bioassay

Edge and James (1982) have described the bioassay procedure used in detail. Briefly, the method requires young adult female mites to be transferred from culture to French bean leaf discs. With the aid of a Potter spray tower mites and leaf disc were sprayed with insecticide or water only control. Numbers of alive and dead mites were counted pre treatment and then at 48, 72 and 96 h after treatment

Results and Discussion

Table 1. Percent control corrected mortality for various aqueous concentrations of DC-026 and BSN-2060 at 48, 72 and 96 h withholding periods

Concentration	BSN-2060 240 g / L SC				DC-026 240 g / L SC			
	n=	@48h	@72h	@96h	n=	@48h	@72h	@96h
0.1%W/V	25	41	78	81	22	64	81	86
0.01%W/V	21	30	52	66	24	46	70	78
0.001%W/V	26	31	53	77	27	44	46	50
0.0001%W/V	24	3	0	26	23	9	5	9
Water only	21	5	10	15	24	0	4	4

Where: n= pre treatment total number of mites, @48h = percent mites dead at 48 hours after treatment, @72h = percent mites dead at 72 hours after treatment, and @96h = percent mites dead at 96 hours after treatment.

When sprayed against susceptible two-spotted mite DC-026 and BSN-2060 both gave poor control with increasing concentration. However, as withholding period was extended so efficacy increased and the bioassay may not have reached a stable end point mortality within the 96 hour test period.

Acknowledgments

Mrs Jeanette Rophail, Senior Technical Officer Scientific, provided technical assistance.

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Edge, V.E. and James, D.G. 1982. Detection of cyhexatin resistance in two-spotted mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) in Australia. *Journal of the Australian Entomological Society*, **21**: 198.

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3. Provide a conclusion as to research outcomes compared with objectives. What are the “take home messages”?

Aims:

- Monitor resistance levels in mites and aphids.
- Establish baseline for new acaricides and aphicides.
- Cross-resistance profiles for current and experimental chemicals.
- To elucidate mechanisms of resistance in cotton aphid.
- Use all the above information to develop and/or refine resistance management strategies for mites and aphids in cotton

Take home messages:

Aphids

- Thiodan®, Confidor®, Actara®, Intruder® and Pegasus® resistance was not detected.
- Rescue® is not the magic silver bullet for aphid control. Rescue® resistance was consistently detected in 30-40% of strains.
- Pirimor® resistance was more abundant than Rescue® resistance with a low of 52% resistant strains detected in 2002-2003 to a maximum of 77% resistant strains in 2003-2004. Pirimor® resistance continued at 61% of strains in 2004-2005 despite additional use restrictions introduced in season 2003-2004. It is likely that the continuing Pirimor® resistance was caused by a known cross-resistance to Folimat® or Rogor® and their use against other pests, possibly mirids, with coincident selection of concurrent aphids. The 2004-2005 management strategy for aphids included a warning to that effect and aphid resistance should be considered when applying Folimat® or Rogor® for mirid control.
- Aphids were additionally collected from hosts other than cotton and monitoring detected resistant populations in farm backyards and on weeds. These rogue overwintering aphid populations have the potential to provide a nucleus of future control problems and so should be eliminated where practical.
- Biochemical studies have allowed aldicarb to be considered a unique rotation group. For the 2004-2005 cotton season the aphid rotation groupings were Temik®, organophosphates and carbamates, neonicotinoids, Pegasus®, Thiodan® and Fullfil®.

Mites

- No resistance was detected to Agrimec® or Pegasus®.
- Incipient Comite® resistance was detected in 2003-2004 but not in the following season. This suggests that the resistance management strategy for this product is working well and gives confidence to the strategy as a whole
- Talstar® resistance was common during the study making the product at best unreliable for two-spotted mite control.
- Intrepid® resistance was common and continued to be detected despite a modification to the resistance management strategy that further restricted product use from two to one spray per season. However, I don't consider this indicates a flaw with the management strategy because Intrepid® failed in horticulture after a single application. In contrast in cotton it survived for some 5 seasons before Intrepid® resistance was detected in two-spotted mite
- Curacron® resistance in two-spotted mite was ubiquitous and often at high frequencies. Resistance was such that Curacron could no longer be considered a useful control for two-spotted mite

4. Detail how your research has addressed the Corporation's three Outputs - Economic, Environmental and Social?

Economic: Aphids and mites have the potential to cause substantial loss to yield and to reduce lint quality. The capacity to effectively manage aphids and mites is critical to the profitability and competitiveness of cotton production. Effective proactive insecticide resistance monitoring will contribute significantly to maintaining the capacity for effective insecticidal control.

Environmental and / or social: Field control failures caused by resistance invariably lead to an increase in pesticide use as growers struggle for field control. Such sprays may well be inappropriate with potential for adverse community impact. The ability to effectively detect and consequently manage resistance in sucking pests will prevent the unnecessary application of ineffective controls, thereby potentially reducing the risk of any adverse community impact.

5. Provide a summary of the project ensuring the following areas are addressed:

a) technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.)

The resistance management strategy for aphids and mites has been technically improved by including use limits for Intrepid® use against two-spotted mite and strategic use of Pirimor®, Folimat® and Rogor® against cotton aphid.

b) other information developed from research (eg discoveries in methodology, equipment design, etc.)

Aphid strains with confirmed resistance were forwarded to colleagues (DAN164c) for further biochemical analysis. Chemical assays showed Temik® target site resistance was different from Pirimor® or OP target site resistance allowing the creation of a Temik® only rotation group within the cotton IRMS.

c) are changes to the Intellectual Property register required?

Nil

6. Detail a plan for the activities or other steps that may be taken:

(a) to further develop or to exploit the project technology.

The target site resistances identified above will be examined with molecular methods. The aim to see if resistance is as described in overseas studies and ascertain the viability of a rapid diagnostic molecular based technique to detect Pirimor® resistance.

(b) for the future presentation and dissemination of the project outcomes.

Cotton resistance extension tour, TIMS committee, and cotton conference

(c) for future research.

- Aphid resistance monitoring to continue and include new chemicals such as thiamethoxam (for weed and backyard samples linked to CSP165C).
- Mite resistance monitoring to continue and may include new chemistries such as etoxazole
- Methods investigated for mirid bioassay and culture (Linked to M. Khan at QDPI)
- Existing collaborative links to continue
- See above for molecular methods. In addition, French researchers are looking at cotton aphid using micro satellites. They consider there to be one major clone on cotton worldwide that causes resistance. As this study has generated strains with known resistance status from cotton and weeds it may be useful to subject the local strains to micro satellite analysis. A simple project for a French /Australian collaboration could be instigated explores specific questions on clone variability. We could tap into this for more specific questions / answers related to resistance.

7. List the publications arising from the research project and/or a publication plan. (NB: Where possible, please provide a copy of any publication/s)

- Rossiter, L., Wilson, L., Larsen, L., Pyke, B., Gunning, R., **Herron, G.** and Kelly, D. (2003) Insecticide Resistance Where Are We Now? Dec 2003. Cotton Information Sheet TRC 43 12/03. Australian Cotton Cooperative Research Centre, Narrabri. 4 pp.
- Wilson L., **Herron G.** and Heimoana S. (2004) Secondary Pests in the Bollgard II era. In: Cotton Consultants Australia Inc, Annual General Meeting May 2004, 18 – 19th May 2004, Narrabri RSL
- Wilson, L., **Herron, G.** and Heimoana, S (2004) Research Comments Pest Management pp. 81-83. In: Variety Trial Results 2004. Cotton Seed Distributors, Wee Waa and Dalby.
- **Herron, G.A.** and Wilson, L.J. (2004) The management of cotton aphid (*Aphis gossypii* Glover) in Australian cotton p 29 In: LaSalle, J., Pattern, M. and Zalucki, M. eds. Book of Titles of Presentations, Entomology Strengths in Diversity, XXII International Congress of Entomology, 15-21 August 2004, Brisbane, Queensland, Australia.
- Wilson, L., Heimoana, S., Smith, T., **Herron, G.** and Franzman, B. (2004) Research on aphid ecology and management. In: (Crop Protection) “Quality Cotton” – A Living Industry. 12th Australian Cotton Conference 10 – 12th August 2004, Gold Coast Convention and Exhibition Centre, CD ROM.
- **Herron, G.**, Rophail, J. and Wilson. (2004) Resistance monitoring in two-spotted mite: cotton seasons 2001/2002 and 2002/2003. In: (Crop Protection) “Quality Cotton” – A Living Industry. 12th Australian Cotton Conference 10 – 12th August 2004, Gold Coast Convention and Exhibition Centre, CD ROM.
- **Herron, G.**, Cottage, E., Wilson, L. and Gunning, R. (2004) Insecticide resistance in cotton aphid (*Aphis gossypii*): results and management options after seasons

2002/2003 and 2003/2004. In: (Crop Protection) "Quality Cotton" – A Living Industry. 12th Australian Cotton Conference 10 – 12th August 2004, Gold Coast Convention and Exhibition Centre, CD ROM.

- **Herron, G.A.**, Gullick, G., and Holloway, J. (2004) Amparo®: a new cotton seed-dressing mixture of imidacloprid and thiodicarb. *General and Applied Entomology*, **33**: 25-28.
- Rossiter, L., **Herron, G.** and Gunning, R. (2004) Review of insecticide resistance management strategy principals. *The Australian Cottongrower*, October –November, **25(6)**: 20-26
- **Herron, G.A.**, Rophail, J. and Wilson, L. (2004) Chlorfenapyr resistance in two-spotted spider mite (Acari: Tetranychidae) from Australian cotton. *Experimental & Applied Acarology*, **34**:315-321.
- Lewis Wilson, Mary Whitehouse, **Grant Herron**, and Simone Heimoana (2005) Aphids and mites in the Bollgard II era. In: Cotton Consultants Australia Inc, Annual General Meeting 2005, 16-17 May 2005, Goondiwindi Community Centre.

8. Have you developed any online resources and what is the website address?

No

9. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. Where possible include a statement of the costs and potential benefits to the Australian cotton industry or the Australian community.

Key Points

1. Resistance monitoring showed a high proportion of aphid populations showing Pirimor resistance despite a change to the management strategy. I consider it likely this relates to high omethoate or dimethoate use against other pests, possibly mirids, with coincident selection of concurrent aphids.
2. Aphids were also collected from hosts other than cotton where monitoring detected resistant populations in farm backyards and on weeds. These rogue overwintering aphid populations have the potential to provide a nucleus of future control problems and so should be eliminated where practical.
3. Biochemical studies using post-bioassay aphids sourced from DAN163c have allowed aldicarb to be considered its own rotation group. For the 2004-2005 cotton season the aphid rotation groupings were Temik®, organophosphates and carbamates, neonicotinoids, Pegasus®, Thiodan® and Fulfil®.

IRACGroup	Chemical	Sold as
	Aldicarb	Temik
1A 1B	Carbamate Organophosphates	Foliar: Pirimor, Aphidex At planting or side dress: Thimet Foliar: Dimethoate, omethoate, chlorpyrifos, chlorpyrifos-methyl, parathion-methyl profenofos
4A	Neonicotinoids	Seed treatments: Gaucho, Amparo, Cruiser Foliar: Confidor, Intruder, Actara
12B	Diafenthiuron	Pegasus
2A	Endosulfan	Thiodan <i>etc</i>
9A	Pymetrozine	Fulfil

4. Intrepid® resistance in two-spotted mite increased quickly in both level and proportion of resistant strains detected in season 2002-2003. In response, the 2003-2004 resistance management strategy for Intrepid® use in cotton was altered and recommended a further restriction of use from two to one spray per season.

Part 4 – Final Report Executive Summary

Provide a one page Summary of your research that is not commercial in confidence, and that can be published on the World Wide Web. Explain the main outcomes of the research and provide contact details for more information. It is important that the Executive Summary highlights concisely the key outputs from the project and, when they are adopted, what this will mean to the cotton industry.

Up until the introduction of transgenic cotton the cotton bollworm has been the major pest of the Australian cotton industry. However following the introduction of the single gene Ingard® transgenic cotton sucking insect pests such as aphids, mites and mirids have become more troublesome, so requiring increased targeted insecticide control. Targeted control has caused resistance to develop with resultant control problems.

Cotton aphid and two-spotted mite were collected from Australian cotton growing regions and tested in the laboratory for resistance. The two-spotted spider mite *Tetranychus urticae* was tested over three years for resistance against a range of chemicals registered in cotton for its control. Encouragingly, Agrimec® and Pegasus® resistance was not detected at all during the study. Comite® resistance was detected in season 2003–2004 but was absent in the subsequent 2004-2005 season. We consider the 2004-2005 result for Comite® together with that of Agrimec® support the effectiveness of the resistance management strategy. However, during season 2001-2002 Intrepid® survivors were detected for the first time in two-spotted mite. Season 2002-2003 produced an alarming trend of increasing level and abundance of Intrepid® resistance. In response the mite management strategy for Intrepid was modified from season 2003-2004 with a reduction in total Intrepid sprays to one per season for either *Helicoverpa* spp or two-spotted mite control. Unfortunately during season 2004-2005 resistance was again detected in 3 out of 7 strains tested but encouragingly resistance frequencies in each strain were generally less than in seasons 2003-2004. The modified strategy restricting Intrepid® use will be maintained so it will be interesting to see if the trend continues with Intrepid® resistance frequencies per strain continuing to drop.

The cotton aphid *Aphis gossypii* was also screened for resistance against several chemicals used for its control in cotton. Earlier screening included Pirimor®, Rescue®, Pegasus®, Confidor®, Endosulfan and Talstar®. Pegasus® and Confidor® resistance was not detected and endosulfan resistance rarely detected. Pirimor® resistance was common as was resistance to Rescue®. Interestingly, Talstar® resistance was detected in some 10-20% of strains although the product is not registered for that use. The result highlights the issue of accidental selection with concurrent pest species. Pirimor® resistance was detected in 61% of cotton aphid populations and as Pirimor® is known to cause cross resistance to Folimat® / Rogor® those products would also be compromised by resistance. Pirimor® resistance remained despite a change to the management strategy in 2003-2004 when Pirimor® and Folimat® use was restricted by the implementation of chemical use windows. It is possible that control of mirids with Folimat® or Rogor® may be selecting concurrent aphids so producing Pirimor®, Folimat® and Rogor® resistance in aphids. The resistance strategy for aphids in cotton has now been amended to include reference to mirids

Collaborative cross-resistance studies with Drs Gunning and Cottage caused the carbamate Temik® to be split from Pirimor® for cotton aphid control (see also DAN164c). This has given growers access to an extra rotation group for the purposes of resistance management.

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