Project Title :	Insecticide resistance in cotton aphid
Project Number:	DAN 93C
Research Organisation:	NSW Agriculture
Principal Researcher:	Dr. Grant Herron (phone 02 9843 777)
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SUMMARISED REPORT

Cotton aphid is a global pest of cotton. It is the major aphid pest of cotton in many countries but as yet has not caused widespread problems in Australia. Recently, Australian cotton aphid proved difficult to control with some field control failures being reported. There have also been reports received that cotton aphid caused problems at seasons end in some plots of Bt-cotton, requiring several insecticide sprays. However, at the moment it is not possible to determine if those field control failures are due to resistance, because base-line data for resistance monitoring are not available.

To address that problem the aims of this study were to: obtain base-line data against 12 pesticides and to conduct an initial evaluation of the current resistance status of field-collected cotton aphid strains. To that end laboratory studies were required to establish base-line data for Australian cotton aphid. Susceptible strains of cotton aphid were established and breed in purpose built cages. When sufficient numbers of aphids were available they were assayed against a range of established and potential pesticides to establish base line-data. Once generated, the base-line data was used to screen a number of field-collected strains for resistance.

A total of 24 registered or potential pesticides were evaluated, twice the number initially envisaged. Base-line data was generated to 21 of the pesticides, with three chemicals abandoned due to lack of efficacy. The base-line data was used to detect resistance in three field populations of cotton aphid. Resistance was detected to endosulfan and pyrethroid pesticides but not to carbamates or organophosphates. Two of the three chemicals proved to have modes of action incompatible with the testing procedure. Those chemicals will require further evaluation at a later date requiring intricate and time consuming methods development. Such methods development is beyond the scope of the present study.

A new project should monitor a large number of field-collected strains of cotton aphid to determine the variability in response to key chemicals. Methods to test novel pesticides require development and further work is needed to refine each discriminating-dose to avoid false positive results. Finally, resistance management of cotton aphid needs to be considered under the specific insecticide use requirements of transgenic cotton.

Addendum to summarised report: abstract- Two strains of cotton aphid (*Aphis gossypii* Glover) were collected from an unsprayed source and tested against 24 registered or experimental pesticides by laboratory bioassay. Full log-dosage probit regressions were completed for 21 chemicals and LC99.9s calculated for the least tolerant strain which was used as a discriminating-dose. The discriminating dose was used to monitor and detect resistance in three field populations of cotton aphid. In contrast to overseas studies, discriminating-dose evaluation of the three field strains suggested resistance in Australian cotton aphid was present to endosulfan and pyrethroids but not to carbamates or organophosphates.

INTRODUCTION

Cotton aphid is a worldwide pest of many plant species including cotton (Blackman and Eastop, 1984). It is the main aphid pest of cotton throughout the world causing significant problems in Thailand, The Sudan, USSR and the USA (Schepers, 1989). In Australia, cotton aphid has not yet proved difficult to control.

Overseas studies have found cotton aphid resistant to organophosphate, pyrethroid and carbamate insecticides. Kerns and Gaylor (1992) found organophosphate (80x) and pyrethroid (50x) resistance in cotton aphid from cotton fields in Texas and Alabama. O'Brien *et al.*, (1992) found carbamate and organochlorine resistance in cotton aphid from Mississippi. In Hawaii, where cotton aphid is a major pest of cucurbits, resistance levels to the organophosphate insecticide oxydemeton-methyl were >2,000x (Hollingsworth *et al.*, 1994). Such levels of resistance invariably lead to complete control failure.

The reduction of chemical use and/or changes in the patterns of chemical use associated with the introduction of transgenic cotton may raise the pest status of cotton aphid in Australia. Reports have already been received that cotton aphid caused problems late in the growing season in some transgenic cotton plots. Those plots required several insecticide sprays.

In Australia it has not been proved that reported field control failures of insecticides are due to resistance. Base-line data to detect resistance and determine resistance levels have not been available for cotton aphid. Establishing base-line data for cotton aphid will allow the resistance status of cotton aphid in Australia to be determined.

THE OBJECTIVES

1. To obtain base-line data for approximately twelve pesticides against a susceptible strain(s) of cotton aphid.

2. To conduct an initial evaluation of the current resistance status of field-collected strains of cotton aphid.

MATERIALS & METHODS

Products tested

Products tested included: Orthene, Lorsban, MetaSystox, Rogor, Endosulfan, Confidor, Lannate, Nuvacron, Folimat, Folidol, Pirimor, Curacron, Ekatin, Talstar, Karate, Ekalux, Helthion, Decis Forte, Aztec, CGA-140408, Mitac, Pegasus and Chess . The chemical supplier, common name, chemical group, formulation and formulation concentration are given in Table 1. Not all aphid strains were tested against all chemical products.

Strains tested

Two strains of cotton aphid collected from unsprayed backyards in Sydney were designated "susceptible" and subsequently used to generate base-line data. Four field-collected strains were obtained from cotton growing areas in NSW, one of which subsequently died out before it could be tested. The susceptible stains are designated susceptible A and susceptible B. The surviving field collected strains are designated Neil Forrester, Robert Mensah and Lewis Wilson (after their respective collectors).

Bioassay

Aphid culturing. Aphids were cultured on cotton variety Deltapine 90 which was grown from seed in a pesticide free growth cabinet at 28 °C. Aphids were cultured in a mass culture room maintained at 25±4 °C and under natural light. Aphid strains were isolated in purpose built aphid proof cages (Plate 1).

Bioassay method. The method of testing green cotton aphid is similar to green peach aphids (Herron *et al.*, 1993) except for Petri dish utilisation. For cotton aphid Petri dish diameter is reduced from 85 to 35 mm. Additionally, excised cotton plant leaf discs were placed onto 3 mL of liquid but still cooling agar contained within the Petri dish (Plate 2). When the discs had cooled, batches of aphids were transferred to the Petri dishes and then pesticide sprayed with the aid of a Potter spray tower. Aphids were exposed to a range of serially diluted aqueous insecticide emulsions (log-dose probit assays) or a single dose (discriminating-concentration). Calculated LC99.9 values for the "susceptible" A strain was used as a basis for a discriminating-concentration. Each test included a water only sprayed control. After spraying, Petri dishes were covered with clear cling wrap and perforated with a fine needle to prevent condensation. tests were maintained under conditions of constant light and temperature for 24 hours until

Plate 1. Purpose built aluminium cages used to contain cotton aphid.

[illegible]

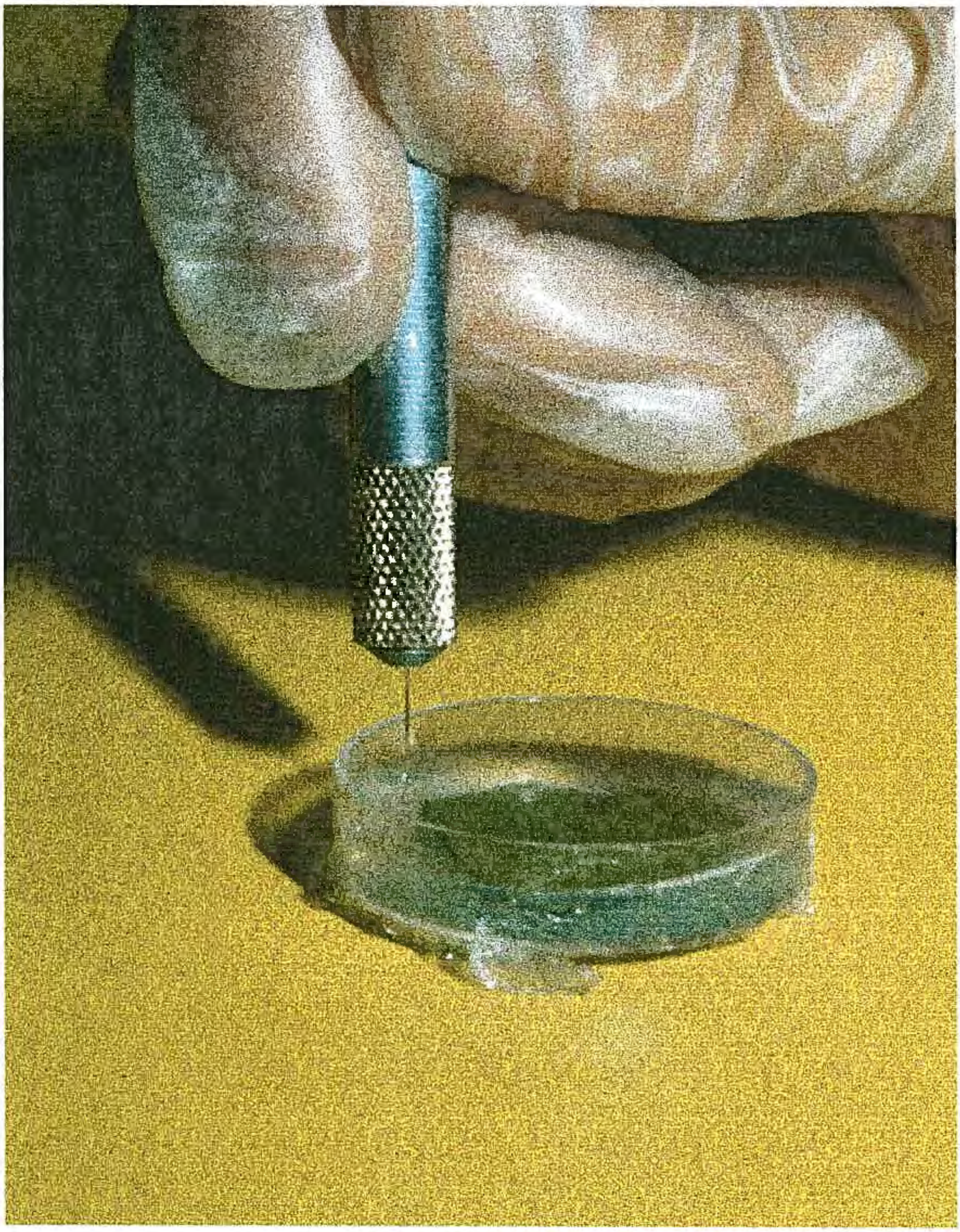
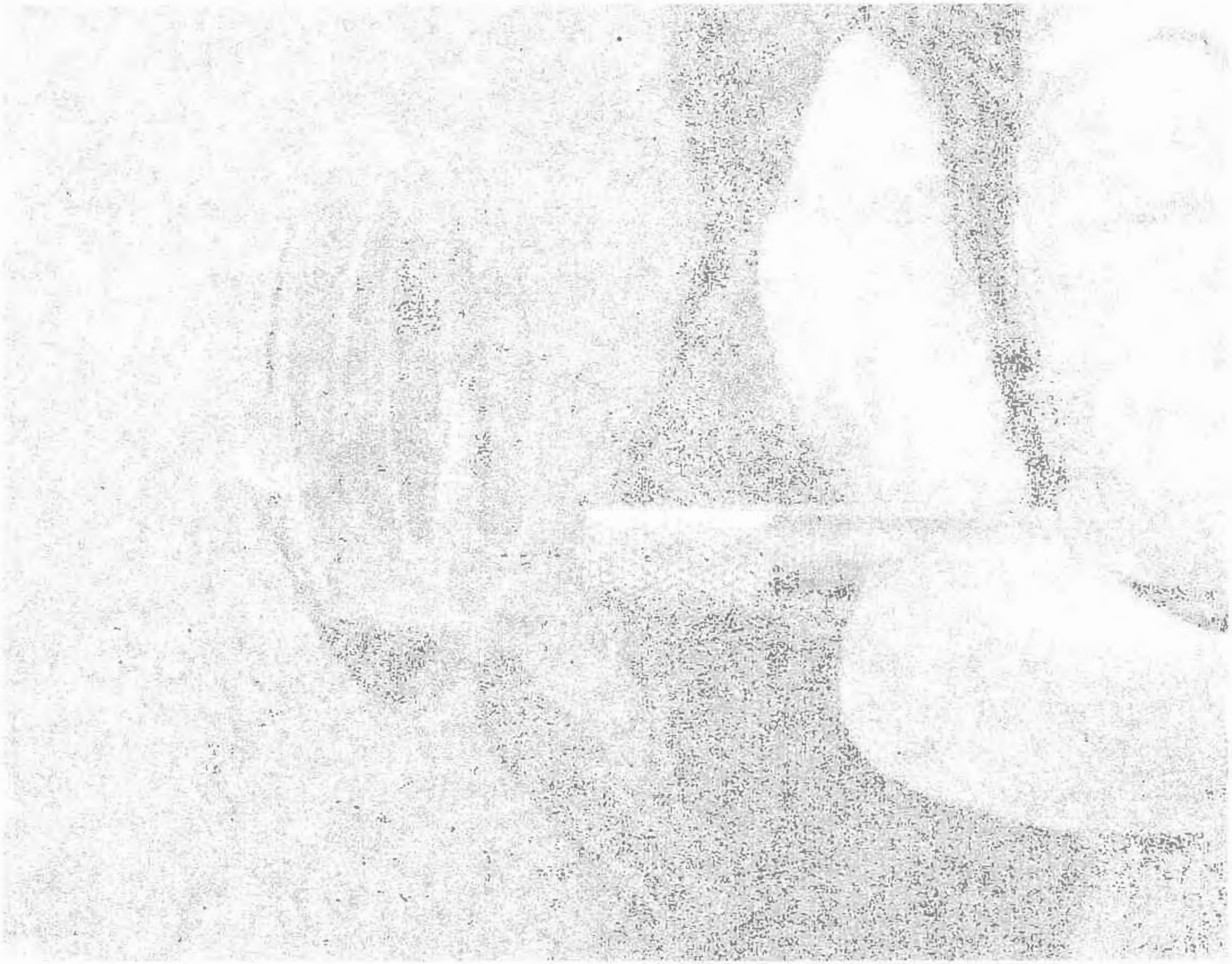


Plate 2. Detail of a Petri dish used in the bioassay of cotton aphid.



mortality was assessed.

Analysis. Probit regressions were computed using Probit 5 for Windows (Gillespie, 1995). Discriminating-concentrations are expressed as percent control corrected mortality (Abbott, 1925).

RESULTS

Twenty chemicals from five known and four chemicals from unknown chemical groups were examined, approximately twelve more than originally anticipated (Table 1). There was little variation in response between susceptible A and B at the LC50 level or the LC99.9 level. Generally differences in response were less than 2x at the LC50 level except Nuvacron which just exceeded 3x (Tables 2 and 3). Response between the strains A and B was slightly more variable at the LC 99.9 level but differences were usually less than 3x, except Pirimor (3.1x), Confidor (3.2)x and Endosulfan (5.6x) (Tables 2 and 3).

Pyrethroids were most active against the susceptible strains with LC 50s as low as 0.0000061 % ai (Tables 2 and 3). Organophosphate performance was variable, with the most efficacious organophosphate, Nuvacron, having LC 50s of 0.000027 and 0.000091 % ai respectively for susceptible strains A and B (Tables 2 and 3). On the other hand the least efficacious organophosphate was Acephate with LC 50s of 0.0025 and 0.0019 % ai respectively for strains A and B. The organochlorine endosulfan was not particularly efficacious (Tables 2 and 3). Of the newer chemicals, Confidor performed best, with LC 50s less than 0.00003 % ai for both susceptible strains. Aztec and CGA-140408 were about one order of magnitude less efficacious than Confidor (Tables 2 and 3) with similar efficacy to the carbamates Lannate and Pirimor.

There is prima-facia evidence of pesticide resistance in Australian cotton aphid (Table 4). Australian cotton aphid appear resistant to endosulfan and pyrethroids but susceptible to organophosphates and carbamates.

DISCUSSION

The data provide a good basis for the continuing study of cotton aphid in Australian cotton. There is now strong evidence of pyrethroid and endosulfan resistance in Australian cotton aphid but more work is needed to determine the range of resistance levels. The discriminating-dose (Table 4)used was based on the response of susceptible A (Table 2) which was the more susceptible of the two field-collected strains. In some instances the discriminating-dose based on susceptible A may be too low leading to false positive results. More research is needed to document the range of tolerance of cotton aphids against the chemicals tested to lessen the chance of false positives.

O'Brien *et al*., (1992) states that cotton aphid from the mid-south USA went from just organophosphate resistant to a broader resistance to several classes of insecticide. In contrast Australian cotton aphid is resistant to other classes of insecticide and susceptible to organophoshates. Kerns and Gaylor (1992) found moderate level Curacron (8.5x) and low Rogor (2.5x) resistance plus very high level Monitor (85x) resistance. They also found 22x resistance to Talstar which is approximately the same as the 19x found in strain Neil Forrester (Table 4). Clearly the resistance situation in Australia is different from overseas. However, overseas experience suggests that if cotton aphid is specifically targeted for control with organophoshates resistance will develop.

Research should now concentrate on monitoring a large number of field-collected strains from a range of cotton growing areas to determine the variability in response to key chemicals. Specifically, the variability in response of pyrethroids and endosulfan to cotton aphid requires quantification and, based on overseas experience organophosphate and carbamate resistance requires careful monitoring. Finally, resistance management of cotton aphid needs to be considered under the specific insecticide use requirements of transgenic cotton. If not, overseas experience clearly indicates that overuse of organophosphate or carbamate aphicides in cotton will lead to resistance and control failure.

Additional effort is also required to evaluate the newer experimental compounds under laboratory conditions. In this study both Pegasus and Chess were tested and gave very poor results. Subsequent discussions with the supplier elucidated the likely cause, which was likely due to the novel action of both

chemicals. Chess was an antifeedant so the lethal effects of the compound were not visible within the 24 hours. Pegasus is a light activated and the fluorescent lights in the laboratory could not activate the chemical. Unfortunately, extra methods development for individual chemicals was outside the scope of this study, but is essential in future work.

RECOMMENDATIONS

The study should be continued and:

1. Monitor a large number of field-collected strains from a range of cotton growing areas to determine the variability in response to key chemicals.

2. Consider resistance management of cotton aphid under the specific insecticide use requirements of transgenic cotton.

3. Develop methods to test novel pesticides.

4. Further refine each discriminating-dose to avoid false positive results by studying more susceptible strains.

ACKNOWLEDGEMENTS

We gratefully acknowledge the CRDC funding this study (DAN 93C). I thank all the persons involved in forwarding field-collected strains for testing. I wish to acknowledge Mr Kevin Powis for his technical assistance. Finally, I am indebted to Mrs Jeanette Rophail for her unsupervised methods development and for her day to day supervision of Mr Powis.

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Table 1. Supplier, trade name, common name, chemical group, formulation# and concentration for 24 pesticides tested against cotton aphid.

Supplier	Trade Name	Common Name	Chemical Group	Form.	Conc.
Agrevo	Orthene	acephate	organophosphate	SP	750 g/Kg
Dowlanco	Lorsban	chlorpyrifos	organophosphate	EC	500 g/L
Bayer	MetaSystox	demeton-S-methyl	organophosphate	EC	250 g/L
Rhone-Poulenc	Rogor	dimethoate	organophosphate	EC	400 g/L
Crop Care	Endosulfan	endosulfan	organochlorine	EC	350 g/L
Bayer	Confidor	imidacloprid	nitroguanidine	SC	350 g/L
Crop Care	Lannate	methomyl	carbamate	LC	225 g/L
Ciba-Geigy	Nuvacron	monocrotophos	organophosphate	AC	400 g/L
Bayer	Folimat	omethoate	organophosphate	LC	800 g/L
Bayer	Folidol	parathion-methyl	organophosphate	EC	500 g/L
Crop Care	Pirimor	pirimicarb	carbamate	WP	500 g/Kg
Ciba-Geigy	Curacron	profenofos	organophosphate	EC	500 g/L
Sandoz	Ekatin	thiometon	organophosphate	EC	245 g/L
Crop Care	Talstar	bifenthrin	pyrethroid	EC	100 g/L
Crop Care	Karate	lambdacyhalothrin	pyrethroid	EC	50 g/L
Sandoz	Ekalux	quinalphos	organophosphate	EC	223 g/L
Bayer	Helothion	sulprofos	organophosphate	EC	720 g/L
Cyanamid	Hallmark	esfenvalerate	pyrethroid	EC	50 g/L
Agrevo	Decis Forte	deltamethrin	pyrethroid	EC	27.5 g/L
Cyanamid	Aztec	triazamate	unknown	WDG	112 g/Kg
Ciba-Geigy	CGA-140408	unknown	unknown	EC	250 g/L
Agrevo	Mitac*	amitraz	amidine	EC	200 g/L
Ciba-Geigy	Pegasus*	diafenthiuron	unknown	EC	500 g/L
Ciba-Geigy	Chess*	pymetrozine	unknown	WP	250 g/Kg

*Testing not successful- refer to text.

AC=aqueous concentrate, EC=emulsifiable concentrate, LC=liquid concentrate, SC=suspension concentrate, SP=soluble powder, WDG=water dispersable granule and WP=wettable powder.

Table 2. Dose-response data for cotton aphid susceptible strain A tested against a range of experimental and registered pesticides.

Chemical	n=	Chi-square	d.f	Slope (±s.e.)	LC50 (% ai) (95% F.L.)	LC 99.9 (% ai)
Orthene	282	3.2	3	2.5 (0.31)	0.0025 (0.0034-0.0019)	0.045 (0.15-0.0046)
Lorsban	176	0.1	2	4.6 (0.089)	0.00055 (0.00072-0.00042)	0.0026 (0.0062-0.0011)
MetaSystox	207	1.8	2	3.5 (0.38)	0.00018 (0.00025-0.00013)	0.0014 (0.0044-0.00048)
Rogor	177	3.8	2	2.4 (0.59)	0.00027 (0.00042-0.00017)	0.0051 (0.053-0.00048)
Endosulfan	211	0.8	2	4.0 (0.29)	0.0025 (0.0033-0.0019)	0.015 (0.039-0.0058)
Confidor	240	4.2	3	2.0 (0.30)	0.000013 (0.000018-0.0000090)	0.00040 (0.0016-0.00010)
Lannate	268	1.2	3	2.7 (0.17)	0.00025 (0.00032-0.00019)	0.0035 (0.0087-0.0020)
Nuvacron	386	2.2	3	2.4 (0.18)	0.000027 (0.000034-0.000021)	0.00048 (0.0011-0.00021)
Folimat	223	0.5	3	2.5 (0.13)	0.000070 (0.000094-0.000052)	0.0011 (0.0054-0.00037)
Folidol	198	0.7	2	2.6 (0.19)	0.00035 (0.00054-0.00023)	0.0057 (0.033-0.00096)
Pirimor	208	5.6	2	3.4 (0.63)	0.00011 (0.00016-0.000083)	0.00091 (0.0027-0.00030)
Curacron	197	3.9	2	3.9 (0.64)	0.00036 (0.00050-0.00027)	0.0022 (0.0063-0.00078)
Ekatin	219	0.4	2	3.1 (0.16)	0.00038 (0.00053-0.00028)	0.0037 (0.012-0.0015)
Talstar	157	0.5	1	4.0 (0.43)	0.0000089 (0.000026-0.0000031)	0.000053 (0.0033-0.00000083)
Karate	154	0.4	1	4.3 (0.37)	0.0000061 (0.000014-0.0000026)	0.000032 (0.00068-0.0000014)
Ekalux	161	0.8	1	5.4 (0.65)	0.00017 (0.00036-0.000082)	0.00064 (0.0075-0.000055)
Helothion	199	1.6	2	3.9 (0.38)	0.00073 (0.00098-0.00054)	0.0045 (0.019-0.0023)
Hallmark	200	1.2	2	2.6 (0.26)	0.000012 (0.000018-0.0000082)	0.00018 (0.00094-0.000035)

Decis Forte	189	2.4	2	2.9 (0.40)	0.000024 (0.000034-0.000016)	0.00026 (0.0011-0.000064)
Aztec	257	3.7	3	2.7 (0.31)	0.00016 (0.00022-0.00012)	0.0022 (0.0050-0.00097)
CGA-140408	222	1.9	2	3.3 (0.39)	0.00017 (0.00024-0.00012)	0.0015 (0.0054-0.00041)

For all regressions the Chi-squared goodness of fit test indicated a reliable fit to the probit model at the 5% level of significance.

Table 3. Dose-response data for cotton aphid susceptible strain B tested against a range of experimental and registered pesticides.

Chemical	n=	Chi-square	d.f. n-2	Slope (±s.e.)	LC50 (% ai) (95% F.L.)	LC 99.9 (% ai) (95% F.L.)
Orthene	248	1.7	3	3.0 (0.24)	0.0019 (0.0024-0.0015)	0.020 (0.094-0.012)
Lorsban	141	0.3	1	3.0 (0.29)	0.00044 (0.0020-0.000035)	0.0048 (4.66-0.0000050)
MetaSystox	201	2.5	2	3.6 (0.48)	0.00027 (0.00037-0.00020)	0.0019 (0.0051-0.00069)
Rogor	195	3.4	2	2.8 (0.54)	0.00029 (0.00042-0.00019)	0.0036 (0.020-0.00078)
Endosulfan	201	1.0	2	2.2 (0.23)	0.0032 (0.0055-0.0019)	0.085 (0.99-0.0073)
Confidor	241	1.6	3	1.8 (0.17)	0.000028 (0.000040-0.000019)	0.0013 (0.0066-0.00025)
Lannate	201	0.1	2	2.5 (0.07)	0.00053 (0.00080-0.00035)	0.0087 (0.050-0.0015)
Nuvacron	195	1.4	2	2.7 (0.27)	0.000091 (0.00013-0.000062)	0.0013 (0.0055-0.00029)
Folimat	189	0.2	2	2.6 (0.12)	0.00011 (0.00017-0.000075)	0.0016 (0.0087-0.00031)
Folidol	192	2.1	2	4.4 (0.51)	0.00025 (0.00033-0.00019)	0.0013 (0.0029-0.00056)
Pirimor	192	0.2	2	2.2 (0.10)	0.00011 (0.00018-0.000072)	0.0028 (0.024-0.00033)
Curacron	153	5.3*	1	4.9 (1.50)	0.00035 (0.00081-0.00015)	0.0015 (0.025-0.000089)
Ekatin	201	1.6	2	2.8 (0.31)	0.00051 (0.00075-0.00035)	0.0066 (0.031-0.0014)
Talstar	159	3.1	1	3.1 (0.88)	0.000012 (0.000033-0.0000041)	0.0001 (0.014-0.00000092)
Karate	152	0.6	1	3.5 (0.41)	0.0000064 (0.000017-0.0000024)	0.000050 (0.0036-0.00000068)
Ekalux	209	0.8	2	4.3 (0.29)	0.00025 (0.00033-0.00019)	0.0013 (0.0030-0.00056)
Helothion	207	1.6	2	5.2 (0.55)	0.0011 (0.0014-0.00081)	0.0041 (0.0087-0.0019)
Hallmark	206	0.2	2	2.9 (0.11)	0.000012 (0.000017-0.0000082)	0.00014 (0.00055-0.000034)
Decis Forte	208	0.9	2	3.5 (0.28)	0.000031 (0.000042-0.000023)	0.00023 (0.00068-0.000079)

Aztec	266	8.4*	3	2.3 (0.46)	0.00016 (0.00022-0.00011)	0.0034 (0.0098-0.0012)
CGA-140408	213	4.4	2	2.6 (0.49)	0.00016 (0.00026-0.00010)	0.0025 (0.015-0.00042)

For all regressions, except those marked with *, the Chi-squared goodness of fit test indicated a reliable fit to the probit model at the 5% level of significance.

Table 4. Percent mortality at the discriminating dose with resistance factors in brackets (if known) for four field collected strains of cotton aphid.

Chemical	DD % ai	Strain Neil Forrester	Strain Robert Mensah	Strain Lewis Wilson	Prima-facia Resistance
Orthene	0.045	*	100	100	no
Lorsban	0.0025	100 (RF 0.6x)	100	100	no
MetaSystox	0.0015	*	100	100	no
Rogor	0.0050	100	100	100	no
Endosulfan	0.015	73 (RF 3.5x)	46	20	yes
Confidor	0.00040	*	100	100	no
Lannate	0.0035	*	100	100	no
Nuvacron	0.00050	100	100	100	no
Folimat	0.0010	100	100	100	no
Folidol	0.0060	*	100	100	no
Pirimor	0.0010	100 (RF 1.9x)	100	100	no
Curacron	0.0020	100	100	100	no
Ekatin	0.0035	*	100	100	no
Talstar	0.000050	11 (RF 19x)	100	10	yes
Karate	0.000030	0 (RF 28x)	*	*	yes
Ekalux	*	*	*	*	*
Helothion	*	*	*	*	*
Hallmark	*	*	*	*	*
Decis Forte	0.00026	11	*	*	yes
Aztec	*	*	*	*	*
CGA-140408	*	* (RF 0.7x)	*	*	no

COMMUNICATION OF RESULTS

Herron, G.A., Rophail, J. and Powis, K. (1996) Insecticide resistance in cotton aphid. *In*: Proceedings of the 8th Australian Cotton Conference, Hotel Conrad and Jupiters Casino, Broad Beach, Gold Coast, August 13-16, 1996. (in press).

APPENDIX

REQUESTED BUDGET 1995-96

ITEM	**March-June 95**	**1995-96**
	Original Estimates	
A. STAFFING		
Technical Officer (Sci.) (Grade 1, Year 5) 3 days per week.	$5,446.34	$16,339.19
Payroll tax 7.0%	$381.24	$1,143.74
Workers Compensation 2.5%	$136.15	$408.47
Leave Loading 1.5%	$81.69	$245.08
Superannuation 6.0%	$326.68	$980.35
TOTAL STAFFING	**$6,372.11**	**$19,116.85**
B. TRAVEL		
TOTAL TRAVEL		
C. OPERATING		
Q-Fleet: Holden Commodore wagon at 28c km	$56	$140
Maintenance: Gilson pipettes and growth cabinets.	$250	$750
Consumables: Pipette tips, acetone, petri dishes, agar, plastic film etc.	$375	$1125
Cages: Five cages to contain and isolate strains of cotton aphid.	$2,500	
3% library services support	$95.43	$60.45
TOTAL OPERATING	**$3,276.43**	**$2,075.45**
D. ASSETS		
TOTAL ASSETS		
TOTAL REQUESTED	**$9,648.54**	**$21,192.30**