Cotton *Research and Development Corporation*

FINAL REPORT

"Insecticide resistance management in *Bemisia tabaci*"

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INSECTICIDE RESISTANCE AND RESISTANCE MANAGEMENT IN B-BIOTYPE *BEMISIA TABACI*

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SUMMARY

a) BACKGROUND OF THE PROJECT

The cotton whitefly *Bemisia tabaci* is a serious pest of fibre, horticultural and ornamental crops world wide. When present in sufficient numbers, it can cause extensive damage through direct feeding, the production of large quantities of honeydew and as a vector of many viruses. Australia has a benign native strain of *Bemisia tabaci.* but recently, a new biotype was identified by Robin Gunning, known as the B-type or poinsettia strain. Overseas, B-type *B, tabaci* is a primary pest on cotton, other vegetable crops (curcubits, tomatoes, rock melons) and ornamentals. This strain is extremely virulent, highly insecticide resistant, adapts to temperate climates and has a host range of over 500 plants. A nation-wide survey has now shown that this whitefly is widely distributed over eastern Queensland and NSW and the Darwin area of the NT (Fig 1).

The spread of this whitefly is expected to result in it becoming a major pest in Australia and a primary pest of cotton. B-biotype *B. tabaci* will effect field crops (such as cotton, maize, lucerne and sunflowers); field grown vegetables (curcubits, cole crops, melons, tomatoes); glasshouse vegetables; fruit crops (grapes) and glasshouse ornamentals (poinsettias, hibiscus, geberas and gloxinia).

Insecticides will be the primary weapon against this insect in Australia. B -type *B. tabaci* are resistant to many insecticides overseas and this insect may be very difficult to control, However, there are new insecticides to which this whitefly may still be susceptible. It is essential that the use of all chemicals is carefully managed to minimise or avoid resistance problems. This can only be achieved by establishing effective resistance detection and monitoring techniques and understanding the underlying mechanisms of resistance.

b) PROJECT OBJECTIVES:

- To use standard bioassay techniques to establish the particular resistance / susceptibility profiles of Australian native and B-biotype *B. tabaci.* Insecticide tested, will be organophosphates, carbamates, pyrethroids, newer pyrethroids, cyclodienes, novel control agents.

- Evaluation the mechanisms of resistance to organophosphates, carbamates and pyrethroids in B-biotype *B. tabaci.*

- Assess the applicability of rapid biochemical resistance monitoring techniques to *B. tabaci.*

- To investigate the relationship between B-biotype characteristics (silverleaf induction and B esterase bands) and insecticide resistance in B-biotype *B. tabaci*, will be investigated.

- Assessment of the potential of B- type *B. tabaci* to interbreed with and thus spread insecticide resistance into native *B. tabaci.*

- To investigate the relationship between "resistance status" and field control of resistant *B. tabaci* .

- Resistance management strategies will be devised and tested in the field and the laboratory.

The objectives of this project have largely been achieved or will shortly be accomplished. This is in spite of difficulties experienced because of a greatly increased workload associated with the *Helicoverpa* resistance monitoring programme.

c) RESULTS

(i) Distribution of B-biotype *B. tabaci* on cotton

Whiteflies were found on cotton in all principal cotton growing areas most NSW and Queensland and it is obvious that numbers of B-biotype *B. tabaci* on cotton in Australia is increasing, season by season. By 1998/99, approximately the number of whiteflies recovered, had increased approximately 15 times. Emerald was identified as an area of particular concern.

(ii) Insecticide bioassays

Bioassays confirm that B-type *B. tabaci* entered Australia with resistance to most pyrethroids (permethrin, cypermethrin, deltamethrin and es-fenvalerate), organophosphates (profenofos, methyl parathion, methamidphos, fenthion, sulprofos, and dimethoate) and carbamates (methomyl , methiocarb). B-type *B. tabaci* were, however, initially susceptible, or virtually susceptible, to endosulfan, bifenthrin, imidacloprid and amitraz but field use of these products on horticultural crops in Queensland has resulted in resistance selection. In particular, high levels of resistance to endosulfan, imidacloprid, and bifenthrin have been recently recorded in Queensland field populations. Native, non-B type *B. tabaci* are susceptible to all insecticides tested.

(iii) Field selection of insecticide resistance

Results show a very rapid build up of resistance, in response to selection at Ayr and Bowen.. Resistance levels in unsprayed populations were low, however, application of bifenthrin and imidacloprid dramatically increased the resistance factor to 714 and 205 fold respectively (and 6 commercial applications of imidacloprid on Okra by a farmer) increased the resistance factor to over 500 fold). Amitraz selection increased the resistance factor from 2 to 22 fold.

iv) Organophosphate and carbamate resistance mechanisms

Studies of organophospbate resistance in B-type *B. tabaci* has shown that resistance is effectively monogenic, conferred by a single insensitive target site acetylcholinesterase (AChE), with differing levels of insensitivity to organophosphates tested. This resistant AChE is diagnosed by insensitivity to paraoxon or methyl paraoxon.

(v) Pyrethroid resistance mechanisms

Pyrethroid resistance in B-biotype *B. tabaci,* is largely to be as a result of metabolism by the B band esterase isoenzyme which may sequester and hydrolyse pyrethroids. B-type *B. tabaci* are resistant to most pyrethroids in Australia.

(vi) Bifenthrin resistance mechanism

In Australia, B-biotype *B. tabaci* were initially susceptible to bifenthrin, however, field use of bifenthrin has resulted in the selection of resistance. Bifenthrin resistant, B-biotype *B. tabaci* have developed a novel form of B band esterase, which binds more readily to bifenthrin than the susceptible form of the enzyme.

(vii) Interbreeding of native non-B type *B. tabaci* and B-biotype *B. tabaci*

While these experiments are as yet, incomplete, interesting data have been obtained. It is obvious that B-biotype *B. tabaci* readily interbreed with native non-B type *B.tabaci*. We are investigating whether insecticide resistance can be spread into the populations of native *B. tabaci* .

(ix) Insect growth regulators and juvenile hormone analogues

Insect growth regulators and juvenile hormone analogues, have been used successfully overseas against B-biotype *B. tabaci.* These insecticides act by disrupting the insect moulting process and were initially considered to be almost resistance proof. While B-biotype *B. tabaci* were initially susceptible to buprofezin and fenoxycarb in Australia, there has been no difficulty in the laboratory selection of resistant strains, within 2 generations. Buprofezin resistant B-biotype *B. tabaci* have extra esterase isoenzymes, (not occurring in susceptible insects)., which appear to bind to and metabolise buprofezin .

2. BACKGROUND OF THE PROJECT

The cotton whitefly *Bemisia tabaci* is a serious pest of fibre, horticultural and ornamental crops world wide. When present in sufficient numbers, it can cause extensive damage through direct feeding, the production of large quantities of honeydew and as a vector of many viruses. Australia has a benign native strain of *Bemisia tabaci*. but recently, a new biotype was identified by Robin Gunning, known as the B-type or poinsettia strain. Overseas, B-type *B, tabaci* is a primary pest on cotton, other vegetable crops (curcubits, tomatoes, rock melons) and ornamentals. This strain is extremely virulent, highly insecticide resistant, adapts to temperate climates and has a host range of over 500 plants. A nation-wide survey has now shown that this whitefly is widely distributed over eastern Queensland and NSW and the Darwin area of the NT (Fig 1).

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3. PROJECT OBJECTIVES:

- To use standard bioassay techniques to establish the particular resistance / susceptibility profiles of Australian native and B-biotype *B. tabaci*. Insecticide tested, will be organophosphates, carbamates, pyrethroids, newer pyrethroids, cyclodienes, novel control agents.

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- To investigate the relationship between "resistance status" and field control of resistant *B. tabaci*

- Resistance management strategies will be devised and tested in the field and the laboratory.

The objectives of this project have largely been achieved or will shortly be accomplished. This is in spite of difficulties experienced because of a greatly increased workload associated with the *Helicoverpa* resistance monitoring programme.

4. METHODOLOGY

The methods employed in this project are appropriate for this type of study and are described in detail in the individual sections of this report.

5. RESULTS

(a) Distribution of B-biotype *B. tabaci* on cotton

Introduction

As an adjunct to the *Helicoverpa* resistance monitoring program, cotton crops in Queensland and NSW were also surveyed for whiteflies, 1996 - 1999. Immature or adult whiteflies on cotton leaves sent to Tamworth were removed and identified by polyacrylamide gel electrophoresis. The naphthyl esterase banding patterns in individual female, adults whiteflies were characterised after separation of the esterases on 7.5% polyacrylamide gels Following staining with 1-naphthyl butyrate, bands were identified by measuring their electrophoretic mobility relative to the buffer front on the gel. B-biotype *B. tabaci* are indicated by the distinctive esterase at 0.14 Rm (designated as $E_{0.14}$).

Results
Numbers of B-biotype *B. tabaci* found on cotton are plotted in Fig 2.. These whiteflies were found on cotton in all principal cotton growing areas most NSW and Queensland and it is obvious that numbers of B-biotype *B. tabaci* on cotton in Australia is increasing, season by season. By 1998/99, approximately the number of whiteflies recovered, had increased approximately 15 times. Emerald was identified as an area of particular concern.

Discussion
During the same period, B-type *B. tabaci* has had explosive population increases in horticultural crops (Ayr, Bowen, Bundaberg and Tweed heads) and has assumed major pest status there.
While the conditions under which populations of this whitefly forms rapidly increase are not well understood, overseas experience shows that after invasion of a new habitat, the whitefly appears to gradually increase in numbers for several years before a population explosion.

(b) Insecticide Bioassay

Introduction and methods
Insects used for bioassay were B-biotype *B. tabaci* from varying locations in NSW, Queensland and the Darwin,NT. An insecticide susceptible strain of native non-B type *B. tabaci* was established from whiteflies collected on weeds near Darwin NT.

Leaf dip bioassay methods used were similar to those used by the Insecticide Resistance group at IACR-Rothamsted in the UK. We chose to use the Rothamsted methods because it was important that our data was comparable with that obtained by the major whitefly insecticide resistance research centre overseas. Pyrethroids, organophosphates , carbamates , endosulfan and imidacloprid were bioassayed against adults as contact insecticides. Insect growth regulators were bioassayed on leaves using immature whiteflies.

Formulated insecticides were used in bioassays. Dics were cut from cotton leaves and dipped into serial dilutions (in distilled water), of formulated product and the discs were placed into a thin layer of Agar gel , inside a plastic petri dish of a similar diameter. Approximately 20 female whitefly were then confined on each disc with controls confined to leaves dipped in distilled water only. All bioassays were done at 25 ^{o}C, using three replicates with at least 5 concentrations per test. Mortality was assessed at 48 h and results analysed by Probit Analysis.

Results and discussion
B-biotype *B. tabaci* were bioassayed against a range of contact insecticides, however, because of difficulties in obtaining and culturing native, non-B type *B. tabaci* some data for this strain has yet to be obtained. In addition, bioassays of insect growth regulators and juvenile hormone analogues, which form part of Emma Cottage's CRDC funded Ph.D. studies, are also incomplete.

Bioassays so far (Table 1), confirm that B-type *B. tabaci* entered Australia with resistance to most pyrethroids (permethrin, cypermethrin, deltamethrin and es-fenvalerate), organophosphates (profenofos, methyl parathion, methamidphos, fenthion, sulprofos, and dimethoate) and carbamates (methomyl , methiocarb). B-type *B. tabaci* were, however, initially susceptible, or virtually susceptible, to endosulfan, bifenthrin, imidacloprid and amitraz but field use of these products on horticultural crops in Queensland has resulted in resistance selection. In particular, high levels of resistance to endosulfan, imidacloprid, and bifenthrin have been recently recorded in Queensland field populations. Native, non-B type *B. tabaci* are susceptible to all insecticides tested.

Bioassays (endosulfan, methomyl. methamidiphos, bifenthrin, es-fenvalerate, imidacloprid), have failed to find any differences in the resistance profiles of B-type *B. tabaci* which originated from

Table 1

Response of adult B-type and non B-type *Bemisia tabaci* to insecticides. Leaf dip bioassays.

Insecticide	type	location	selection status	Slope	LD_{50} (fiducial limits) (pmm)	Chi square	Bioassay date	RF
endosulfan	B	Qld	nil	3.1	1.2 (0.9 - 1.6)	2.4	3/96	1
	B	NSW	nil	3.2	1.1 (0.8 - 1.50	2.4	4/96	1
	B	NT	nil	3.1	1.2 (0.9 - 1.5)	2.2	3/96	1
	B	Ayr	field sel.	1.2	19 (13 - 29)	4.7	6/97	14.6
	B	Bundaberg	field sel.	0.76	500 (200 - 1200)	10.3	4/99	385
	non -B	NT	nil	3.3	4.3 (3.5 - 5.4)	2.6	4/99	-
triazimate	B	Qld	nil	1.1	188 (120 - 300)	5.2	3/96	
fenproximate	B	Qld	nil	1.5	58 (39 - 84)	2.3	3.96	
methomyl	B	Qld	nil	0.9	245 (130 - 450)	7.9	3/96	
	B	NSW	nil	1.2	256 (145 - 458)	8.2	4/96	
	B	NT	nil	1.3	220 (130 - 405)	7.5	4/96	
	B	Ayr	field sel	1.5	> 2500	4.9	10/97	
flurofenoxuron	B	Qld	nil	0.3	>> 1000	3.2	5/96	
diafenthiuron	B	Qld	nil	1.5	36 (25 - 52)	11.7	6/96	
amitraz	B	Qld	nil	2.4	65 (48 - 85)	3.7	3/96	3.2
	B	Ayr	field sel	4.9	300 (290 - 590)	1.3	4/99	15
	non-B	NT	nil	4.4	20 (17 - 25)	5.7	4/99	-
profenofos	B	Qld	nil	1.2	>5000		6/96	>6000
	non-B	NT	nil	1.7	0.83 (0.6 - 12.0)	6.9	5/99	
methiocarb	B	Qld	nil	1.2	248 (170 - 312)	4.1	6/96	388
	non-B	NT	nil	2.1	0.064 (0.052 - 0.0780	8.5	5/99	-
methamidiphos	B	Qld	nil	1.5	102 (89 - 125)	4.6	6/96	
	B	NSW	nil	1.8	112 (93 - 134)	3.8	6/96	
	B	NT	nil	1.6	106	4.4	6/96	
fenthion	B	Qld	nil	1.4	150 (128 - 179)	4.8		
sulprofos	B	Qld	nil	1.3	33 (22 - 51)	5.8	3/96	
dimethoate	B	Qld	nil	1.4	140 (60 - 200)	7.5	3/96	

bifenthrin	B	Qld	nil	1.9	1.7 (1.2 - 2.4)	8.5	5/96	20
	B	NSW	nil	1.9	1.9 (1.4 - 2.5)	4.8	7/96	23
	B	NT	nil	2.2	1.1 (0.90 - 2.1)	3.3	7/96	13
	B	Bundaberg	field sel	1.3	2.6 (1.2 - 3.8)	6.4	4/99	31
	B	Ayr	nil	2.5	0.093 (0.064 - 0.13)	2.1	4/99	1
	B	Ayr	field sel	1.3	15 (11 - 19)	7.5	4/99	179
	non-B	NT	nil	3.5	0.084 (0.072 - 0.099)	10.2	4/99	-
permethrin	B	Qld	nil	1.4	25 (10 - 400	3.3	3/99	57
	non-B	NT	nil	4.4	0.44 (0.38 - 0.51)	9.1	5/99	-
cypermethrin	B	Qld	nil	1.0	70 (40 - 120)	8.9	6/96	
deltamethrin	B	Ayr	nil	1.2	8.2 (2.7 - 240)	9.4	5/99	63
	non-B	NT	nil	2.7	0.13 (0.11 - 0.17)	1.6	5/99	-
es-fenvalerate	B	Qld	nil	1.4	1.2 (0.8 - 2.0)	3.6	3/96	9.2
	B	NSW	nil	1.4	1.3 (0.7 - 2.1)	3.8	4/96	10
	B	NT	nil	1.5	1.4 (0.9 - 1.9)	3.3	4/99	11
	B	Ayr	field sel.	1.2	8.2 (2.7 - 24)	9.4	5/99	63
	non-B	NT	nil	2.7	0.13 (0.11 - 0.17)	1.6	5/99	-
imidacloprid	B	Qld	nil	2.9	23 (15 - 35)	3.8	6/96	1.2
	B	NSW	nil	2.8	21 (17 - 26)	3.6	7/96	1.1
	B	NT	nil	3.0	20 (17 - 28)	3.9	7/99	1.0
	B	Ayr	nil	1.42	120 (90 - 170)	4.2	4/99	6.0
	B	Ayr	field sel.	0.94	1200 (900 - 1700)	12.1	4/99	60
	non-B	NT	nil	2.6	20 (17 - 29)	3.9	4/99	-

NT (Darwin), Queensland(Cairns) or NSW (Tamworth). These data indicate that a single introduction of B-type *B. tabaci* to Australia most probably occurred.

(c) Field selection of insecticide resistance.

Introduction and methods

The field selection of insecticide resistance was studied in collaboration with Mr John Brown, (DPI, Ayr). Field populations of B-type *B. tabaci* on Okra, were sprayed with two applications (7 days apart), of commercial rates of bifenthrin, amitraz, and imidacloprid. Treated leaves and unsprayed control leaves, carrying immature whiteflies, were transported to Tamworth and adult whiteflies bioassayed on emergence. Other insecticides (buprofezin, acetamprid and pymetrozine,) were also used in this trial, but we were unable to obtain sufficient numbers of survivors for bioassay and this work is continuing.

Results

Insecticide resistance data are shown in Table 2. Results show a very rapid build up of resistance, in response to selection. Resistance levels in unsprayed populations were low, however, application of bifenthrin and imidacloprid dramatically increased the resistance factor to 714 and 205 fold respectively (and 6 commercial application of imidacloprid on Okra by a farmer) increased the resistance factor to over 500 fold). Amitraz selection increased the resistance factor from 2 to 22 fold.

Table 2

The selection of insecticide resistance in B-type *B. tabaci* in the field (Ayr). Field populations of *B. tabaci* were exposed to 2 commercial rate sprays of bifenthrin or amitraz and 2 or 6 commercial rate sprays of imidacloprid

Insecticide	Strain	Resistance Factor[a]
bifenthrin	unsprayed control	7
	bifenthrin sprayed (2)	714
amitraz	unsprayed control	2
	amitraz sprayed (2)	22
imidacloprid	unsprayed control	10
	imidacloprid sprayed(2)	205
	imidacloprid sprayed(6)	~ 500

[a] Resistance factor calculated as the ratio of LD99 [R]/LD99 [S]

Discussion
These data demonstrate the extreme facility of B-type *B. tabaci* to develop insecticide resistance in the face of quite moderate insecticide selection pressure. Field studies with DPI entomologists at Ayr and Bundaberg are still continuing, however, it is obvious that the only insecticide resistance management tactics that could be used against this whitefly is a rotational, single use strategy.

(d) Organophosphate and carbamate resistance mechanisms
Introduction
Overseas, studies of organophosphate resistance in B-type *B. tabaci* has shown that resistance is effectively monogenic, conferred by a single insensitive target site acetylcholinesterase (AChE), with differing levels of insensitivity to organophosphates tested. This resistant AChE is diagnosed by insensitivity to paraoxon or methyl paraoxon.

Methods
Activity of acetylcholinesterase (AChE) in *H. armigera* was measured by the method of Ellman *et. al*, in which the hydrolysis of the substrate analogue acetylthiocholine iodide (ATChI)is measured colorimetically by the absorbance of 2-nitro - 5- thiobenzoate at 405 nm, after the reaction of 5,5'-dithiobis (2-nitrobenzoate) (DTNB) with the liberated thiocholine. Assays were done in a microplate. Mass homogenates of 10, *H. armigera* larvae (each 3-4 mg) were made in 200 µl 0.01 M phosphate buffer, pH 7.5, containing 0.05% Triton X-100 and 10 µl aliquots placed in 96 well microplates. ATChI and DTNB solutions (in buffer) were added (100 µl each), to give final concentrations of 0.5 mM and 0.05 mM respectively. Activity was measured continuously in a Bio-Rad microplate reader for 20 mins, utilising kinetic collector software to fit linear regressions to the kinetic plots. Activity was usually linear up to an absorbance limit of 50 m OD.

For inhibition studies, varying concentrations of methyl paraoxon and methomyl stock solutions prepared in acetone were evaporated and the insecticide re-dissolved in the ATCh I /buffer solution. 100µl was then added to the wells containing homogenate and DTNB with an eight-channel multipipette. Wells containing 100µl of ATChI in buffer served as uninhibited controls. Enzyme activity in the presence of insecticides was calculated as a percentage of the corresponding uninhibited rate. Curves of enzyme activity versus final insecticide concentration in the wells were plotted.

Results and discussion
The inhibitory effects of methyl paraoxon on AChE activity in organophosphate resistant and susceptible *B. tabaci* are shown in Fig. 3. AChE from the resistant strain was clearly less sensitive to inhibition, compared to the susceptible strain. Based on the concentration of metbyl paraoxon required to inhibit all enzyme activity in the susceptible strain (IC_{100} = 20µM), the resistant AChE was >>100 times less sensitive to inhibition. Uninhibited AChE activity was greatly reduced in the resistant strain compared to the susceptible strain and this is consistent with other work which indicates that target site resistance is associated with a change in the kinetic properties of the enzyme.

Since organophosphate and carbamate insecticides share a common target site, organophosphate insensitive AChE may also confer resistance to carbamates. B-biotype *B. tabaci* are resistant to carhamates (Table 1, e.g. methomyl and methiocarb) and it is obvious that the AChE is also somewhat insensitive to methomyl, (Fig 4.). It is not known whether there is any association between forms of insensitive AChE, causing organophosphate and carbamate resistance.

A resistance mechanism, detectable by colorimetic analysis gives the opportunity for rapid field-based biochemical resistance detection. Such methods are already in use for the rapid diagnosis of pyrethroid and carbamate resistance in *H. armigera*. These resistance mechanisms may lead to a rapid field based detection of carbamate and OP resistance in *B. tabaci*.

(e) Pyrethroid resistance mechanisms
Introduction
Pyrethroid resistance in B-biotype *B. tabaci,* overseas, is thought largely to be as a result of metabolism by the B band esterase isoenzyme (R_m 0.14), which may sequester and hydrolyse

pyrethroids. B-type *B. tabaci* are resistant to most pyrethroids (Table 1)in Australia and the objective of this study was to examine the binding of pyrethroids to *B. tabaci* esterases.

Methods
Whiteflies used for these experiments were from unselected, B-biotype *B. tabaci* collected from Ayr in 1999. The whiteflies were resistant to all pyrethroids used, with the exception of bifenthrin. Whiteflies were homogenised in buffer and incubated for 30 mins. with pyrethroids (flucythrinate, fenvalerate, es-fenvalerate, permethrin, zeta-cypermethrin, alpha-cypermethrin, bifenthrin and deltamethrin). Pyrethroid concentrations were within aqueous solubility limits. Esterase activity was detected, using 1-naphthyl acetate as a substrate, using kinetic microplate assays.

Results
Most pyrethroids inhibited esterase activity in resistant B-type *B. tabaci*. (Fig. 5). However, there were considerable differences between pyrethroids. Fenvalerate and flucythrinate resulted in virtually complete inhibition of esterase activity but esterase did not bind as readily to deltamethrin, alpha-cypermethrin, zeta-cypermethrin or permethrin and even at the highest pyrethroid concentrations, only 50 - 90 % of esterase activity was inhibited. Es-fenvalerate was intermediate in esterase binding ability, between these two pyrethroid groups. No bifenthrin/esterase binding was observed at all.

Discussion
Bioassay results indicated that all pyrethroids did not act identically, when binding to esterases of B-type *B. tabaci*. Flucythrinate, fenvalerate and es-fenvalerate, were the most effective while permethrin, deltamethrin, bifenthrin, zeta-cypermethrin and alpba-cypermethrin bound poorly even at higher insecticide concentrations. Esterase binding ability is likely to be correlated to pyrethroid resistance factor and bioassays are underway.

An examination of the structures of the pyrethroids used in this study, indicates marked differences in structure of ester-pyrethroids which interacted more readily with B-biotype *B. tabaci* esterases and those which bound less readily. The former group, (fenvalerate, flucythrinate and es-fenvalerate), are pyrethroids which contain a halogenated benzyl group. While in the latter group of pyrethroids (permethrin, deltamethrin, bifenthrin, Z-cypermethrin and a-cypermethrin) the benzyl ring was replaced by a dihalogenated aliphatic entity.

(f) Bifenthrin resistance mechanism
Introduction
In Australia, B-biotype *B. tabaci* were initially susceptible to bifenthrin, however, field use of bifenthrin has resulted in the selection of resistance. While bifenthrin did bind to esterases in pyrethroid resistant, but bifenthrin susceptible B-biotype *B. tabaci*, in this study, we examined bifenthrin/esterase binding in a strain of B-biotype *B. tabaci* which exhibited a high level of resistance to bifenthrin (179 fold).

Materials and Methods
Homogenates of B-biotype *B. tabaci* and native susceptible *B. tabaci* were incubated with bifenthrin were also run on polyacrylamide gels using electrophoresis techniques and stained for esterase activity using 1-naphthyl butyrate as a substrate.

Results
While there was no evidence that bifenthrin had any interaction with esterases in susceptible *B. tabaci*, in the resistant strain, bifenthrin clearly bound to B band esterases, increasing with bifenthrin concentration (Fig 6).

Conclusions
While results at this stage are preliminary, it is evident that bifenthrin resistant, B-biotype *B. tabaci* have developed a novel form of B band esterase, which binds more readily to bifenthrin than the susceptible form of the enzyme. Detailed studies are currently underway

(g) Mechanisms of resistance to imidacloprid
Introduction
Imidacloprid is one of the newer insecticides that have been developed for the control of sucking insects, including whiteflies. It is a compound which has a novel mode of action, attacking the nicotinoid receptors of the insect nervous system. This compound is registered for whitefly control in Australia and so far, has been used with great success. It is considered to be a most important product for whitefly control and maintaining susceptibility to imidacloprid is vital. However, these studies (Tables 1, 2), have shown rapid selection of resistance to imidacloprid in B-biotype *B. tabaci.*

Methods
Whiteflies used were native, susceptible non-B type *B. tabaci*, susceptible B-biotype *B. tabaci* and resistant B-biotype *B. tabaci.* Both B-biotype *B. tabaci* strains originated from Ayr in 1999. *B. tabaci* homogenates were incubated for 30 mins. with imidacloprid. Esterase activity was detected, using 1-naphthyl acetate as a substrate, using kinetic microplate assays. Final concentration of imidacloprid were from 0.4 - 15μM.

Homogenates were also run on polyacrylamide gels using and stained for esterase activity using 1-naphthyl butyrate as a substrate.

Results
The total esterase assay (Fig 7), showed that esterases in the resistant strain, clearly bound to imidacloprid. At the highest concentration, some 40% of esterase activity was inhibited. There was no significant inhibition of esterase activity in B biotype or non-B type susceptible strains. Polyacrylamide gels indicated that imidacloprid binding in occurred in the region of the B bands in the resistant strain (Fig. 8).

Discussion
While the mechanism of resistance to imidacloprid requires further study, it is evident that resistance is clearly correlated to altered esterase isoenzymes occurring in the B bands of resistant whiteflies.

(h) Interbreeding of native, non-B type *B. tabaci* and B biotype *B. tabaci*
Introduction
In common with some other insect species, *B. tabaci* can reproduce sexually or asexually. Males are produced asexually (haploid), while females are the result of sexual reproduction and have two sets of chromosomes (diploid). While world wide, there are a number of biotypes of *B. tabaci*, B -biotype *B. tabaci* are notoriously difficult to interbreed with other biotypes, which has led some researchers in the US to describe it as a separate species, *Bemisia argentifolia.* Nevertheless, our initial whitefly surveys in the NT, indicated that B -biotype *B. tabaci* could interbreed with native, non-B type *B. tabaci* in the field. Interbreeding of these two whitefly biotypes could be of some concern to cotton in northern Australia, because native non-B type *B. tabaci* are quite common and interbreeding represents an addition means to spread insecticide resistance into the whitefly community.

Methods
Newly emerged, virgin male and female B -biotype *B. tabaci* and native non-B type *B.tabaci*, were used for these experiments. To simulate field conditions, 20 males and females of each biotype each were placed in cages. Whitefly progeny were reared on cotton and newly emerged adults were identified by esterase gel electrophoresis.

Results
Male progeny, which are haploid, carried the genotype and electrophoretic patterns of the respective female parents. Female progeny (e.g. Fig. 8), however, showed evidence that interbreeding had sometimes taken place. The hybrids usually retained the esterase banding pattern of the non-B type parent, however, the B-band was also weakly expressed. In addition the uppermost esterase band, which is common to both biotypes but which is not well expressed in non-B type *B.tabaci*, had become deeply stained, indicating a contribution from the B-biotype. Other hybrids, not illustrated,

showed that the B-biotype characteristics were strongly expressed along with non-B esterase bands. The hybrids were not sterile.

We are further investigating this interbreeding phenomena by further crosses to segregate the respective contributions of each sex,. Two crosses have been made: female B -biotypc *B. tabaci* x male native non-B type *B.tabaci* and male B -biotype *B. tabaci* x female native non-B type *B.tabaci*. Progeny will be assessed for biotype, silverleaf induction and insecticide resistance status.

Conclusions

While these experiments are as yet, incomplete, interesting data bave been obtained. It is obvious that B-biotype *B. tabaci* readily interbreed with native non-B type *B.tabaci*. These data are amongst some few examples which contradict the widely held assumption that B-biotype *B. tabaci* does not interbreed with other biotypes. Descriptions of B-biotype *B. tabaci* as a new whitefly species, *Bemisia argentifolia*, appear to be based on erroneous assumptions.

(i) B-biotype B. tabaci resistance to insect growth regulators and juvenile bormone analogues

Introduction

Insect growth regulators and juvenile hormone analogues such as buprofezin, fenoxycarb, pyriroxyfen, have been used successfully overseas against B-biotype *B. tabaci*. These insecticides act by disrupting the insect moulting process and were initially considered to be almost resistance proof. However, resistance has occurred in *B. tabaci* overseas. In Australia, registrations for these products, are being pursued for B-biotype *B. tabaci*., with the utmost urgency, because of resistance problems to most other conventional insecticides. There is an also an urgent need to asses the resistance status, ease of resistance selection and resistance mechanisms of *B. tabaci* in Australia to these types of insecticides. Enzyme systems, used by insects use to detoxify zenobiotics, are also candidates for detoxification of insect growth regulators and juvenile hormone analogues. Examples of such enzyme systems are: esterase isoenzymes, monooxygenases and glutathione-S-transferases. These topics form the subject of Ms Emma Cottage's Ph. D. studies. (Emma Cottage is a CRDC funded Ph.D. student from the University of New England based at NSW Agriculture's Tamworth Centre for Crop Improvement under the supervision of Robin Gunning). Ms Cottage's studies are progressing well and the following is a summary of major findings, so far.

Results

While B-biotype *B. tabaci* were initially susceptible to buprofezin and fenoxycarb, there has been no difficulty in the laboratory selection of resistant strains, within 2 generations. Bioassays on immature whiteflies, to determine the degree of resistance are continuing.

Studies so far, have shown that buprofezin resistant B-biotype *B. tabaci* have extra esterase isoenzymes, not occurring in susceptible insects. The whiteflies have a higher esterase titre, which can be attributed to the additional esterase bands, (below the B-hands) (Fig.9). Incubation of homogenates of resistant and susceptible B-biotype *B. tabaci* with buprofezin, has demonstrated that buprofezin binds to esterase in the resistant strain. Fig. 10 , shows a clear inhibition of esterase activity, compared to the susceptible control. We are currently trying to isolate the esterase bands which are responsible. The nature of the buprofezin/esterase binding, is unclear, because buprofezin does not possess an ester linkage, nevertheless, esterase isoenzymes are well known for sequestration of insecticides, a process which does not involve the hydrolysis of an ester.

6. LIKELY IMPACT OF THE RESULTS FOR THE COTTON INDUSTRY

It is only a matter of time until B-biotype *B. tabaci* become a significant pest of cotton in Australia. The major weapon against this pest will be insecticides, however, given the resistance history of this insect , there could be severe control problems. However, because of this project, the cotton industry will be prepared. Pre-emptive research, has established baseline information about distribution on cotton, the insecticide resistance status of the insect, speed of resistance selection in the laboratory and in the field, evolution of new insecticide resistances and likely resistance mechanisms. Information has enabled the formulation of a resistance management strategy, which is currently being tested by the horticulture industries. Future research needs are: to continue to monitor whitefly number on cotton and to monitor insecticide resistance development in the field and on horticultural crops.

7. PROJECT TECHNOLOGY

Techniques for biochemical resistance monitoring of organophosphate and carbamate resistance would be adaptable for rapid field based detection of resistance.

8. PUBLICATIONS ARISING FROM THE PROJECT

Gunning, R. V., Byrne F. J. and Devonshire, A. L (1997) - Electrophoretic analysis of B-type and non B- type *Bemisia tabaci* in Australia. Journal *of the Australian Entomological Society* ,**36**: 245 - 249.

9. ACKNOWLEDGEMENTS

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Fig. 1

Locations of B-biotype *B. tabaci*

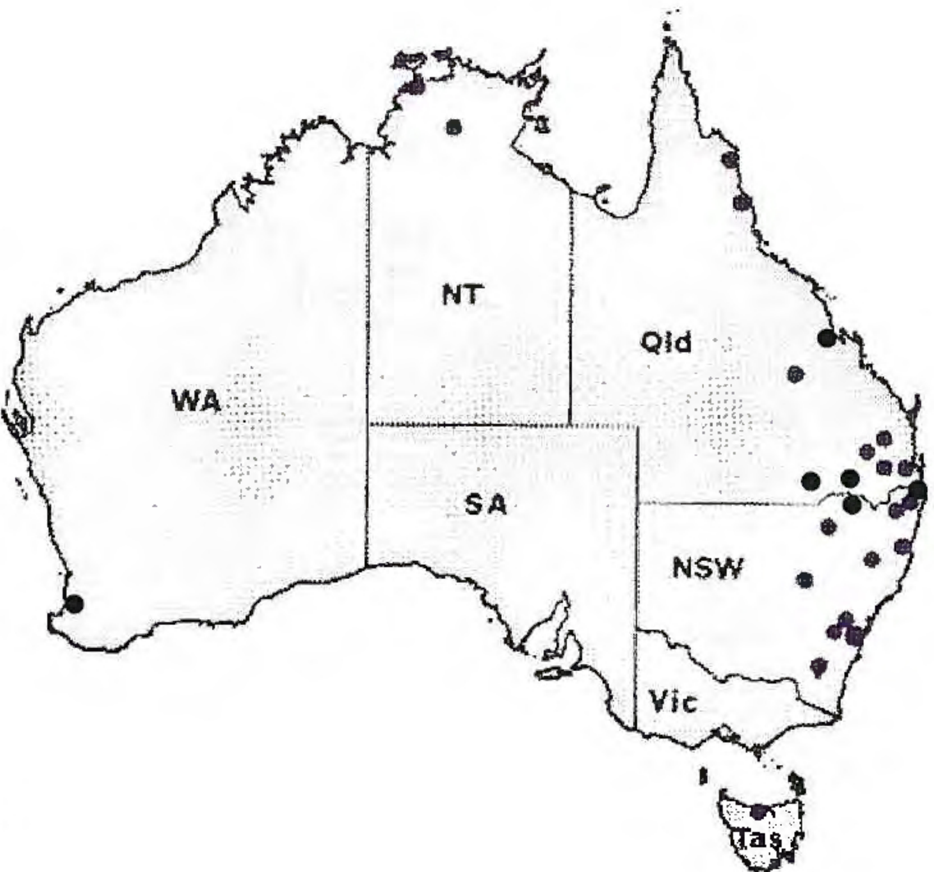


Fig. 2

B-biotype *B. tabaci* recovered from cotton leaves 1995 - 1999

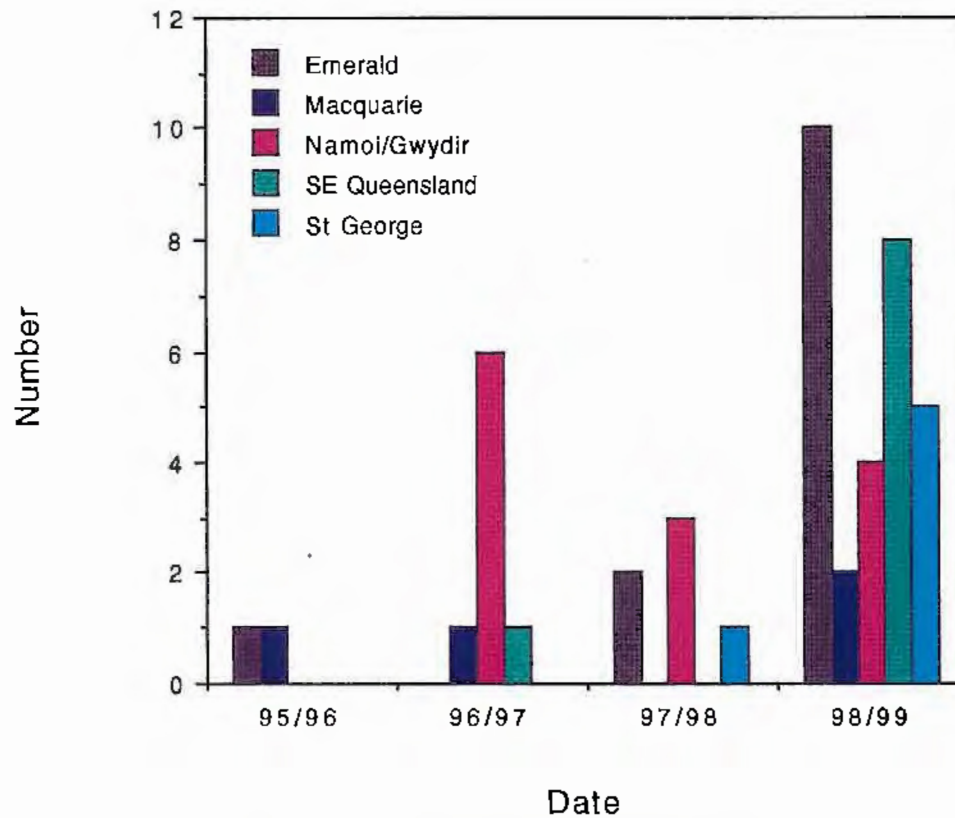


Fig. 3

Paraoxon type, acetylcholinesterae insensitivity in organophosphate resistant B-type *Bemisia tabaci*.

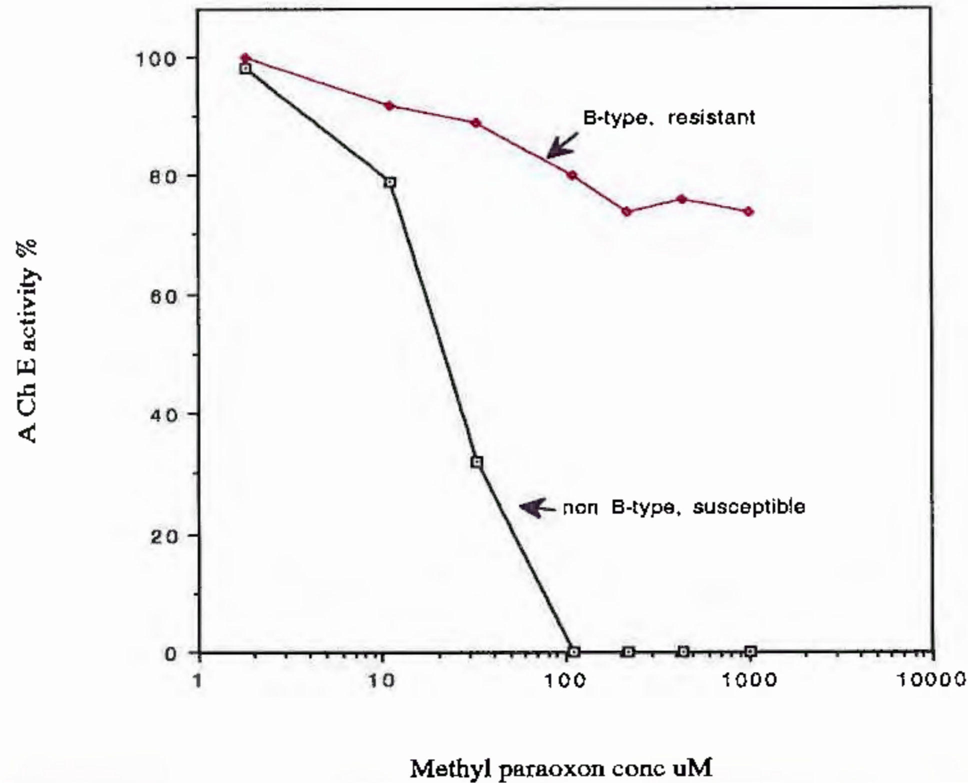


Fig. 4

Acetylcholinesterae insensitivity in methomyl resistant B-type *Bemisia tabaci*.

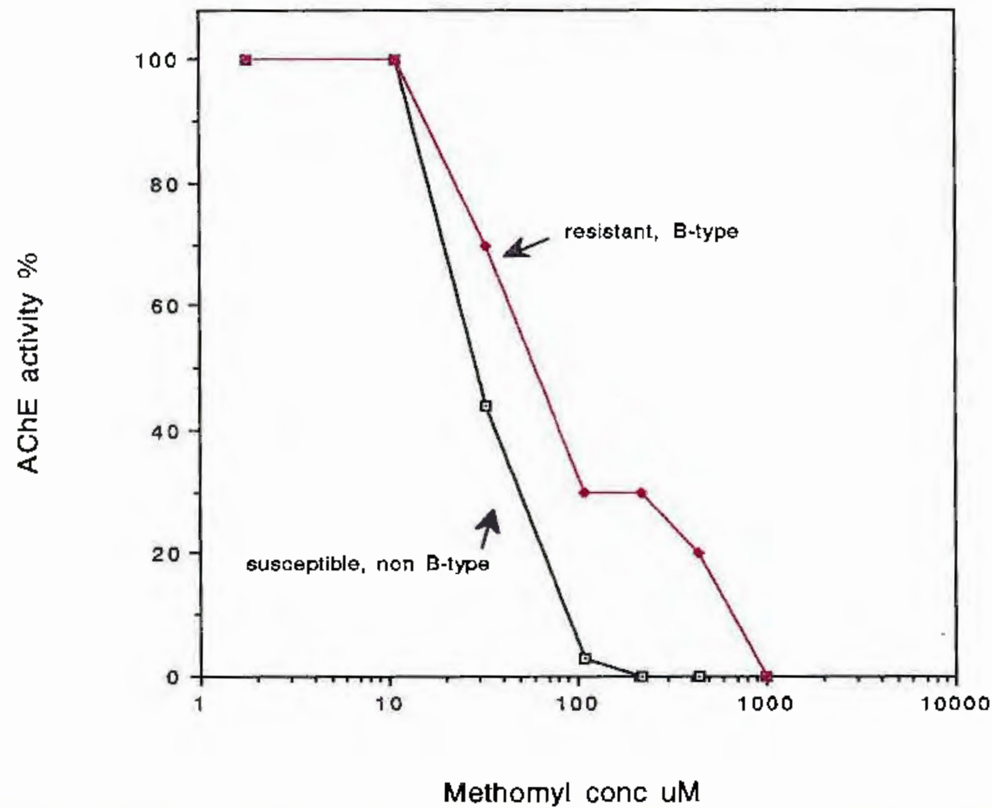


Fig. 5

Pyrethroids bind to esterase iso-enzymes, in pyrethroid-resistant B-type *Bemisia tabaci*

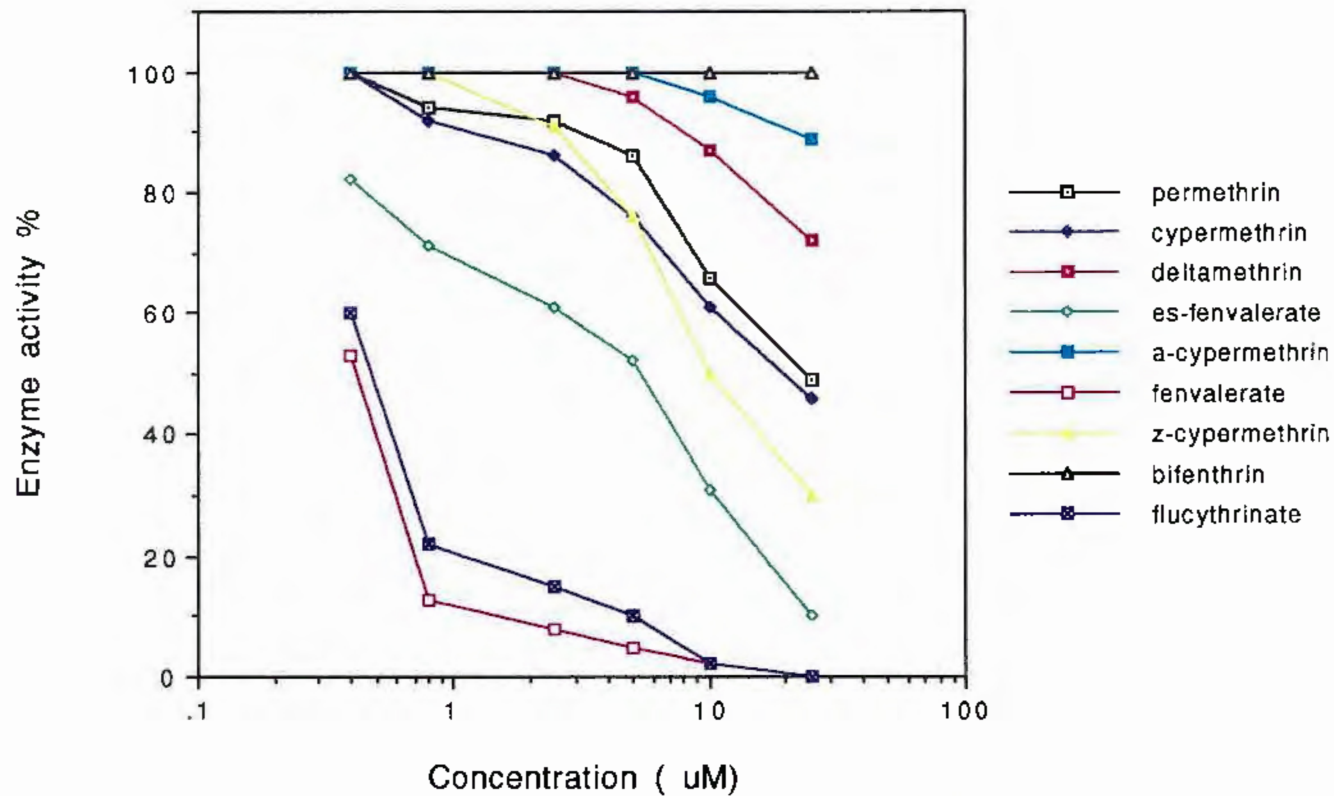
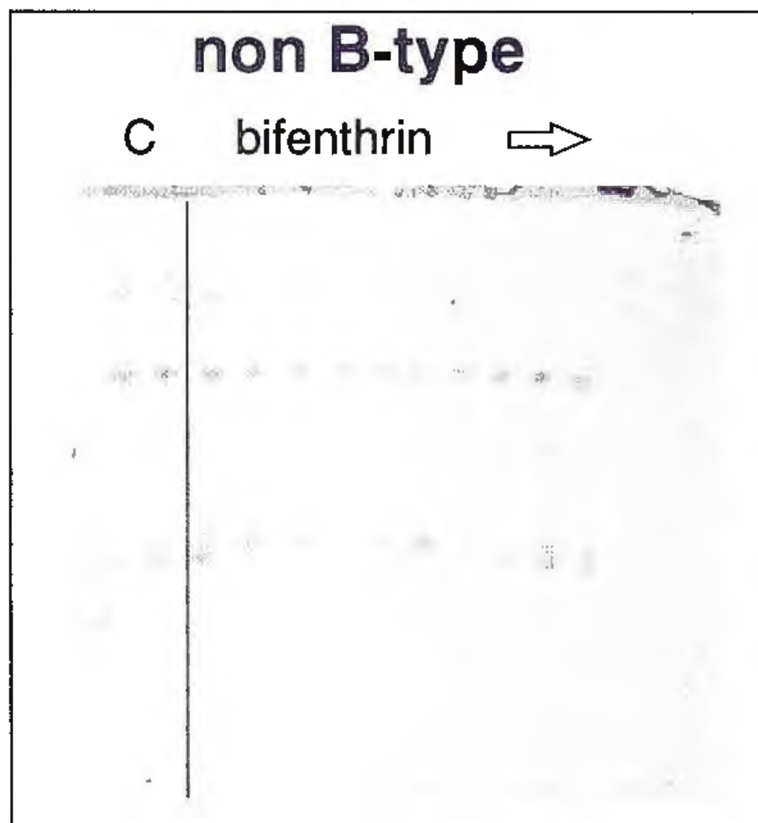


Fig. 6

Bifenthrin binds to esterases in bifenthrin resistant, B-type *Bemisia tabaci*



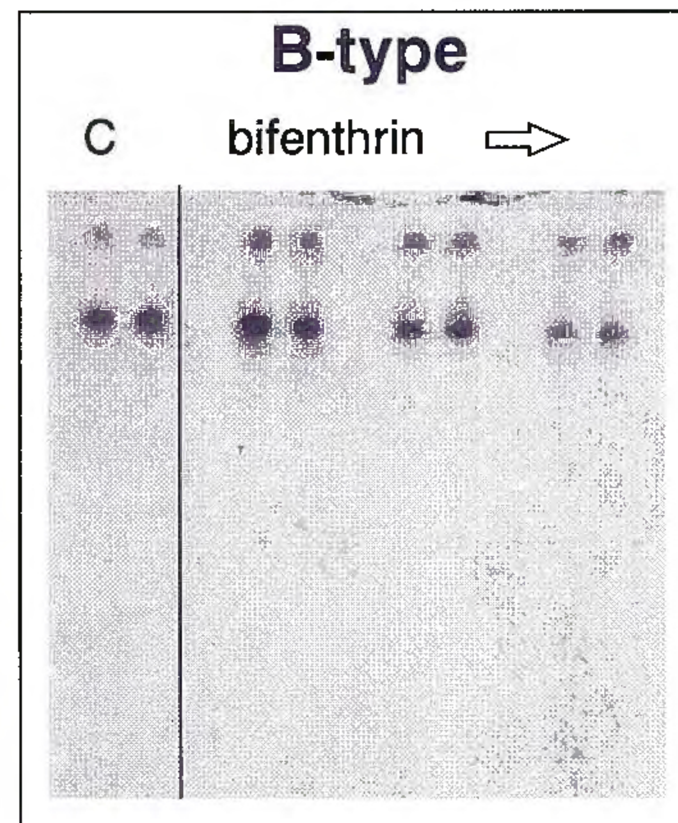


Fig. 7

Imidacloprid binds to esterase iso-enzymes, in imidacloprid-resistant B-type *Bemisia tabaci*

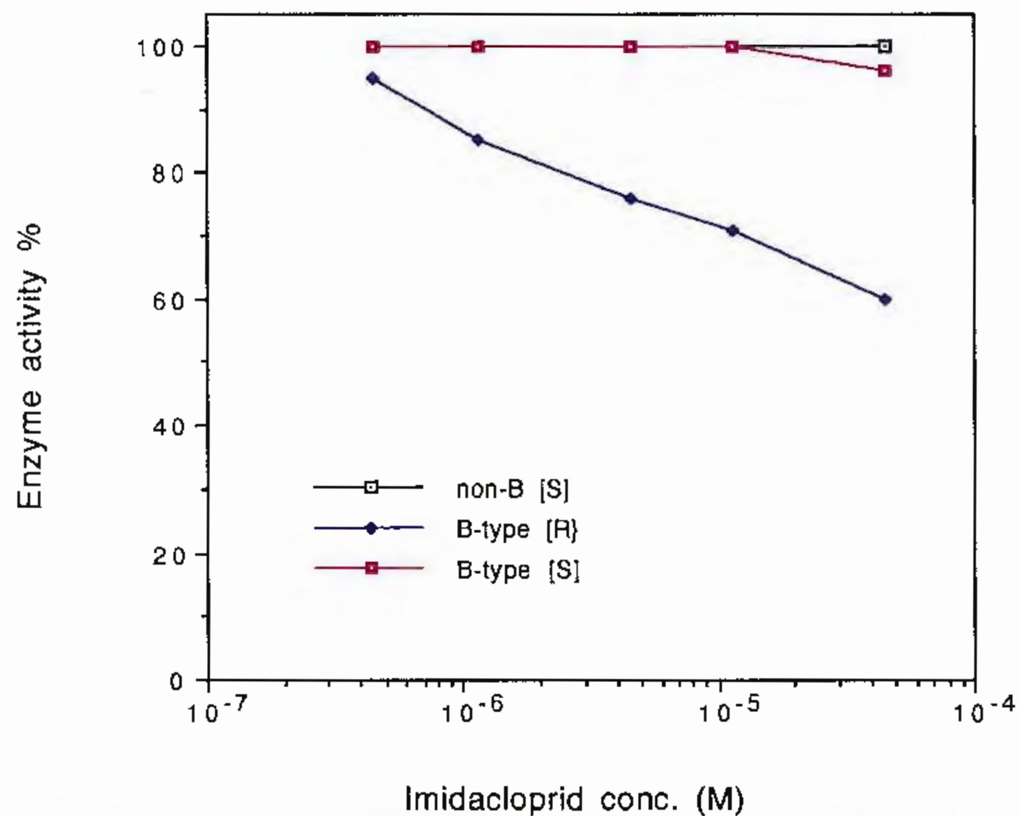
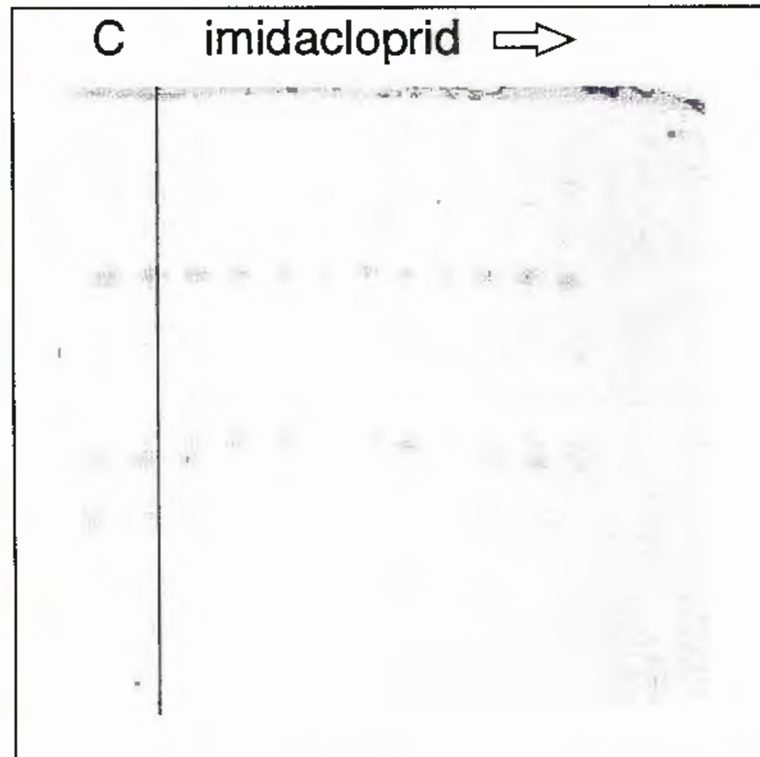


Fig. 8

Imidacloprid binds to esterases in resistant, B-type *Bemisia tabaci*

non B-type



B-type

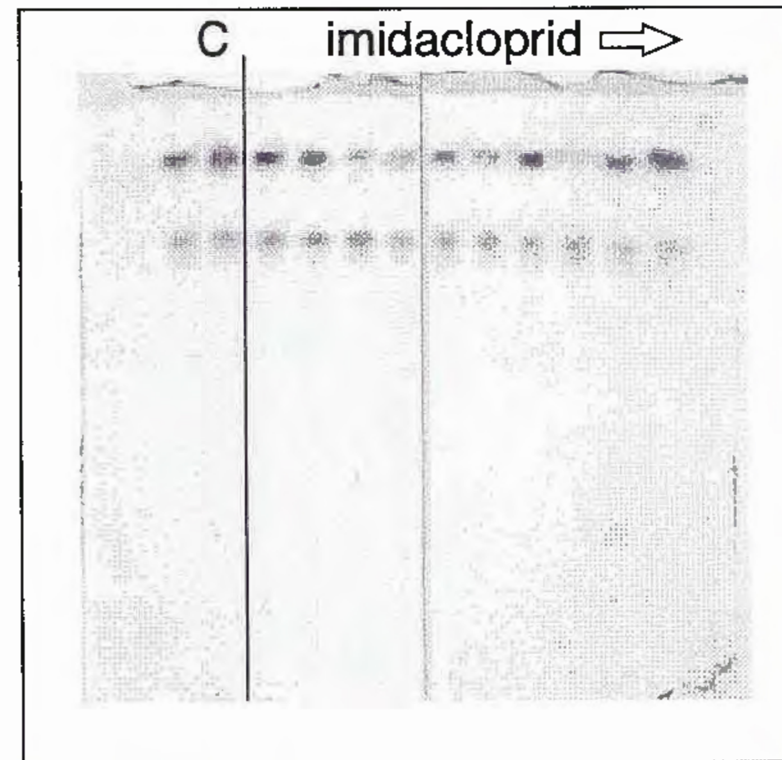


Fig. 9

Native non-B *Bemisia tabaci* will interbreed with B-type *B. tabaci*

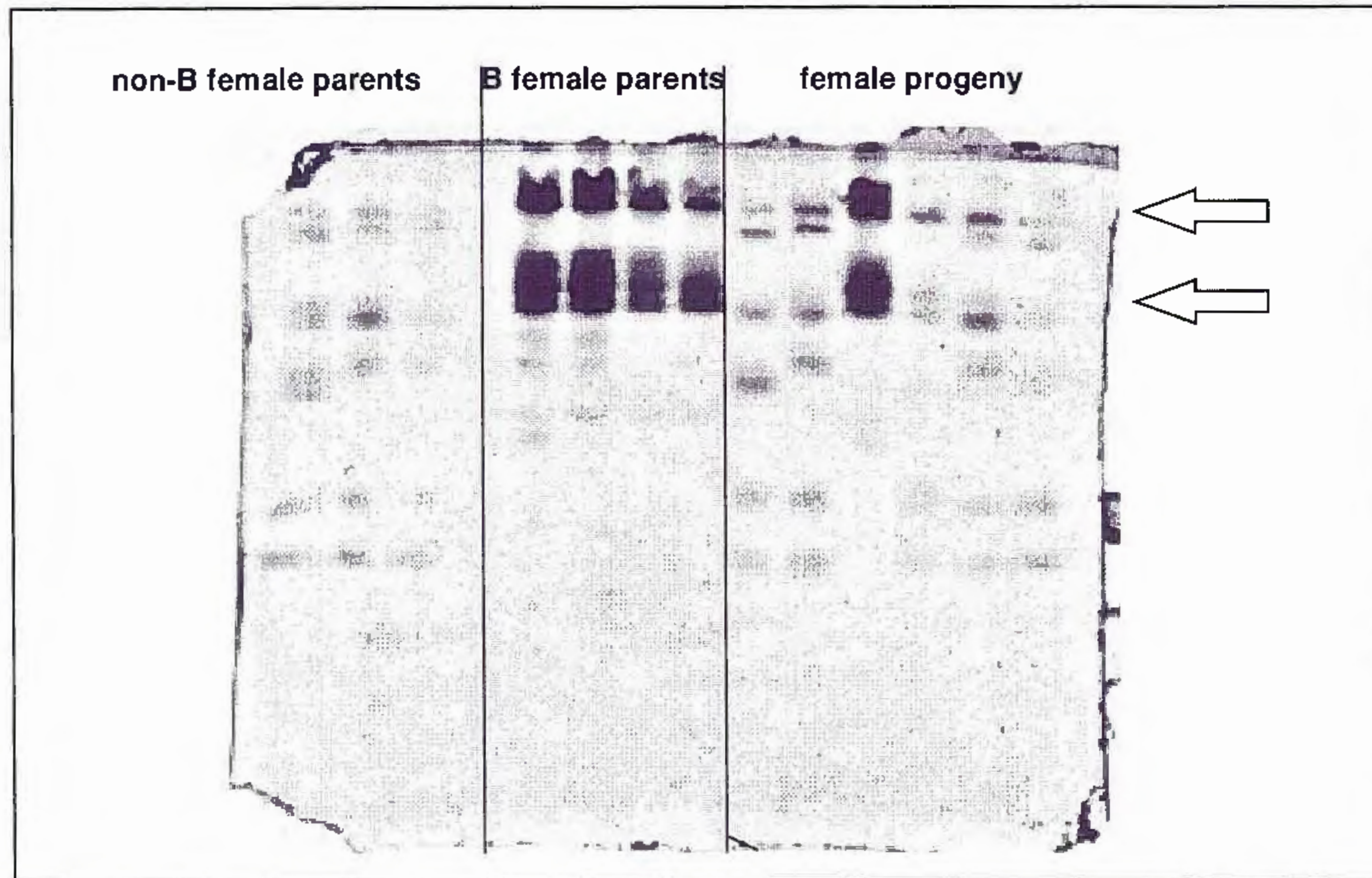


Fig. 10

Buprofezin resistant B-type *Bemisia tabaci*, have additional esterase bands

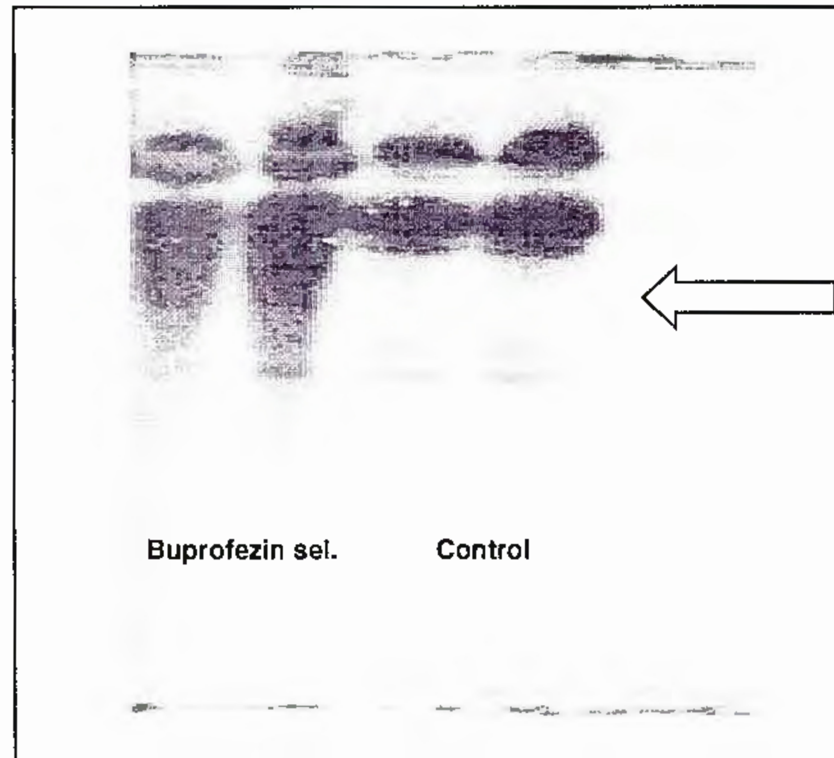


Fig. 11

Esterase iso-enzymes, in buprofezin resistant B-type*Bemisia tabaci*, bind to buprofezin