# CROSS-RESISTANCE STUDIES IN COTTON APHID, *APHIS GOSSYPII* GLOVER FROM AUSTRALIAN COTTON

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## Summary

A number of insecticides were investigated for cross-resistance patterns using reference susceptible and resistant cotton aphid populations and standard bioassay methods. The highest levels of cross-resistance (>1500x at $LC_{50}$) were detected to pirimicarb in omethoate-, and omethoate/bifenthrin resistant populations. Cross-resistance between the organophosphates and carbamates has caused these two groups to be considered the same for the purpose of resistance management. To reduce the likelihood of difficult to control resistant populations we suggest that omethoate, dimethoate or pirimicarb use be avoided and pirimicarb used only in conjunction with Integrated Pest Management. Further study is required to determine if the carbamates aldicarb and carbosulfan should be treated separately from pirimicarb.

**Keywords:** resistance mechanisms, insecticide resistance management

## INTRODUCTION

Cotton aphid, *Aphis gossypii* Glover, is renowned for developing resistance to the chemicals used for its control (Devonshire 1989). Resistance has caused cotton aphid to become the dominant aphid pest in Australian cotton (Herron *et al*. 2001). A major contributor to resistance development is cross-resistance, namely the protection from more than one chemical through the action of a single mechanism (Scott 1990).

We used reference susceptible and resistant cotton aphid strains and standard bioassay methods to screen a number of chemicals for cross-resistance. These data will be used to better manage existing chemicals used against cotton aphid in cotton.

## MATERIALS AND METHODS

### *Insecticides*

The insecticides tested are each used on Australian cotton. CGA-140408, however, was substituted for difenthiuron (Pegasus®). CGA-140408 is the insecticidal derivative formed when difenthiuron is subjected to ultra-violet light. Their supplier, common name, chemical group, formulation and active ingredient concentration are given in Table 1.

### *Aphids*

Aphids were reared on cotton, variety 'Deltapine 90', at 25±4 °C under natural light. Strain integrity was assured by maintaining populations in purpose-built aphid proof cages. Strain Susceptible A was collected from an unsprayed domestic backyard and was used as the susceptible strain (Herron *et al*. 2000). The remaining strains AWF12, Katherine and CQ were collected from cotton crops and known to be bifenthrin (pyrethroid), omethoate (organophosphate) and omethoate/bifenthrin resistant respectively (Herron *et al*. 2001). Strain CQ died and was subsequently replaced by strain JQ that was also confirmed omethoate/bifenthrin resistant (Table 2). Resistant strains were pressured every two to three months with insecticide(s) to maintain resistance. Strains CQ and JQ were sprayed sequentially with omethoate and bifenthrin without allowing the first spray deposit to dry effectively making a mixture.

### *Bioassay method*

The method of testing *A. gossypii* is as described in Herron *et al*. (2000). Briefly, the method utilised 35 mm Petri dishes into which an excised cotton plant leaf disc was placed onto 3 mL of cooling liquid agar (15 g $L^{-1}$). When the agar had set, batches of about 20 adult aphids were transferred onto the leaf discs. Leaf disc and aphids were then sprayed by means of a Potter spray tower (Burkard Scientific, Uxbridge, Middlesex, UK), producing an aqueous deposit of 1.6 ± 0.07 mg $cm^{-2}$ with a 2 mL aliquot. If a discriminating-dose assay was performed five batches of aphids per test were exposed to the discriminating concentration (LC99.9 value for Susceptible strain A) (Herron *et al*. 2000). However, most strains were subject to full probit analysis that required a range of three to five concentrations to produce log-dose probit (dose-response) assays. Discriminating doses were replicated once and log-dose probit assays four times. Each test (replicate) included a water-only sprayed control. After spraying, Petri dishes were covered with finely perforated clear plastic film that maintained high humidity but prevented condensation. Test insects were maintained at 25±0.1 °C in constant light for 24 h until mortality was assessed.

**Table 1. Chemical group, trade name, common name, supplier, formulation, and concentration for pesticides tested for cross-resistance against cotton aphid.**

| Chemical Group | Trade Name | Common Name | Supplier | Formulation | Concentration |
|---|---|---|---|---|---|
| cyclodiene | Endosulfan | endosulfan | Crop Care | EC[1] | 350 g $L^{-1}$ |
| organophosphate | Curacron | profenofos | Syngenta | EC | 500 g $L^{-1}$ |
| | Folimat | omethoate | Bayer | LC[2] | 800 g $L^{-1}$ |
| | Dibrom | naled | Rotam | EC | 898 g $L^{-1}$ |
| | Orthene | acephate | Bayer | GR[3] | 970 g $kg^{-1}$ |
| | Rescue | chlorpyrifos-ethyl | Dow AgroSciences | EC | 500 g $L^{-1}$ |
| | Lorsban | chlorpyrifos-methyl | Dow AgroSciences | EC | 500 g $L^{-1}$ |
| carbamate | Pirimor | pirimicarb | Crop Care | WP[4] | 500 g $kg^{-1}$ |
| | Temik | aldicarb | Aventis | technical | - |
| | Marshall | carbosulfan | Crop Care | EC | 250 g $L^{-1}$ |
| | Lannate | methomyl | Crop Care | SL[5] | 225 g $L^{-1}$ |
| pyrethroid | Talstar | bifenthrin | Crop Care | EC | 100 g $L^{-1}$ |
| | Decis Forte | deltamethrin | AgrEvo | EC | 27.5 g $L^{-1}$ |
| | Hallmark | esfenvalerate | Cyanamid | EC | 50 g $L^{-1}$ |
| Neonicotinoid | Confidor | imidacloprid | Bayer | SC | 200 g $L^{-1}$ |
| Urea | Pegasus | CGA-140408[6] | Syngenta | SC | 500 g $L^{-1}$ |

1- EC = emulsifiable concentrate
2 - LC = liquid concentrate
3 - GR = granular
4 - WP = wettable powder
5 - SL = soluble concentrate
6 - CGA-140408 = the ultra-violet light activated carbodiimide derivative of difenthiuron (Pegasus®)

***Data analysis***

Non-pooled data were analysed using a Probit program written in GENSTAT 5 statistical software (Barchia 2001). $LC_{50}$ and $LC_{99}$ values were calculated using the probit method outlined in Finney (1971) and included control mortality correction (Abbott 1925). These were used to calculate cross-resistance factors (CRF) derived from the ratio of the $LC_{50}$ or $LC_{99}$ value of resistant strain divided by previously published susceptible data (Herron *et al*. 2000). The associated 95% confidence intervals (CI) for $CRF_{50}$ and $CRF_{99}$ were calculated as outlined in Robertson and Preisler (1992).

## RESULTS

There were several instances of high-level (>10x) cross-resistance including acephate against omethoate/bifenthrin resistant aphids, chlorpyrifos-ethyl against omethoate/bifenthrin resistant aphids, deltamethrin against bifenthrin resistant aphids, esfenvalerate against bifenthrin resistant aphids, omethoate against omethoate/bifenthrin resistant aphids, pirimicarb against omethoate resistant aphids and pirimicarb against omethoate/bifenthrin resistant aphids (Table 3).

Lower level cross-resistance (less than 10x) was also

**Table 2. Percentage mortality at the discriminating concentration (percentage susceptible) for reference populations AWF12, Katherine, CQ and JQ immediately after collection.**

| Insecticide | AWF12* | Katherine* | CQ* | JQ |
|---|---|---|---|---|
| Pirimicarb | 100 | 1 | 3 | 4 |
| Profenofos | 100 | 67 | 48 | 0 |
| Omethoate | 100 | 1 | 0 | 5 |
| Endosulfan | 66 | 100 | 99 | Not tested |
| Deltamethrin | 21 | 100 | 100 | Not tested |
| Bifenthrin | 6 | 100 | 91 | 11 |
| Esfenvalerate | 23 | 100 | 95 | Not tested |

*adapted from Herron *et al*. (2001).

detected including aldicarb against omethoate/ bifenthrin resistant aphids, bifenthrin against omethoate/bifenthrin resistant aphids, carbosulfan against omethoate/bifenthrin resistant aphids, CGA-140408 against omethoate-, bifenthrin- and omethoate/bifenthrin resistant aphids, chlorpyrifos-methyl against omethoate/bifenthrin resistant aphids, endosulfan against bifenthrin resistant aphids, methomyl against omethoate/bifenthrin resistant aphids, naled against omethoate/bifenthrin resistant aphids and profenofos against omethoate and omethoate/bifenthrin resistant aphids.

Some degree of negative cross-resistance is possible for acephate and carbosulfan against the individual resistant aphid strains omethoate and bifenthrin and naled against bifenthrin only. All other chemical strain combinations did not show cross-resistance.

## DISCUSSION

Until recent Australian control failures (Herron *et al.* 2001) organophosphates and carbamates were very effective at controlling cotton aphid. Following chemical failures, growers often tried to manage troublesome populations by alternating organophosphates such as omethoate with the carbamate pirimicarb. However, failures continued and it is now confirmed such failures were caused by cross-resistance. Moores *et al.* (1996) previously documented the existence of at least two insecticide-insensitive forms of acetylcholinesterase resistant cotton aphid. These conferred different resistance spectra to pirimicarb and specific organophosphates. The Australian management strategy for cotton aphid has now been modified to account for organophosphate/carbamate cross-resistance (Schulze and Tomkins 2002).

There are at least five known resistance mechanisms that can each cause resistance in *A. gossypii* (Han *et al*. 1998). Individual mechanisms have the potential to interact in multiple resistant aphids producing unpredictable resistances (Han *et al*. 1998) and therefore cross-resistance. Such an interaction appears in the omethoate and bifenthrin resistant populations against aldicarb, chlorpyrifos-ethyl and chlorpyrifos-methyl. For these chemical-population combinations there was no significant difference in response with individual resistances but there was a significant difference in response against the multiple resistant population. Confoundingly, high-level bifenthrin resistance was detected in the bifenthrin resistant strain but not in the omethoate/bifenthrin resistant strain. This is likely due to the low initial frequency of bifenthrin resistant aphids in strain CQ.Testing was done five weeks after strain collection before routine strain pressuring had begun. However, it is interesting that despite regular pressuring strain JQ currently gives a response to bifenthrin similar to that initially seen in strain CQ (unpubl. data) suggesting a very significant fitness impost against multiple resistant aphids.

Chemical management of all Australian cotton pests is based on a ‘window’ strategy developed by NSW Agriculture (Schulze and Tomkins 2002). The strategy has evolved around the major lepidopteran pests *Helicoverpa* spp. and their control overrides all other chemical use. However, the strategy does include aphids but with control options limited to organophosphates, carbamates, imidacloprid, diafenthiuron, endosulfan and pymetrozine. The *Helicoverpa* spp. chemical application window restricts endosulfan use to early in the season with organophosphates restricted to late in the season.

**Table 3. Cross-resistance factor (CRF) at $LC_{50}$ or $LC_{99}$ plus associated 95% confidence intervals (CI) for a range of insecticides evaluated against three reference resistance strains (omethoate, bifenthrin or omethoate/bifenthrin) of cotton aphid.**

| Strain tested against: | Strain resistant to: | CRF at $LC_{50}$ | 95% CI | CRF at $LC_{99}$ | 95% CI |
|---|---|---|---|---|---|
| acephate | omethoate | 0.2 | 0.1 – 0.3 | 0.3 | 0.1 – 1.0 |
| | bifenthrin | 0.07 | 0.03 – 0.1 | 0.3 | 0.04 – 1.8 |
| | both chemicals[3] | 12.5 | 6.7 – 23.4 | 14.6 | 3.1 – 69.0 |
| aldicarb | omethoate | 1.2 | 0.6 – 2.3 | 0.8 | 0.1 – 6.8 |
| | bifenthrin | 1.5 | 0.7 – 2.9 | 0.8 | 0.1 – 4.6 |
| | both chemicals[2] | 2.4 | 1.1 – 5.3 | 3.4 | 0.4 – 26.3 |
| bifenthrin | omethoate | 1.0 | DD[1] only | 1.0 | DD[1] only |
| | bifenthrin | 41 | 33.6 – 49.9 | 45.3 | 28.3 – 72.4 |
| | both chemicals[3] | 1.5 | 1.2 – 1.9 | 3.7 | 2.1 – 6.5 |
| carbosulfan | omethoate | 0.4 | 0.2 – 0.9 | 2.0 | 0.2 – 16.9 |
| | bifenthrin | 0.3 | 0.1 – 0.5 | 0.7 | 0.1 – 3.7 |
| | both chemicals[3] | 1.9 | 1.1 – 2.5 | 2.0 | 0.7 – 5.5 |
| CGA-140408 | omethoate | 3.2 | 1.5 – 6.9 | 4.6 | 0.7 – 29.9 |
| | bifenthrin | 1.9 | 1.3 – 2.8 | 2.9 | 1.1 – 7.4 |
| | both chemicals[3] | 2.7 | 1.6 – 4.7 | 6.1 | 1.5 – 24.8 |
| chlorpyrifos-ethyl | omethoate | 1.0 | 0.6 – 1.5 | 1.0 | 0.3 – 3.2 |
| | bifenthrin | 1.3 | 0.9 – 1.9 | 0.9 | 0.3 – 2.3 |
| | both chemicals[2] | 3.8 | 1.0 – 14.4 | 19.8 | 0.4 – 865 |
| chlorpyrifos-methyl | omethoate | 1.6 | 0.8 – 3.3 | 0.8 | 0.1 – 4.5 |
| | bifenthrin | 1.4 | 0.6 – 3.3 | 0.8 | 0.1 – 6.3 |
| | both chemicals[2] | 9.8 | 4.2 – 22.5 | 9.4 | 0.9 – 94.0 |
| deltamethrin | omethoate | 1.0 | DD[1] only | 1.0 | DD[1] only |
| | bifenthrin | 16.0 | 12.4 – 20.6 | 24.1 | 12.9 – 45.2 |
| | both chemicals[3] | 1.0 | DD[1] only | 1.0 | DD[1] only |

**Table 3 cont.**

| Strain tested against: | Strain resistant to: | CRF at $LC_{50}$ | 95% CI | CRF at $LC_{99}$ | 95% CI |
|---|---|---|---|---|---|
| endosulfan | omethoate | 1.0 | DD[1] only | 1.0 | DD[1] only |
| | bifenthrin | 8.4 | 5.3 - 13.4 | 9.6 | 3.0 - 30.9 |
| | both chemicals[3] | 1.3 | 0.9 - 1.8 | 1.5 | 0.6 - 3.6 |
| esfenvalerate | omethoate | 1.0 | DD[1] only | 1.0 | DD[1] only |
| | bifenthrin | 21.5 | 17.6 – 26.2 | 19.3 | 11.7 – 31.9 |
| | both chemicals[3] | 2.3 | 1.8 – 3.0 | 6.6 | 3.4 – 13.1 |
| imidacloprid | omethoate | 1.0 | DD[1] only | 1.0 | DD[1] only |
| | bifenthrin | 1.0 | DD[1] only | 1.0 | DD[1] only |
| | both chemicals[3] | 1.2 | 0.9 – 1.7 | 0.8 | 0.3 – 1.9 |
| methomyl | omethoate | 1.7 | 0.8 – 3.5 | 2.7 | 0.6 – 11.2 |
| | bifenthrin | 0.6 | 0.2 – 1.4 | 1.2 | 0.3 – 5.3 |
| | both chemicals[2] | 2.4 | 0.6 – 9.3 | 6.7 | 0.4 – 111 |
| naled | omethoate | 0.8 | 0.6 – 1.1 | 1.3 | 0.6 – 2.8 |
| | bifenthrin | 0.3 | 0.2 – 0.5 | 5.4 | 1.8 – 15.7 |
| | both chemicals[3] | 3.2 | 2.3 – 4.4 | 6.3 | 2.8 – 13.9 |
| omethoate | omethoate | 42.1 | 20.6 – 85.8 | 46.6 | 7.5 – 291.4 |
| | bifenthrin | 1.0 | DD[1] only | 1.0 | DD[1] only |
| | both chemicals[3] | 59.1 | 44.1 – 79.3 | 59.4 | 28.4 – 124.4 |
| pirimicarb | omethoate | 1546 | 170 – 3275 | 4292 | 575– 32017 |
| | bifenthrin | 1.0 | DD[1] only | 1.0 | DD[1] only |
| | both chemicals[3] | 1699 | 1126 – 2562 | 15,830 | 4053-61824 |
| profenofos | omethoate | 4.7 | 2.9 – 7.4 | 3.5 | 1.1 – 10.7 |
| | bifenthrin | 1.0 | DD[1] only | 1.0 | DD[1] only |
| | both chemicals[3] | 5.7 | 3.7 – 8.8 | 8.6 | 3.1 – 23.9 |

[1]DD only = strain evaluated using a discriminating-dose technique producing 100% mortality and so giving a cross-resistance factor approximately equal to 1.0 times.
[2]Data produced using strain JQ
[3]Data produced using strain CQ

Omethoate and pirimicarb are not affected by the *Helicoverpa* spp. treatment windows and can be used throughout the growing season. The newer chemistries imidacloprid, diafenthiuron and pymetrozine have very long withholding periods of 13 weeks, five weeks and four weeks respectively limiting late season use. When concurrent aphids are exposed to late season pyrethroid applications for *Helicoverpa* spp. control omethoate/bifenthrin resistant aphids can be selected. Cross-resistance data suggest these multiple resistant populations will be difficult to control with remaining organophosphate or carbamate options. Organophosphate resistance is known to be stable in cotton aphid while pyrethroid and endosulfan resistances are not (O'Brien *et al*. 1992). Therefore only organophosphate carbamate resistant populations are unlikely to survive season to season. Early season carbamate or organophosphate use risks reselecting organophosphate and carbamate resistant aphids. Therefore early season use of organophosphates and carbamates against aphids is probably best avoided with pirimicarb used only in conjunction with Integrated Pest Management.

Routine monitoring for diafenthiuron (CGA-140408) resistance in Australian cotton aphid has never produced a positive result. Yet the results for CGA-140408 show small yet significant differences in response. Therefore, these differences likely indicate population vigour tolerance rather than cross-resistance. Clearly, the statistically significant result for aldicarb may also be vigour tolerance. Therefore the early season use of aldicarb against aphids requires further consideration. Cross-resistance is often conferred at levels lower than can ultimately be selected (Cranham and Helle 1985). We intend to pressure the omethoate/bifenthrin resistant population with aldicarb to ascertain if there is any change in response. Additionally, survivors of the aldicarb pressuring will be challenged on aldicarb treated cotton to determine practical field implications. If tests do not support cross-resistance then aldicarb should be separated out from other organophosphates and carbamates and its early season use incorporated as another option in the resistance strategy.

Negative cross-resistance has the potential to assist effective resistance management (Tabashnik 1990). Villatte *et al*. 1999 showed negative cross-resistance between the carbamates pirimicarb and bendiocarb. Interestingly, our study may also have found negative cross-resistance but conferred by omethoate and bifenthrin against carbosulfan. Carbosulfan is effective against cotton aphid (Cauquil *et al*. 1983) with potential to give better control than either dimethoate or methamidophos (Alfeu and Ernesto 1999). Although not currently used for aphid control on Australian cotton, the possibility of negative cross-resistance may make carbosulfan useful for controlling organophosphate or pyrethroid resistant strains.

## ACKNOWLEDGMENTS

Tanya James and Idris Barchia are thanked for data entry and probit analysis respectively. Lewis Wilson and Emma Cottage commented on an early draft.

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