Development and use of a method to measure aldicarb resistance in *Tetranychus urticae* Koch (Acari: Tetranychidae) from cotton in Australia

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INTRODUCTION

Aldicarb is a carbamate pesticide with insecticidal and acaricidal properties (Anon., 1991) which was first commercially used in Australian cotton late in the 1987/88 season (B. Howie, pers. com.). Although toxic to *Tetranychus urticae* Koch, aldicarb is predominantly used in Australian cotton to control the thrips *Frankliniella schultzei* (Trybom) and *Thrips tabaci* Lindeman (Wilson and Bauer, 1993). Aldicarb provides additional benefits by reducing concurrent populations of *T. urticae* infesting early season cotton. Consequently, early season aldicarb use may select for aldicarb resistance in those *T. urticae* populations established in early cotton crops (Wilson and Morton, 1993).

Rophail (1984) tested a susceptible and organophosphate (OP) resistant strain of *T. urticae* from cotton. Strains of *T. urticae* from Australian cotton carry OP resistance at varying levels (Herron *et al.*, 1998). The OP resistant strain tested appeared to be resistant to aldicarb, but the method was not particularly sensitive and cross-resistance could not be confirmed. There was concern that aldicarb selection over a number of years could be reducing its efficacy due to the development of resistance, particularly if there was a degree of cross-resistance from OP resistance.

Resistance can potentially be measured by a diverse range of methods (Busvine, 1971). However, methods for tetranychid mites have become relatively standardised (Dittrich *et al.*, 1980) with those techniques being adapted to other mite genera (Overmeer, 1985). The standard techniques for measuring resistance to pesticides in mites involve the spraying of

aqueous insecticide solutions or the use of aqueous insecticide dips. Obviously, these techniques are used because they can usually produce reliable and reproducible data. However, the reliability of resistance testing is influenced by insecticide formulation (Dittrich, 1962).

Aldicarb (Temik®) is extremely toxic to mammals (Anon., 1991) and so is available commercially only as a polymer coated granular formulation. Emulsifiable concentrate or wettable powder formulations, which are compatible with our standard method using a Potter spray tower, are not available for sale anywhere in the world (B. Howie, Pers. Com.). The toxicity of aldicarb can be evaluated by treating whole plants with granules (French and Ludlam, 1972; Gould and Jessop, 1981) or treated plant material can be excised for use in laboratory bioassays (Rophail, 1984). Rophail (1984) showed formulated aldicarb (Temik®) taken up by French bean plants could only provide a crude dose-response for *T. urticae* and the artificial infestation of treated plants gave only an indication of resistance.

In order for us to investigate possible cross-resistance between OPs and aldicarb in *T. urticae,* and the influence of aldicarb use on resistance, we required an accurate and reproducible bioassay method, particularly allowing direct comparison with our previous studies (Herron *et al.*,1998). Rhône-Poulenc Rural Australia Pty Ltd supplied us with a sample of technical aldicarb and we developed a test method based on a standard Potter spray tower. We also present base-line dose-response data for aldicarb against a laboratory susceptible strain of *T. urticae* and strains from Australian cotton.

MATERIALS AND METHODS

Chemicals

Technical grade aldicarb was supplied by Rhône-Poulenc, Alexander Drive, Research Triangle Park, North Carolina, 27709, USA. A stock solution of aldicarb was made by dissolving the chemical into A.R. grade acetone. When not being used the stock was stored in a fridge at 4 ^{o}C, but always allowed to warm to room temperature before use. Test solutions were made using several aqueous dilutions. Formulated profenofos (Curacron 500 EC) was

supplied by Ciba-Geigy Australia Limited (now Novartis Australasia Limited). Its preparation and use is described in detail by Herron *et al.* (1998).

Mites

A susceptible reference strain of *T. urticae* was used to refine the assay. The susceptible strain was collected from an unsprayed source in the Sydney region in 1987. It has subsequently been maintained on pesticide-free French bean, *Phaseolus vulgaris* L., in an isolated rearing unit at 28 ^{o}C, and under constant illumination. Eighteen strains with some previous aldicarb exposure were collected from NSW cotton and tested with aldicarb. The field-collected strains were maintained in separate rearing units under similar conditions to the reference strain.

Bioassay method

Methods for profenofos are given in Herron *et al.* (1998). Working solutions of aldicarb were prepared in a fume cabinet (with glass screen) by a person wearing full protective clothing (gloves, laboratory coat and eye shield) in a safety shower equipped laboratory. The stock solution of aldicarb (in acetone) was diluted in a volumetric flask (with water) so that the working concentration was equivalent to the highest concentration sprayed. The bioassay range was then bracketed (10 x) by preliminary testing. Once the bioassay range was known a working solution of aldicarb was then serially diluted four or five times with water to achieve the range of concentrations required for a full assay. Containers were always capped to prevent evaporation.

Aldicarb and profenofos were sprayed by a Potter spray tower (Busvine, 1971). For both chemicals the spray tower was calibrated to produce an aqueous insecticide deposit of 1.6 ± 0.07 mg/cm^2 with a 2 mL spray aliquot. The Potter spray tower was housed in a stainless steel and glass containment cabinet that was extracted by down draft air movement to avoid turbulence in the spray tower (Figure 1). The tower was operated by means of an lever external to the cabinet which was attached to the side of the spray tower and manipulated the standard on-off valve (Figure 1). Pesticide over-spray was extracted through a perforated grid at the base of the cabinet resulting in an even exhaust air flow from all sides of the spray tower which avoided turbulence in the spray tube (Figure 1). Extraction efficacy has been verified by the relevant Australian authorities and was sufficient to draw all over-spray back

down into the cabinet to prevent exposure of the operator. Run-off was collected in an integrated receptacle below the cabinet and was drained after each experiment with a drainage tap.

Mites were tested by the methods described in Edge and James (1982). Briefly that required the transfer of 22 to 25 young adult female mites to French bean leaf discs. The discs were then placed under the spray tower and sprayed with aqueous pesticide solution or water (control). Sprayed discs were then transferred to gauze covered moistened cotton wool and maintained for 48 hours at 28 ± 0.4 ^{o}C, 70% RH, under constant illumination. Mortality, defined as the inability to walk when prodded, was then assessed. Probit regressions, corrected for control mortality, were calculated using Probit 5 for Windows (Gillespie, 1995). Control mortality did not exceed 10%. Strain specific LC_{50} s for profenofos were interpolated from the data of Herron *et al.* (1998), although such detail was not given in that publication.

RESULTS

There was little variation in slope values (3.1-3.3) between individual dose-response replicates for the susceptible strain (Table 1). Non-significant Chi-squared values ($P<0.05$) for each replicate indicate a good fit to the probit model (Table 1). LC50 and LC99.9 values are 0.020 and 0.20 g aldicarb / L (Table 1) after pooling of replicates.

There was little variation in response between the susceptible and field-collected strains. The maximum difference detected was 2.5x (Table 2). Thirteen of the field-collected strains had significant ($P<0.05$) chi-squared values indicating a systematic departure from the probit model which could indicate incipient resistance (Table 2).

Strains BD-94/95 and AE-94/95 had slope values of <2.0x and higher RFs (Table 2) but low slope or higher RF value was not associated with extreme profenofos resistance. Consequently, cross-resistance between aldicarb and profenofos was not indicated (Table 2)

DISCUSSION

We have demonstrated that technical aldicarb, applied with a standard Potter spray tower in an efficient down draft extraction cabinet (Figure 1), safely produces precise and reproducible bioassay data (Table 1). However, we stress that the method we describe must only be used in a well equipped laboratory with strict adherence to the described procedure.

The method reported can delineate the small differences in level of response necessary for detecting and quantifying aldicarb resistance (Table 2). Based on the data in Table 1, we consider a concentration of 0.5 g ai aldicarb / L should be used as the discriminating-dose for routine resistance monitoring. A dose higher than the theoretical LC99.9 level of the laboratory susceptible strain is necessary because base-line testing during the 1995/96 season showed there were survivors at the usual discriminating-dose of 0.2 g ai aldicarb / L (i.e. LC99.9 level susceptible strain), in strain ME-95/96, and we consider that a likely false positive (Table 2).

Data generated against *T. urticae* are directly comparable to our earlier resistance studies (Herron *et al.*, 1998) and cross-resistance to aldicarb from OP use was not apparent (Table 2). This is a positive finding for the Australian cotton industry, as the current level of aldicarb use for thrips control appears to be a sustainable management tool for *T. urticae* populations, particularly as aldicarb has been used in Australian cotton for several years.

SUMMARY

We suspected organophosphate (OP) resistance in *T. urticae* from cotton in Australia may confer cross-resistance to aldicarb. However, there was no accurate method available to test aldicarb that was compatible with our standard method using a Potter spray tower. We report a laboratory-based method to detect aldicarb resistance in *T. urticae*, using technical grade aldicarb dissolved in acetone, mixed with water and sprayed through a Potter spray tower. The spray tower is housed in an efficient down draft extraction cabinet. The method proved sufficiently sensitive to detect small differences in level of response and was useful for detecting and monitoring aldicarb resistance. Base-line data produced for *T. urticae* indicate a concentration of 0.5 g ai aldicarb / L should be used to discriminate resistance. Cross-resistance to aldicarb from OP resistance in *T. urticae* from cotton was not detected. Aldicarb

has been used for several years in Australian cotton to control thrips which also gives early season control of *T. urticae*. Our data indicate that this practice is currently effective for *T. urticae* control and is likely to remain so unless there is a significant change in use patterns.

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Table 1. Three dose-response replicates for a standard susceptible strain of *Tetranychus urticae* tested against aldicarb.

Rep	n=	•2 (df)	slope (±se)	LC50* (95% F.L.)	LC99.9* (95% F.L.)
1	210	1.4 (2)	3.1 (0.3)	0.017 (0.026 - 0.012)	0.18 (0.56 - 0.059)
2	204	5.3 (2)	3.0 (0.6)	0.019 (0.028 - 0.013)	0.21 (0.75 - 0.060)
3	198	4.3 (2)	3.3 (0.6)	0.023 (0.032 - 0.016)	0.20 (0.71 - 0.058)
1+2+3	612	3.4 (2)	3.1 (0.3)	0.020 (0.024 - 0.016)	0.20 (0.38 - 0.10)

* g aldicarb / L

Table 2. Full dose-response data against aldicarb for field-collected (1994/95 - 1996/97) strains of *Tetranychus urticae* from cotton. Strain specific LC_{50} data for profenofos was interpolated from Herron *et al.* (1998).

Year	strain	n=	•2 (df)	slope (± se)	LC50* (95% F.L.)	RF #	LC99.9* (95% F.L.)	Profenofos RF (LC50 level)
94/95	BD	745	21.8+ (3)	1.9 (0.37)	0.051 (0.065 - 0.040)	2.5	2.15 (5.74 - 0.81)	37
	PM	724	39.9+ (3)	2.1 (0.52)	0.044 (0.054 - 0.035)	2.2	1.40 (3.27 - 0.59)	111
	M	558	7.9+ (2)	3.4 (0.54)	0.030 (0.037 - 0.025)	1.5	0.25 (0.52 - 0.12)	54
	AE	760	39.3+ (3)	1.9 (0.52)	0.045 (0.056 - 0.035)	2.2	1.8 (4.8 - 0.67)	26
	SG	692	8.2 (3)	3.4 (0.38)	0.021 (0.025 - 0.019)	1.0	0.18 (0.29 - 0.11)	87
	GK	561	9.0+ (2)	2.9 (0.49)	0.027 (0.034 - 0.022)	1.3	0.32 (0.76 - 0.14)	49
95/96	TO	603	6.5+ (2)	3.1 (0.39)	0.027 (0.033 - 0.022)	1.3	0.29 (0.60 - 0.14)	29
	TN	594	16.3+ (2)	2.7 (0.64)	0.030 (0.037 - 0.024)	1.5	0.41 (1.05 - 0.16)	17

	GR	571	16.1+ (2)	2.9 (0.64)	0.028 (0.035 - 0.023)	1.4	0.34 (0.81 - 0.14)	15
	ME	729	15.6+ (2)	2.8 (0.40)	0.029 (0.034 - 0.025)	1.4	0.37 (0.61 - 0.22)	24
	WL	747	2.0 (4)	2.9 (0.12)	0.026 (0.029 - 0.023)	1.3	0.30 (0.46 - 0.19)	17
	MV	752	6.1 (3)	3.4 (0.32)	0.023 (0.027 - 0.020)	1.1	0.19 (0.31 - 0.12)	14
	MT	670	30.6+ (3)	2.8 (0.62)	0.019 (0.023 - 0.016)	1.0	0.24 (0.40 - 0.14)	15
96/97	CD	734	7.11 (4)	3.1 (0.26)	0.018 (0.021 - 0.016)	0.9	0.19 (0.27 - 0.13)	108
	AP	723	1.41 (4)	3.1 (0.11)	0.017 (0.020 - 0.015)	0.9	0.18 (0.24 - 0.12)	20
	EL	671	7.90+ (3)	3.2 (0.34)	0.016 (0.019 - 0.014)	0.8	0.16 (0.25 - 0.099)	54
	AE	698	11.3+ (3)	2.6 (0.42)	0.0099 (0.012 - 0.0081)	0.5	0.15 (0.29 - 0.081)	16
	BA	714	9.5+ (3)	2.8 (0.34)	0.012 (0.014 - 0.0099)	0.6	0.16 (0.27 - 0.091)	11

* g ai aldicarb / L

#RF LC50 of field-collected strain / LC50 of susceptible strain

+ •2 values indicate a systematic departure from the probit model (P<0.05).

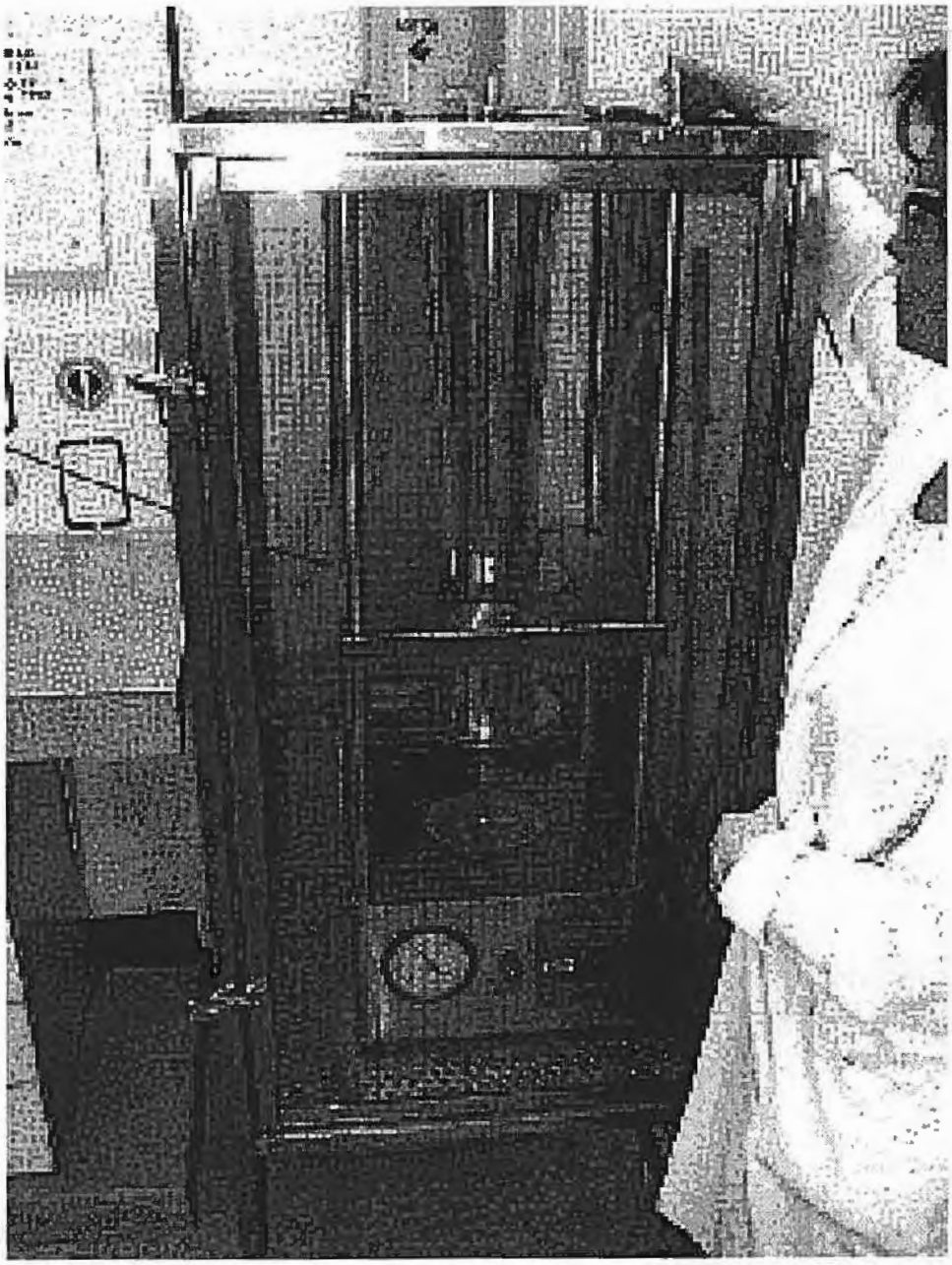

Figure 1. A purpose built pesticide over-spray extraction cabinet housing a Potter spray tower.