
Experimental and Applied Acarology **34:** 315–321, 2004.

Chlorfenapyr resistance in two-spotted spider mite (Acari: Tetranychidae) from Australian cotton

G.A. HERRON[1,*], J. ROPHAIL[1] and L.J. WILSON[2]
*[1]NSW Agriculture, EMAI, PMB 8, Camden 2570, Australia; [2]CSIRO LB 59, Narrabri 2390, Australia; *Author for correspondence (e-mail: grant.herron@agric.nsw.gov.au; phone: +61-46293333; fax: +61-46293300)*

Received 25 February 2004; accepted in revised form 24 May 2004

Key words: Chlorfenapyr, Miticide bioassay, Negative cross-resistance, Resistance management, *Tetranychus urticae*

Abstract. The responses of *Tetranychus urticae* Koch from Australian cotton to chlorfenapyr has been monitored since the 1997–1998 growing season. Resistance was first detected in the 2001–2002 season and then increased quickly in both level and proportion of resistant strains detected. In response, the resistance management strategy for chlorfenapyr use in cotton was altered and now recommends a further restriction of use from two to one spray per season. There was no evidence of negative cross-resistance to the pyrethroid bifenthrin, but chlorfenapyr was associated with an undefined negative cross-resistance.

Introduction

The Insecticide–miticide chlorfenapyr (AC-303-630) was first synthesised and characterised by the American Cyanamid Company in 1988 (Treacy et al. 1994) and registered for use in Australia in September 1998 on cotton. However, resistance causing control failure against two-spotted spider mite *Tetranychus urticae* Koch was first detected in Australian horticulture following a single use of the product on nectarines in 2001 (Herron and Rophail 2003).

Chlorfenapyr is used in Australian cotton to control the primary crop pests *Helicoverpa* spp. (Noctuidae) plus the secondary pest *T. urticae* (Johnson and Farrell 2003). A total of two chlorfenapyr applications per season were allowed until 2003–2004 when total sprays approved was further reduced to one including both *T. urticae* and *Helicoverpa* spp. sprays (Johnson and Farrell 2003). We have monitored *T. urticae* responses to chlorfenapyr in cotton since its introduction. Here we present these data and discuss the practical implications of the monitoring outcomes to the ongoing management of chlorfenapyr use in Australian cotton.

Materials and methods

Chemical

Mites were tested against commercially available chlorfenapyr 360 g/l Suspension Concentrate (SC) (Secure 360 SC Insecticide–miticide, BASF Australia Ltd).

Mites

The susceptible strain of *T. urticae* used in this study was collected from an unsprayed Sydney backyard in 1987 and its response to several chemicals has been previously published (Herron et al. 1998) The field-collected strains of *T. urticae* were randomly sampled from randomly selected Australian cotton fields between 1998 and 2003. Exposure histories to chlorfenapyr were unknown.

Bioassay

Mites were tested using a standard adulticidal method (Edge and James 1982) that was adapted from the standard FAO leaf residue method for spider mites (Dittrich et al. 1980). Briefly, this entailed transferring 20–25 young adult female mites to 30 mm diameter bean leaf discs. Mites on the leaf discs were then sprayed with 2 ml of aqueous product using a Potter spray tower (Burkard Scientific, Uxbridge, Middlesex, UK) operating with a 48 kPa inlet pressure and producing a deposit of 1.6 ± 0.07 mg cm^{-2} after a 3-s settling time. Mites from each strain were sprayed with a range of serial concentrations of which one was a discriminating concentration (0.4 g ai/l chlorofenapyr) and one a water only control. Sprayed leaf discs were maintained under constant light on moistened cotton wool for 48 h at 28 ± 0.4 °C, 70% RH, after which mortality was assessed. Each bioassay consisted of two leaf discs per concentration, and was replicated three times giving six treated leaf discs per dose. If control mortality exceeded 15%, results from the bioassay were rejected.

Analysis

Individual replicate data were analysed using a Probit program written in GENSTAT 5 statistical software (Barchia 2001). LC_{50} and $LC_{99.9}$ 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) at the LC_{50} and $LC_{99.9}$ percent level (RF_{50} and $RF_{99.9}$)

plus their associated 95% confidence intervals (CI) were calculated as outlined in Robertson and Preisler (1992).

Results

Monitoring continued for four seasons without resistance detection, although minimum probit regression slope values progressively declined season to season (Table 1). In the fifth season chlorfenapyr resistance was detected in a single strain (BE) but the frequency of resistant mites was such that it did not significantly alter the RF_{50} value from 1-fold. Resistance then increased quickly in both level (8.7-fold in strain G) and abundance (four of six strains tested) during the following 2002–2003 season.

Discussion

Unlike the situation in nectarine crops where resistance was detected after a single application, chlorfenapyr resistance in *T. urticae* was not detected until after five seasons of use for *Helicoverpa* spp. and *T. urticae* control in cotton. Resistance took several seasons to be detected, despite multiple applications annually for *T. urticae* and *Helicoverpa* spp. control, compared to a single application in nectarines (Herron and Rophail 2003). This difference in time to detection may be related to differences in chemical use between cotton and nectarines, as *T. urticae* on the two crops are largely controlled with different products. Dicofol is the only common product, with fenbutatin-oxide and tetradifon additionally registered for *T. urticae* control on nectarines and abamectin, propargite, diafenthiuron and bifenthrin registered in cotton. The 'nectarine' strain reported by Herron and Rophail (2003) was tested for cross-resistance against three unregistered products used by the nectarine grower at that time (propargite, tebufenpyrad and pyridaben) but cross-resistance was not detected. As the 'nectarine' strain was resistant to fenbutatin-oxide (unpublished data) it may be useful to further evaluate fenbutatin-oxide and tetradifon for cross-resistance against chlorfenapyr resistant and susceptible *T. urticae* strains from cotton.

Chlorfenapyr is a pyrrole class pro-insecticide activated by the *in vivo* oxidative removal of its *N*-ethoxymethyl group (Treacy et al. 1994). Consequently, pests with elevated levels of oxidases such as the synthetic pyrethroid-resistant tobacco budworm reported by Pimprale et al. (1997) exhibit negative cross-resistance. Bifenthrin (a synthetic pyrethroid) resistance in *T. urticae* from Australian cotton was first detected in the 1996–1997 season (Herron et al. 2001). However, there was no evidence of negative cross-resistance to bifenthrin but chlorfenapyr was associated with an undefined negative cross-resistance. For instance, strain Col-B had $>100\times$ bifenthrin resistance (Herron et al. 2001) but did not exhibit the highest level of negative cross-resistance for

Table 1. Log-dose probit regression summaries for chlorfenapyr tested against two-spotted spider mite from Australian cotton, 1997–1998 to 2002–2003.

Season	Strain	Slope (s.e.)	LC_{50} g/l (95% FL[a])	RF (95% CI[b])	$LC_{99.9}$ g/l (95% FL)	RF (95% CI)
	Susc.	3.2 (0.45)	0.017 (0.014–0.021)	–	0.16 (0.12–0.25)	–
97/98	M	3.5 (0.93)	0.016 (0.012–0.019)	0.9 (0.6–1.4)	0.12 (0.081–0.23)	0.7 (0.2–2.5)
	AW	3.0 (2.30)	0.015 (0.0079–0.021)	0.9 (0.3–2.7)	0.15 (0.081–0.88)	0.9 (0.02–34.3)
	MF	3.0 (0.70)	0.015 (0.011–0.018)	0.8 (0.6–1.3)	0.15 (0.10–0.31)	0.9 (0.3–3.4)
	JQ	3.7 (2.65)	0.027 (0.016–0.036)	1.5 (0.6–3.9)	0.18 (0.11–0.75)	1.1 (0.06–21.0)
	WS	3.4 (0.91)	0.0094 (0.0064–0.012)	0.5 (0.4–0.8)	0.074 (0.050–0.15)	0.4 (0.2–1.2)
	TO	3.3 (0.64)	0.015 (0.011–0.018)	0.8 (0.6–1.2)	0.13 (0.09–0.22)	0.8 (0.3–2.3)
98/99	AW	2.9 (0.36)	0.012 (0.0096–0.014)	0.7 (0.5–0.9)	0.13 (0.096–0.21)	0.8 (0.3–1.9)
	AC	2.5 (1.21)	0.015 (0.0096–0.021)	0.9 (0.4–2.0)	0.27 (0.13–1.52)	1.7 (0.1–29.0)
	K	2.4 (0.43)	0.0049 (0.0033–0.0064)	0.3 (0.2–0.4)	0.099 (0.063–0.21)	0.6 (0.2–2.0)
	EL	2.7 (0.41)	0.012 (0.0091–0.014)	0.7 (0.5–0.9)	0.16 (0.11–0.30)	1.0 (0.4–2.7)
	MO	2.6 (0.30)	0.015 (0.011–0.019)	0.9 (0.6–1.2)	0.24 (0.17–0.41)	1.5 (0.6–3.7)
	W	3.2 (0.34)	0.0050 (0.0040–0.0059)	0.3 (0.2–0.4)	0.047 (0.039–0.061)	0.3 (0.1–0.6)
99/00	Y	2.0 (0.42)	0.0066 (0.0049–0.0086)	0.4 (0.2–0.6)	0.22 (0.11–0.70)	1.3 (0.3–6.8)
	CO	2.8 (0.50)	0.0082 (0.0059–0.010)	0.5 (0.3–0.7)	0.10 (0.069–0.19)	0.6 (0.2–1.9)
	ColA	1.8 (0.35)	0.015 (0.011–0.019)	0.8 (0.5–1.4)	0.70 (0.32–2.52)	4.2 (0.7–24.3)
	ColB	2.0 (2.60)	0.0070 (0.0042–0.011)	0.4 (0.024–6.7)	0.24 (0.073–22.84)	1.5 (0.00034–6458.9)
	KU	2.3 (0.61)	0.0068 (0.0042–0.0093)	0.4 (0.2–0.6)	0.14 (0.084–0.35)	0.9 (0.2–4.3)
	KA	2.0 (0.39)	0.0059 (0.0037–0.0081)	0.3 (0.2–0.5)	0.20 (0.12–0.49)	1.2 (0.3–4.9)
	WA	2.3 (0.50)	0.0071 (0.0050–0.0092)	0.4 (0.3–0.6)	0.15 (0.084–0.43)	0.9 (0.2–3.7)
	W	2.8 (0.67)	0.0077 (0.0054–0.0098)	0.4 (0.3–0.7)	0.098 (0.061–0.22)	0.6 (0.2–2.4)
	MV	2.3 (0.92)	0.0023 (0.0015–0.0031)	0.1 (0.06–0.3)	0.047 (0.021–0.26)	0.3 (0.02–3.4)
	TY	1.1 (0.88)	0.0020 (0.00031–0.0049)	0.1 (0.01–1.0)	1.34 (0.014–17928.5)	0.3 (0.00045–149826.0)
00/01	NA	2.7 (0.81)	0.0048 (0.0034–0.0061)	0.3 (0.2–0.5)	0.068 (0.038–0.20)	0.4 (0.07–2.1)
	CU	2.7 (0.42)	0.0038 (0.0030–0.0046)	0.2 (0.1–0.3)	0.055 (0.036–0.10)	0.3 (0.1–0.9)
	AE	1.8 (1.32)	0.0060 (0.0030–0.0096)	0.3 (0.07–1.7)	0.32 (0.010–6.88)	1.7 (0.005–586)
	MI	1.7 (0.36)	0.0015 (0.00092–0.0021)	0.1 (0.05–0.2)	0.093 (0.051–0.25)	0.5 (0.1–2.5)
	EL	2.2 (0.59)	0.0027 (0.0017–0.0037)	0.1 (0.09–0.3)	0.070 (0.040–0.2)	0.4 (0.07–2.1)
	WH	1.6 (1.56)	0.0053 (0.0010–0.0095)	0.3 (0.03–2.7)	0.46 (0.11–128.13)	2.5 (0.001–5,141)

01/02	ST	2.3 (0.17)	0.017 (0.014–0.019)	1.0 (0.7–1.3)	0.38 (0.30–0.53)	2.1 (0.9–4.9)
	BE[c]	1.3 (0.55)	0.014 (0.0054–0.025)	0.8 (0.2–3.2)	3.06 (1.03–28.93)	16.7 (0.4–732)
	WN	2.6 (0.64)	0.0084 (0.0049–0.011)	0.5 (0.3–0.8)	0.12 (0.083–0.26)	0.7 (0.2–2.7)
	AC	2.6 (0.48)	0.034 (0.026–0.041)	1.9 (1.3–2.9)	0.54 (0.34–1.12)	2.9 (0.8–10.2)
	MI	2.8 (0.85)	0.024 (0.018–0.030)	1.4 (0.8–2.4)	0.30 (0.17–0.77)	1.6 (0.3–9.1)
	PE	3.0 (1.13)	0.011 (0.0066–0.015)	0.6 (0.4–1.1)	0.12 (0.076–0.31)	0.7 (0.1–4.7)
	SO	2.0 (0.62)	0.011 (0.0071–0.016)	0.7 (0.4–1.2)	0.37 (0.18–1.41)	2.0 (0.3–15.6)
	KU	3.1 (0.57)	0.0091 (0.0061-0.011)	0.5 (0.4–0.7)	0.087 (0.065–0.14)	0.5 (0.2–1.3)
02/03	G[c]	1.4 (0.35)	0.15 (0.082–0.23)	8.7 (4.0–18.6)	24.80 (10.75–100.20)	151.8 (13.8–1663.3)
	HA	2.8 (0.23)	0.0066 (0.0055–0.0075)	0.4 (0.3–0.5)	0.079 (0.065–0.10)	0.5 (0.2–1.0)
	RA[c]	1.0 (0.42)	0.0022 (0.00016–0.0075)	0.1 (0.002-0.9)	2.04 (0.85–12.51)	12.5 (0.2–687.4)
	TO[c]	1.7 (0.08)	0.0087 (0.0067–0.012)	0.5 (0.4–0.6)	0.59 (0.47–0.76)	3.5 (1.7–7.7)
	W[c]	1.7 (0.35)	0.039 (0.022–0.057)	2.2 (1.3–4.0)	2.76 (1.58–6.60)	16.9 (3.0–95.9)
	Y	2.3 (0.17)	0.0086 (0.0071–0.010)	0.5 (0.4-0.6)	0.18 (0.14–0.23)	1.1 (0.5–2.3)

[a]FL = fiducial limit.
[b]CI = confidence interval.
[c]Survivors at the discriminating concentration of chlorfenapyr (0.4 g ai/l).

that season. In contrast, the study of Gotoh et al. 2001 found eggs of the Tetranychid *Oligonychus coffeae* (Nietner) highly chlorfenapyr and bifenthrin resistant. Consequently, bifenthrin resistance in Australian *T. urticae* from cotton may not be caused by increased oxidases but by another resistance causing mechanism.

When bifenthrin resistance was detected in *T. urticae* from Australian cotton it was not possible to modify product use, as it was essential for *Helicoverpa* spp. Control (Herron et al. 2001). This was not the case for chlorfenapyr and beginning in the 2003–2004 season cotton growers are restricted to using chlorfenapyr only once per season for either *Helicoverpa* spp. or *T. urticae* control.

Chlorfenapyr resistance in Australian *T. utricae* may be incompletely dominant and monogenic (Uesugi et al. 2002). A single locus resistance would be expected to evolve faster with increased insecticide-use (Tabashnik 1990) so the halving of the chlorfenapyr selection pressure could extend the useful life of the product.

Acknowledgements

The Cotton Research and Development Corporation provided funding (DAN139c, DAN163c, CSP74C and CSP103C). Idris Barchia is thanked for the probit analysis and Garry Levot is acknowledged for providing critical comment on an early draft.

References

Abbott W.S. 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Barchia I. 2001. Probit analysis and fiducial limits in Genstat. Genstat 2001 Conference, Goldcoast, Australia.

Dittrich V., Cranham J.E., Jepson L.R. and Helle W.1980. Revised method for spider mites and their eggs (e.g. *Tetranychus* spp. and *Panonychus ulmi* Koch), FAO method No. l0a. FAO Plant Production and Protection Paper 21. FAO, Rome, pp. 49–53.

Edge V.E. and James D.G. 1982. Detection of cyhexatin resistance in two-spotted mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) in Australia. J. Aust. Entomol. Soc. 21: 198.

Finney D.J. 1971. Probit Analysis, 3rd ed. Cambridge University Press, Cambridge.

Gotoh T., Kitashima Y., Goka K. and Nagata T. 2001. Susceptibility of the red spider mite, *Oligonychus coffeae* (Acari: Tetranychidae), to acaricides on tea plants in Japan. Int. J. Acarol. 27: 303–307.

Herron G.A. and Rophail J. 2003. First detection of chlorfenapyr (Secure (R)) resistance in two-spotted spider mite (Acari: Tetranychidae) from nectarines in an Australian orchard following a single application of product to nectarines. Exp. Appl. Acarol. 31: 131–134.

Herron G.A., Edge V.E., Wilson L.J. and Rophail J. 1998. Organophosphate resistance in spider mites (Acari: Tetranychidae) from cotton in Australia. Exp. Appl. Acarol. 22: 17–30.

Herron G.A., Rophail J. and Wilson L.J. 2001. The development of bifenthrin resistance in two-spotted spider mite (Acari: Tetranychidae) from Australian cotton. Exp. Appl. Acarol. 25: 301–310.

Johnson A. and Farrell T. 2003. Cotton Pest Management Guide, 2003–2004. NSW Agriculture, Orange.

Pimprale S.S., Besco C.L., Bryson P.K. and Brown T.M. 1997. Increased susceptibility of pyrethroid-resistant tobacco budworm (Lepidoptera: Noctuidae) to chlorfenapyr. J. Econ. Entomol. 90: 49–54.

Robertson J.L. and Preisler H.K. 1992. Pesticide Bioassays with Athropods.CRC Press, Boca Raton.

Tabashnik B.E. 1990. Modelling and evaluation of resistance management tactics. In: Roush R.T. and Tabashnik B.E. (eds), Pesticide Resistance in Arthropods. Chapman and Hall, New York, pp. 153–182.

Treacy M., Miller T., Black B., Gard I., Hunt D. and Hollingworth R.M. 1994. Uncoupling activity and pesticide properties of pyrroles. Colloquium on the design of mitochondrial electron transport inhibitors as agrochemicals. Biochem. Soc. Trans. 22: 244–247.

Uesugi R., Goka K. and Osakabe M.H. 2002. Genetic basis of resistance to chlorfenapyr and etoxazole in the two-spotted spider mite (Acari: Tetranychiae). J. Econ. Entomol. 95: 1267–1274.