Baseline susceptibility and cross-resistance in *Aphis gossypii* Glover (Aphididae: Hemiptera) to phorate and sulfoxaflor

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Abstract

Susceptible discriminating doses of phorate (0.2 g/L) and sulfoxaflor (0.01 g/L) against cotton aphid *Aphis gossypii* Glover were determined by laboratory bioassay where aphids were sprayed with insecticide with the aid of a Potter spray tower. All of the populations tested were susceptible to sulfoxaflor and only a pirimicarb resistant strain had cross resistance to phorate. If phorate is used as a side dressing in Australian cotton for insect control, neither pirimicarb, or any other chemical associated with *ACE*1 type resistance, should be used as the first foliar spray for any subsequent aphid control.

INTRODUCTION

With the introduction of transgenic cotton in Australia to control *Helicoverpa* spp., an overall reduction in chemical insecticide usage has progressively occurred (Constable *et al*. 2011). However, there has been an increase in populations of sucking insect pests such as green mirid *Creontiades dilutus* (Stål) and cotton aphid *Aphis gossypii* Glover (Fitt *et al*. 1994) with consequent increase in their pest status (Herron *et al*. 2001). Control of these once secondary pests with broad-spectrum insecticides depletes beneficial insect populations and also selects for insecticide resistant strains (Herron *et al*. 2001; Herron and Wilson 2011). If future control problems with secondary pests are to be averted monitoring for resistance to key insecticides is essential.

Aphis gossypii has a long history of developing resistance to a range of insecticides in many crops and countries (Devonshire 1989). In the year 2000 high-level resistance to the organophosphates omethoate and dimethoate and to the

carbamate pirimicarb developed in some *A. gossypii* populations causing control failures (Herron *et al*. 2001). Resistance was conferred by an insensitive acetylcholinesterase (*ACE*1)(Benting and Nauen 2004). However, in recent years the efficacy of products belonging to both insecticide classes has been recovered.

In 2008 neonicotinoid resistance was detected in Australian *A. gossypii* (Herron and Wilson 2011). Subsequent increase in both resistance level (LC_{50}) and number of resistant populations caused control failures (Herron and Wilson 2011). Neonicotinoid class insecticides in Australian cotton are under enormous pressure because most commercial cotton seed is coated with a neonicotinoid (thiamethoxam) insecticide seed dressing (Herron and Wilson 2011). Neonicotinoid resistance is now a serious concern to the Australian cotton industry and alternative chemistries are needed for its management.

Alternative chemistries for aphid control in Australian cotton include amitraz, pymetrozine, spirotetramat, carbamates and organophosphates (Mass 2012). Cross resistance between some organophosphates and carbamates (Herron *et al*. 2003) limits the usefulness of these insecticides. Nonetheless the organophosphate phorate has potential as an alternative to the ubiquitous neonicotinoid cotton seed dressing but only if cross resistance either does not occur or if it can be managed effectively. To date there has been no efficacy data for phorate against Australian *A. gossypii* populations. Sulfoxaflor is currently being developed by Dow AgroSciences Australia Ltd for possible use in Australian cotton. In the future it may also be a useful alternative to the neonicotinoid foliar sprays targeting aphids. Sulfoxaflor is a new sulfoximine class of insecticide with activity in the nicotinic acetylcholine receptor (nAChR) but it is sufficiently different from the neonicotinoids that there appears to be no cross resistance (Zhu *et al*. 2011). Again no Australian baseline susceptibility data is available for sulfoxaflor against *A. gossypii*.

Here we present baseline data for sulfoxaflor and phorate against laboratory reference and field collected *A. gossypii* to test for cross resistance and to establish reliable discriminating doses for resistance monitoring.

MATERIALS AND METHODS

Insecticides

Phorate was supplied by the AMVAC Chemical Corporation Los Angeles USA via Barmac Industries Pty. Ltd. Brisbane Australia as technical grade material (89.9%). Sulfoxaflor was supplied by Dow AgroSciences Sydney Australia Ltd. as formulated (240 g/kg) Transform® WG insecticide. Thiamethoxam was supplied by Syngenta Crop protection Pty. Ltd. Sydney Australia as formulated (250 g/kg) Actara® WG and clothianidin was supplied by Sumitomo Chemical Sydney Australia Pty. Ltd. as formulated (200 g/L) Shield® SC.

Aphids

Reference susceptible strains SB and F 96 were maintained on cotton plants under insecticide free conditions. Most of the field collected aphids were sourced from commercial cotton fields or cotton plants in the vicinity of commercial crops but strains 'Both' and 'Chill' were collected from rockmelon and zucchini respectively (Table 1). Aphids were sent to the bioassay laboratory at the Elizabeth McArthur Agricultural Institute (EMAI) at Camden in New South Wales where they were reared as discrete strains in separate insect proof cages on pesticide-free cotton at 25 ± 4 ^{o}C under natural light (Herron *et al*. 2001).

At EMAI field strains were screened for pirimicarb/omethoate and neonicotinoid resistance several weeks prior to the baseline testing (Table 1). The pirimicarb resistant 'Mon P' strain was pressured monthly using a dose that was 10 fold the pirimicarb discriminating dose. This regimen ensured its resistance remained at a high level during the screening for susceptibility to sulfoxaflor and phorate.

PCR

Pirimicarb and omethoate resistance were detected via an established DNA based method (M^{C}Loon and Herron 2009). Briefly, DNA isolated from 20 individual aphids from each of the field strains was subjected to PCR amplification of the *ACE*I gene followed by restriction enzyme digests with the enzyme *Ssp*I.

Bioassay

Aphids were tested by placing them in a 35 mm Petri dish on an excised cotton plant leaf disc fixed in agar (Herron *et al*. 2001). Aphids were transferred individually from the leaves on which they were grown with the aid of a fine paint brush in batches of approximately ten adult female aphids to leaf discs that were then sprayed via a Potter

spray tower with technical grade phorate diluted in reverse osmosis water as outlined in Herron *et al*. (1998) or with formulated clothianidin, thiamethoxam or sulfoxaflor (Herron *et al*. 2001). Strains were tested against several serial concentrations (selected to achieve $0 < x < 100\%$ mortality) of phorate and sulfoxaflor but only the discriminating concentrations of clothianidin and thiamethoxam (Herron and Wilson 2011) were tested. After spraying, clear plastic film was used to cover the Petri dishes, which were then maintained at $25 \pm 0.1\ ^{o}C$ in 16:8 L:D for 24 h. After this time mortality, defined as the inability to walk when probed, was assessed. All tests were replicated and included a water-only sprayed control that did not exceed 15% mortality. Bioassay data were analysed without replicate pooling using a stand-alone probit program developed by Barchia (2001) that ensures variability between replicates is taken into account during the analysis. The program applies the method of Finney (1971) including data adjustment for natural mortality (Abbott 1925). Significant heterogeneity is identified using a χ^2 test and if significant at the 5% level the variance of the estimated parameter is scaled by the corresponding heterogeneity factor equal to the residual mean deviance (Finney 1971). Lethal concentration ratios plus their associated 95% confidence intervals are calculated as described in Robertson *et al*. (2007) with the latter used to determine significance defined as the non overlap of the 95% confidence intervals.

A minimum effective concentration (MEC) was determined directly from the experimental bioassay data. As distinct from a calculated lethal concentration above it is the actual observed single highest insecticide concentration observed directly from the serial concentration dose response data required to kill all insects tested across all replicates and so has no variance.

RESULTS

Of the 16 field collected strains screened for resistance 10 showed some degree of resistance to thiamethoxam, and 11 showed some clothianidin resistance (Table 1). In contrast, only a single strain, known as ‘Mon P’, was resistant to pirimicarb resistant (Table 1). Phorate was tested against seven field collected strains (Table 2) and sulfoxaflor against eleven (Table 3). Phorate and sulfoxaflor strains that showed a

poor fit to the probit model ($P<0.05$) had their fiducial limit calculation scaled by a heterogeneity factor equal to the residual mean deviance.

The LC_{50} level responses sulfoxaflor and phorate generated from the field strains were not significantly different from the reference strains (as indicated by overlapping 95% FLs) except that for strain 'Mon P' against phorate (Table 2). In addition, a high 1.0 g/L $LC_{99.9}$ estimate was found in the 'Mon P' strain. This strain contained 100% pirimicarb resistant individuals and up to 8% neonicotinoid resistant aphids (Table 1).

In the remaining strains that were not pirimicarb resistant or neonicotinoid resistant the maximum $LC_{99.9}$ estimate was 0.21 g/L. For phorate, the minimum effective concentration (MEC) to control all insects tested ranged from a minimum of 0.025 g/L in strain 'Wis' to a maximum of 1.6 g/L in strain 'Mon P'. For sulfoxaflor the $LC_{99.9}$ level response ranged from a minimum of 0.0018 g/L (strain 'Mon P') to a maximum of 0.0069 g/L (strain 'Glen twn S'). A minimum effective concentration of 0.005 g/L was required to kill all insects tested.

DISCUSSION

Strain 'Mon P' was collected from cotton in the small settlement of Clare in the Burdekin region of Queensland. The cotton initially received multiple spray applications to control green vegetable bug, *Nezara viridula* (L.) and was additionally in close proximity to major melon production that was controlling *A. gossypii* (P. Grundy Pers. Com.). It is not surprising then that control issues with *A. gossypii* did develop and strain 'Mon P' was 100% pirimicarb resistant when initially tested. The LC_{50} of phorate to the 'Mon P' (pirimicarb pressured) strain was significantly different to those of the other strains tested. This suggests that pirimicarb (*ACE*1 type) resistance confers cross resistance to phorate. Such cross resistance is not unexpected because *ACE*1 type resistance may be caused by the carbamate pirimicarb or several organophosphate insecticides including omethoate and dimethoate. Dimethoate and phorate are structurally similar belonging to the phoshorodithioate (double sulphur atoms) class of organophosphates (Yu 2008). In contrast, the LC_{50} dose response data against sulfoxaflor indicate no cross resistance to either pirimicarb or the neonicotinoids clothianidin and thiamethoxam despite activity in the nicotinic acetylcholine receptor (nAChR)(Zhu *et al*. 2011).

A discriminating dose should be lethal to susceptibles in a population without affecting the resistant types. It is an empirical compromise based on a two stage approach; 1. firstly, define the limits of tolerance 2. based on stage one select a dose that accounts for all of the susceptibles. Other than the clearly pirimicarb resistant strain 'Mon P', the field strain showing the most tolerance to phorate (strain 'Both') produced an $LC_{99.9}$ estimate of 0.21 g/L and a MEC of 0.05 g/L. Assuming that this strain's response represents the upper limit of the 'susceptible' range, a suitable theoretical $LC_{99.9}$ level discriminating dose of approximately 0.2 g/L (double the maximum MEC recorded in Table 2) is indicated. In contrast, the $LC_{99.9}$ of sulfoxaflor to the most tolerant strain tested ('Glen twn S') was 0.0069 g/L. This suggests that approximately 0.007 g/L sulfoxaflor would be a suitable discriminating dose. It is noteworthy however, that the MEC of sulfoxaflor in this study was 0.005 g/L. For this reason, to lessen the chance of producing a false positive diagnostic discriminating dose response, a further increase in the sulfoxaflor discriminating dose to 0.01 g/L is warranted.

If phorate is used as an alternative to the ubiquitous neonicotinoid cotton seed dressing then phorate/pirimicarb cross resistance must be carefully considered in the context of the cotton insecticide resistance management strategy (Maas 2012). If phorate was to be used as a side dressing at planting, it would be unwise to apply pirimicarb (or any other chemical associated with *ACE*1 type resistance (i.e. omethoate and dimethoate)) as the first foliar spray for aphid control. However, as there is no apparent cross resistance between phorate and the neonicotinoids the latter could be used for this purpose as could spirotetramat, pymetrozine, parrafinic oil or diafenthiuron (after canopy closure).

ACKNOWLEDGMENTS

Dr Idris Barchia is thanked for the statistical analysis and Kate Marshall for providing technical assistance. Lisa Bird and Garry Levot are thanked for providing critical comment on an early draft. The many researchers, Regional Extension Officers, consultants and growers who collected aphids are thanked. This study was funded by the Cotton Research and Development Corporation (DAN1203).

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Table 1 Percent pirimicarb and omethoate (OP) susceptibility using molecular diagnosis plus bioassay determination of clothianidin (Shield®), and thiamethoxam (Actara®) susceptibility via percent mortality at the discriminating concentration (DC)(ie percent susceptible) for field collected *Aphis gossypii*.

Strain	Host if other than cotton	Percent pirimicarb/omethoate susceptibles (*Ssp*I enzyme)	Percent clothianidin susceptibles (DC 0.05 g ai/L)	Percent thiamethoxam susceptibles (DC 0.02 g ai/L)
Alch		100	81	75
And		100	98	100
Bal		100	90	90
Both	Rockmelon	100	82	44
Bud		100	100	100
Car F3		100	100	100
Car Gin		100	98	92
Carring		100	92	82
Chill	Zucchini	100	87	83
Cly		100	100	100
Cor		100	95	85
Glen twn S		100	96	67

Kilm U	100	93	87
Mon P	0	99	92
Wise	100	100	100
Zig	100	100	100

Table 2 Phorate dose-response data for laboratory reference and field sourced *Aphis gossypii*.

Strain	Chi-Square (df)	Slope (se)	LC_{50}* (95% FL)	$LC_{99.9}$* (95% FL)	MEC*#
Susc. F 96	15.5^{+} (6)	5.9 (1.2)	0.021 (0.017-0.028)	0.069 (0.043- 0.23)	0.05
Susc. SB	47.5^{+} (8)	3.4 (0.8)	0.013 (0.0049-0.020)	0.11 (0.059-0.82)	0.1
Carring	14.6^{+} (6)	8.1 (1.9)	0.025 (0.021-0.043)	0.061 (0.038-0.33)	0.05
Car F3	8.3 (6)	5.6 (0.8)	0.014 (0.013-0.016)	0.051 (0.041- 0.072)	0.05
Mon P	46.1^{+} (12)	5.3 (0.9)	0.26 (0.21-0.31)	1.00 (0.70-2.04)	1.6
Both	53.1^{+} (6)	2.8 (1.0)	0.016 (0.0067-0.032)	0.21 (0.032-33.12)	0.05
Cly	15.2^{+} (6)	6.3 (1.1)	0.013 (0.0092-0.015)	0.040 (0.030-0.078)	0.05
Wis	6.2 (4)	5.5 (0.8)	0.011 (0.0099-0.012)	0.040 (0.032-0.057)	0.025

Zig	21.2[+] (6)	6.2 (1.3)	0.019 (0.014-0.024)	0.060 (0.039-0.22)	0.05

* g ai / L

Minimum effective concentration to kill all test insects

[+] Chi square test significant at $P<0.05$

Table 3 Sulfoxaflor lethal concentration dose-response data for laboratory reference and field sourced *Aphis gossypii*.

Strain	Chi-Square (df)	Slope (se)	LC_{50}* (95% FL)	$LC_{99.9}$* (95% FL)	MEC*#
Susc. F 96	30.7^{+} (6)	6.0 (1.5)	0.00091 (0.00067-0.0012)	0.0029 (0.0018-0.017)	0.0025
Susc. SB	22.6^{+} (5)	3.6 (1.3)	0.00058 (0.000096- 0.0013)	0.0042 (0.0015-0.058)	0.0031
Alch	10.1 (8)	3.1 (0.3)	0.00062 (0.00049-0.00074)	0.0063 (0.0047-0.0096)	0.005
Car Gin	6.8 (8)	3.1 (0.3)	0.00056 (0.00043-0.00068)	0.0056 (0.0042-0.0087)	0.005
Bal	35.5^{+} (6)	3.0 (0.93)	0.00050 (0.00013-0.00086)	0.0052 (0.0017-0.16)	0.0025
Cor	45.2^{+} (6)	5.0 (1.5)	0.00064 (0.00028-0.00093)	0.0027 (0.0014-0.023)	0.0025
Glen twn S	15.7^{+} (6)	2.9 (0.6)	0.00060 (0.00035-0.00082)	0.0069 (0.0034-0.046)	0.0025
Kilm U	7.6 (8)	3.0 (0.3)	0.00058 (0.00044-0.00071)	0.0064 (0.0046-0.010)	0.005

And	50.5^{+} (4)	4.4 (2.0)	0.00063 (0.00028-0.0014)	0.0032 (0.00028-0.32)	0.0012
Car F3	32.0^{+} (6)	5.1 (1.2)	0.00085 (0.00057-0.0012)	0.0035 (0.0020-0.027)	0.0025
Chill	26.1^{+} (6)	4.3 (1.1)	0.00057 (0.00026-0.00082)	0.0030 (0.0017-0.026)	0.0025
Mon P	16.4^{+} (6)	4.9 (1.1)	0.00043 (0.00015- 0.00059)	0.0018 (0.0013- 0.0052)	0.0025
Bud	5.5 (6)	3.8 (0.4)	0.00065 (0.00057-0.00073)	0.0042 (0.0030- 0.0068)	0.0025

* g ai / L

Minimum effective concentration to kill all test insects

$^{+}$ Chi square test significant at $P<0.05$