# Insecticide Resistance in Cotton Aphid (*Aphis gossypii*): Results and Management Options after Seasons 2002-2003 and 2003-2004

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## Key Points

1. Resistance monitoring detected an unwelcome rise in the proportion of populations showing pirimicarb resistance. We 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 have allowed aldicarb and carbosulfan to be considered their own rotation group. For the 2004-2005 cotton season the aphid rotation groupings will be aldicarb and carbosulfan, organophosphates and carbamates, neonicotinoids, diafenthiuron, endosulfan and pymetrozine.

## Introduction

Resistance in the cotton aphid (*Aphis gossypii*) is a major new threat to Australian cotton production (Herron *et al.* 2001). Insecticide monitoring underpins the management of resistant populations and collections of aphids are made from cotton fields, weeds farm gardens and residential backyards. These are tested against a range of the control options allowing us to identify emerging resistance problems as well as keeping track of existing problems. This information then contributes to the development of the aphid component of the Insecticide Resistance Management Strategy (IRMS) for cotton.

Management is underpinned by rotation between insecticide groups and restriction in the number of applications of product from any one insecticide group. At present, there are five chemical groups to use in rotation against *A. gossypii.* In the current insecticide groupings the carbamate aldicarb is in the same resistance management group with pirimicarb and the organophosphates, due to likely cross resistance between these insecticides which fall into the 1A and 1B Groups. However, there is evidence from testing of resistant aphid strains that cross-resistance within the carbamates and organophosphates cannot be assumed. Hence, aphids that are resistant to pirimicarb or organophosphates may be susceptible to aldicarb or carbosulfan and this would change resistance management options. It was decided to investigate biochemically the potential for cross resistance between these two carbamates and other members of Group 1A and 1B. It was hoped to be able to separate aldicarb and carbosulfan into their own separate group for resistance management in order to give the growers more flexible control options.

Here we report the results of the 2002-2003 and 2003-2004 season's research on aphids and out line the practical implication of those findings for resistance management.

## Materials and methods

### Strains tested

Aphids are collected by researchers, CRC Industry Development Officers, consultants and growers from cotton fields, weeds and domestic back yards. These are sent to our laboratory at Camden (EMAI) and each field strain is cultured separately on pesticide-free cotton (Deltapine 90) at 25 ±

4°C under natural light. Strain integrity was assured by maintaining populations in purpose built aphid proof cages. When bioassay was complete the strain was then forwarded from Camden to Tamworth for biochemical analysis.

## A. Monitoring resistance to insecticides

Insecticides screened for resistance include a range of control options as well as one pyrethroid, bifenthrin (Table 1). The pyrethroid was included so that potential multiple or cross-resistance could be explored. Although it is not currently registered for use on aphids carbosulfan was also included to investigate cross-resistance more completely.

### Bioassay method

The method for testing is outlined in (Herron *et al.* 2000). Briefly, 35 mm Petri dishes were used and excised cotton plant leaf discs were placed onto 3 mL of liquid cooling agar within the Petri dish. When the agar had cooled and set, 5-6 batches (batch = 10) of adults female aphids were transferred onto the leaf discs and sprayed with the aid of a Potter spray tower producing an aqueous deposit of 1.6 ± 0.07 mg $cm^{-2}$ with a 2 mL aliquot. Aphids were exposed to a single discriminating concentration of chemical. Each test was replicated and included a water only sprayed control. After spraying, Petri dishes were covered with clear plastic film and perforated with a fine needle to prevent condensation. Tests were maintained at 25 ± 0.1 °C in constant light for 24 h after which mortality was assessed.

### Bioassay analysis

Mortality at the discriminating concentration was corrected for control mortality (Abbott 1925) which did not exceed 10%.

## B. Investigating Cross Resistance

There are two main resistance mechanisms identified in cotton aphids in Australia. These are elevated levels of esterases which can break down or bind-to insecticides, and target site insensitivity, which occurs when the target of the insecticide, in this case acetylcholinesterase, is modified so that the insecticide can no longer bind to it. We investigated both of these mechanisms with respect to aldicarb or carbosulfan.

### Esterase Activity

Total esterase activity was detected in aphid homogenates by measuring the hydrolysis of an artificial substrate, 1-naphthyl acetate. The esterase-substrate complex produced forms a composite with the dye, fast blue salt producing a colour reaction. The kinetic assay of colour formation is then read on a micro plate reader and esterase activity determined by comparison to a non insecticide inhibited control. If there is no cross resistance between the current carbamate / organophosphate resistance and aldicarb then we would expect that esterase activity in response to aldicarb would be similar for the susceptible strain and resistant strain.

### Acetylcholinesterase (AChE) Activity

AChE activity is determined by putting esterase aliquots with varying concentrations of the insecticides. AChE activity is again measured colorimetrically but in this instance using the Ellman reaction where the hydrolysis of the acetylthiocholine iodide substrate analogue interacts with the dye, 2-nitro-5-thiobenzoate. As above, AChE activity was determined with respect to a non-insecticide inhibited control. If there is no cross resistance between the current carbamate / organophosphate resistance and aldicarb we would again expect that the susceptible and resistant strains would show similar AChE activity.

**Table 1** Common name, trade name, chemical group, formulation[1], concentration and supplier for insecticides tested.

| Common Name | Trade Name | Chemical Group | Form | Conc. | Supplier |
|---|---|---|---|---|---|
| aldicarb | Temik | carbamate | GR | 150 g/kg | Bayer |
| bifenthrin | Talstar | pyrethroid | SC | 100 g/L | FMC |
| carbosulfan | Marshal | carbamate | EC | 250 g/L | FMC |
| CGA-140408 | carbodiimide of Pegasus | thiourea | EC | 250 g/L | Syngenta |
| chlorpyrifos-methyl | Rescue | organophoshate | EC | 500 g/L | Dow AgroSciences |
| endosulfan | Endosulfan | organochlorine | EC | 350 g/L | Crop Care |
| pirimicarb | Pirimor | carbamate | WP | 500 g/Kg | Syngenta |

[1]EC=emulsifiable concentrate, SC = suspension concentrate, GR = granule and WP=wettable powder.

## Results

No resistance was detected against imidacloprid, diafenthiuron or endosulfan in either seasons 2002-2003 or 2003-2004 (Tables 2 and 3). Bifenthrin and chlorpyrifos methyl resistance was detected and remained relatively static with about 10-20 and 40 percent respectively of populations showing some resistance. The abundance of pirimicarb resistance increased from season 2002-2003 to 2003-2004 with resistance increasing from 52 to 100 percent of populations tested. Resistant aphids were found in farm backyards, on weeds and cotton regrowth.

Pirimicarb / omethoate / dimethoate insensitive AChE did not confer resistance to aldicarb or carbosulfan. An aldicarb specific AChE was detected which means there is a separate AChE mechanism of resistance to this insecticide that needs to be considered in the future. There was also no significant difference found between susceptible and resistant strains for esterases against aldicarb or carbosulfan so it is unlikely that an esterase mediated resistance mechanism is involved. The results support moving carbosulfan and aldicarb into their own group independent of the current organophosphate / carbamate rotation grouping (Table 4).

## Discussion

This most recent monitoring has included aphids from farm backyards, weeds and cotton regrowth. In every instance resistant aphids have been detected that may form the nucleus of future on farm control problems. These rogue overwintering aphids are potentially a problem to cotton farmers because:

1. Cotton aphid can develop a winged form that can disperse if food quality drops or aphids become too crowded so they can go from the farm gardens, weeds or domestic backyards to the field. 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.

2. Australian cotton aphids reproduce parthogenically – that is female aphids give birth to live young that are all females and clones of themselves. Resistance is not diluted by out crossing with susceptible aphids.

**Table 2.** Percent population susceptible to each chemical tested from cotton during season 2002-2003

| Population | Pirimor | Rescue | Pegasus | Confidor | Talstar | Endo |
|---|---|---|---|---|---|---|
| N Territory 1 | 100 | 100 | 100 | 100 | 100 | 100 |
| N Territory 2 | 100 | 100 | 100 | 100 | 100 | 100 |
| N Territory3 | 100 | 94 | 100 | 100 | 100 | 100 |
| Narrabri; town nursery | 100 | 100 | 100 | 100 | 100 | 100 |
| Farm Garden; ex Chrysanthemum | 99 | 81 | 100 | 100 | 92 | 100 |
| Farm garden; ex hibiscus | Strain | did | not | establish | DOA | |
| Farm garden; ex *Gazania*. | 28 | 100 | 100 | 100 | 100 | 100 |
| ACRI; ex cotton regrowth Namoi | 100 | 83 | 100 | 100 | 100 | 100 |
| Tes Emerald | 100 | 100 | 100 | 100 | 40 | 100 |
| Tink Emerald | 2 | 100 | 100 | 100 | 100 | 100 |
| Car Emerald | 45 | 80 | 100 | 100 | 100 | 100 |
| ACRI Field 2 Namoi | 29 | 95 | 100 | 100 | 100 | 100 |
| ACRI Field 2 buffer Namoi | 20 | 33 | 100 | 100 | 100 | 100 |
| Weed, ex *Bladder ketmia* | 16 | 68 | 100 | 100 | 100 | 100 |
| Carb Goondiwindi | 96 | 100 | 100 | 100 | 100 | 100 |
| Und Goondiwindi | 100 | 100 | 100 | 100 | 95 | 100 |
| Kil Goondiwindi | 100 | 100 | 100 | 100 | 100 | 100 |
| Mor Goondiwindi | Strain | did | not | establish | | |
| Alc Goondiwindi | 100 | 100 | 100 | 100 | 100 | 100 |
| Won Goondiwindi | 100 | 100 | 100 | 100 | 100 | 100 |
| Neil Goondiwindi | 100 | 100 | 100 | 100 | 100 | 100 |
| SGG Goondiwindi | 90 | 100 | 100 | 100 | 100 | 100 |
| Nor Goondiwindi | 100 | 100 | 100 | 100 | 100 | 100 |
| Par Goondiwindi | 86 | 100 | 100 | 100 | 88 | 100 |
| Tog Namoi | 11 | 38 | 100 | 100 | 100 | 100 |
| Wil Namoi | 100 | 77 | 100 | 100 | 42 | 100 |
| Yar Namoi | 36 | 18 | 100 | 100 | 100 | 100 |
| Glen Namoi | 30 | 34 | 100 | 100 | 100 | 100 |
| War Goondiwindi | Strain | did | not | establish | DOA | |
| Alc Goondiwindi | Strain | did | not | establish | DOA | |
| GG Goondiwindi | Strain | did | not | establish | DOA | |
| Boo Goondiwindi | Strain | did | not | establish | DOA | |
| Mor Goondiwindi | Strain | did | not | establish | DOA | |
| Eur Gwydir | Strain | did | not | establish | | |
| Lyn Dalby | 89 | 100 | 100 | 100 | 100 | 100 |
| Percent populations resistant | 52 | 41 | 0 | 0 | 18 | 0 |

**Table 3.** Percent population susceptible to each chemical tested from cotton during season 2003-2004

| Population* | Pirimor | Rescue | Pegasus | Confidor | Talstar | Endo |
|---|---|---|---|---|---|---|
| Narrabri; garden ex *Pandorea jasminoides* | 8 | 74 | 100 | 100 | 100 | 100 |
| Tink Emerald | 98 | 100 | 100 | 100 | 100 | 100 |
| Par Emerald | 98 | 100 | 100 | 100 | 100 | 100 |
| Farm garden; ex Lantana | 16 | 23 | 100 | 100 | 100 | 100 |
| ACRI F18 Namoi | 96 | 100 | 100 | 100 | 100 | 100 |
| ACRI F18 Namoi | 98 | 100 | 100 | 100 | 100 | 100 |
| ACRI F18 Namoi | 97 | 100 | 100 | 100 | 100 | 100 |
| ACRI F18 Namoi | 93 | 100 | 100 | 100 | 100 | 100 |
| Wat Emerald | 34 | 79 | 100 | 100 | 100 | 100 |
| Way Emerald | 88 | 100 | 100 | 100 | 100 | 100 |
| Bre Emerald | 43 | 100 | 100 | 100 | 100 | 100 |
| Jab Emerald | 80 | 74 | 100 | 100 | 96 | 100 |
| Orv Emerald | 74 | 100 | 100 | 100 | 100 | 100 |
| Shan Emerald | 10 | 100 | 100 | 100 | 100 | 100 |
| Den Emerald | 59 | 100 | 100 | 100 | 96 | 100 |
| ACRI; ex *Helianthus annus* Namoi | 41 | 81 | 100 | 100 | 100 | 100 |
| ACRI 1936 Namoi | 36 | 73 | 100 | 100 | 100 | 100 |
| ACRI 1938 Namoi | Strain | did | not | establish | DOA | |
| ACRI 1939 Namoi | 19 | 62 | 100 | 100 | 100 | 100 |
| Percent populations resistant | 100 | 39 | 0 | 0 | 11 | 0 |

*Testing for 2003-2004 is incomplete at the time of writing; a total of 53 strains collected

**Table 4.** Now six chemical groups for rotation against aphids

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

3. Aphids have a fast life cycle and will develop from 1st instar through to adult in 5-6 days, and will produce 4-6 live young per day for several weeks thereafter. One resistant aphid quickly becomes a field of resistant aphids, especially if beneficials have been suppressed by insecticide sprays.

4. Cotton aphid has a wide host range including malvaceous, cucurbitaceous, solanaceous, and asteraceae and legume hosts and they are easily found in farm gardens on plants such as hibiscus where they survive winter.

Comparison with earlier data suggests the proportion of strains showing resistance to pirimicarb is rising with 59 % resistant in 1999-2000, 79 % resistant in 2000-2001,and 80% in 2001-2002, 52% in 2002-2003 and 100% in 2003-2004. The rise in pirimicarb resistance has come despite the 2003-2004 change to the IRMS where pirimicarb and dimethoate / omethoate use was restricted. From season 2003-2004 pirimicarb use was restricted to the beginning of the season due to its IPM fit and omethoate / dimethoate restricted to the end of the season where it is applied with defoliant.

Despite our best efforts resistance levels have increased and we suspect this may be attributable to coincident product use against other pests, especially omethoate / dimethoate. It is clear from the data that coincident product use is a problem, for instance, bifenthrin is not registered for aphid control yet 10-20 percent of populations show some level of bifenthrin resistance. Omethoate and dimethoate are popular chemicals that unfortunately confer cross-resistance to pirimicarb in aphids but are also used to control mirids. It is notable that the 2003-2004 cotton season required many cotton growers had to spray to control mirids and many of those mirid sprays were undoubtedly dimethoate or omethoate. It seems reasonable to suspect that mirid control is now adversely affecting the management of *A. gossypii.*

However, even though pirimicarb may eventually succumb to resistance, news is not all bad because biochemical studies have made available a new rotation group independent from the standard Insecticide Resistance Action Committee (IRAC) grouping. Data support the moving of carbosulfan and aldicarb out into their own group independent from the current organophosphate / carbamate rotation grouping (Table 4). However, as an insensitive AChE mechanism is involved in aldicarb, specific resistance the product should not be overused; an unlikely scenario as aldicarb is currently used only as an in furrow treatment at planting, effectively limiting use. Resistance could become a problem if this were to change, for instance if aldicarb were used at higher doses, providing a longer period of control or if it were applied as a side-dress during the season to control sucking pests.

## Management options

The management of aphids will continue to be problematic and so will continue to rely on an integrated approach. The strategies for aphid control will include:

1. The management of overwinter hosts on farm to reduce carryover hosts for resistant aphids. Control of cotton and cotton volunteers is also important to reduce the risk of cotton bunchy top (CBT) carrying over. Once infected with CBT a plant will remain infected so will act as a source of inoculum for the disease in the next season if allowed to survive.

2. If the risk of CBT is a major concern plant a resistant variety.

3. Use of an at-planting insecticide or seed treatment that is registered against aphids can reduce the chance of aphids establishing on young cotton and help delay the development of CBT (if the aphids are carrying it). However, some of these treatments will also reduce predators of mites but not control mites, which may lead to earlier mite build-up. Choose carefully bearing in mind effect on predators and on selection for resistance in aphids.

4. Sample for aphids from seedling emergence. Check for the presence of nymphs as this means the aphids are able to establish and likely to be either cotton aphid or green peach aphid. Don't spray if only winged aphids are found.

5. Thresholds – this is complicated by the fact that aphids can spread CBT, otherwise the threshold would be 90% of plants infested up until first open boll, after which it drops to 10%. Early in the season it is useful to know where aphids are and to monitor hotspots for the presence of CBT symptoms. CBT takes about 3-5 weeks to develop. The later it occurs the less potential effect on yield. If CBT symptoms are found early season than control is a good option. If it's late season then control may not be worthwhile.

6. Conserve beneficials - aphids are controlled by a range of beneficials such as hoverfly larvae, ladybeetle adults and larvae, lacewing larvae and parasitoids. Disruption of predators allows aphid hotspots to build quickly, produce winged forms and spread rapidly across fields.

7. Rotation of insecticides – follow the IRMS, taking care to rotate chemistry and taking into account the seed or at planting insecticides used.

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