Cotton
RESEARCH & DEVELOPMENT

Annual, Progress and Final Reports

Part 1 - Summary Details

REPORT

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Please use your TAB key to complete Parts 1 & 2.

CRDC Project Number: **DAN 138C**

Annual Report: ☐ Due 30-September

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Project Title: *Insecticide Resistance Mangement in B-biotype Bemisia tabaci*

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Research Program: A Insect Management

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INSECTICIDE RESISTANCE MANAGEMENT IN

B-BIOTYPE *BEMISIA TABACI*

1. Background To The Project

The cotton whitefly *Bemisia tabaci* is a major pest of cotton and other crops world-wide. *Bemisia tabaci* is comprised of a species complex or biotypes. In Australia, there are two native biotypes of *B. tabaci* that occur in south-east Queensland and northern NSW and northern Australia respectively. A new biotype of B-biotype *B. tabaci* (Poinsettia or Silverleaf whitefly), which is thought to have come from the Middle East in the early 1990's , was first identified in the USA and has spread round the world via the world-wide trade in poinsettia cuttings. Robin Gunning and Frank Byrne first detected the silverleaf whitefly in Australia, in 1994.

The silverleaf whitefly is characterised by a huge host range, high fecundity, the ability to induce physiological responses in plants, transmit plant viruses, the copious production of honeydew, and an extreme ability to develop insecticide resistance. The whitefly damages cotton crops by direct feeding (yield can be reduced by 60% under heavy infestation), copious production of honeydew (which contaminates cotton lint and reduces the photosynthetic efficiency of cotton leaves) and by virus transmission.

B-biotype *B. tabaci* came into Australia with insecticide resistance to most pyrethroids, organophosphates and carbamates. Explosion of the silverleaf whitefly into horticultural crops in north Queensland during the late 1990's ensured development resistance to other insecticides (bifenthrin, endosulfan, amitraz and imidacloprid) to which they initially susceptible. Field selection experiments in horticultural crops in North Queensland (DAN 106C) showed a very rapid rate in the selection of resistance to insecticides.

At the commencement of this project, B-biotype *B. tabaci* was not a pest of cotton but was considered to be a major threat to the Australian cotton industry needing pre-emptive research. The aims of this project were therefore, to monitor silverleaf whitefly numbers on cotton, secondly to monitor insecticide resistance levels and to investigate novel insecticides and insecticides combinations as candidates for whitefly control. In December 2001, the silverleaf whitefly exploded on cotton in central Queensland and reached economically damaging levels.

2. Objectives Of The Project

- To monitor silverleaf whitefly numbers on cotton.
- To use toxicological, biochemical, molecular and genetic techniques to continue investigate insecticide resistance mechanisms in B-biotype *B. tabaci* (silverleaf whitefly), both in cotton and other crops, such as horticultural crops in the Burdekin area of north Queensland.

- To continue a resistance monitoring programme for B- biotype, and native non-B biotype *B. tabaci.*
- To devise and test resistance insecticide management strategies for management of *Bemisia tabaci* in Australia.

3. Results

(a) Whitefly survey on cotton

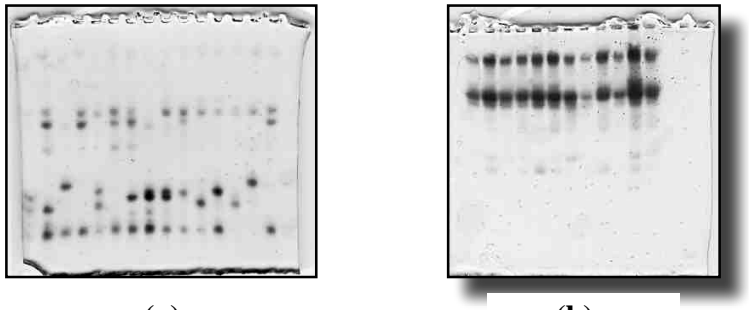
Introduction

Given Australia the ability of silverleaf whitefly to rapidly build up population numbers, after first detection in Australia, it became imperative to closely monitor the whitefly populationson cotton.

Methods and Materials

Each cotton season many thousands of cotton leaves, from every cotton growing area, have been collected and sent to Tamworth as part of the *Helicoverpa* eggs resistance monitoring programme. In addition to bearing *Helicoverpa* eggs these leaves were used a source of whiteflies on cotton. Adult or immature whiteflies were collected and identified (visually) as either *B. tabaci* or the greenhouse whitefly (*Trialerodes vapororium*).

Biotypes of *B. tabaci* are morphologically indistinguishable and we used to use biochemical techniques (esterase isoenzymes) to identify biotypes. Esterase iso-enzyme patterns ar used overseas to identify *B. tabaci* to biotype. Individual adult whiteflies were homogenised in 20μL of 1.6% Triton X-100, containing 10% sucrose and a few grains of bromocresol purple. Aliquots (15μL, 0.75 insect equivalent) were pipetted into wells of polyacrylamide gels. Gels contained 7.5% polyacrylamide with 0.05% Triton X-100, but to achieve optimum resolution, the Triton. Specially designed gel combs that cast wells with 4.5mm spacing in the stacking gel were used. Gels were run at 5°C, in barbitone buffer at 250V maximum current for 1.5 h. Gels were stained for esterase activity, using 0.5mM α-naphthyl butyrate and 0.2% Fast Blue RR, in 0.02M phosphate buffer pH 6.0. Gels were fixed in 5% acetic acid. Electrophoretic mobilities (R_m) of esterase bands were calculated. Typical gels showing esterase bands of B-biotype *B. tabaci* (silverleaf whitefly) and native, non-B biotype *B. tabaci* are shown in Figure 1a and 1b.



(a) (b)

Figure 1 Polyacrylamide gels stowing esterase bands of adult *Bemisia tabaci* (a) native, non-B biotype *B. tabaci* and (b) B-biotype *B. tabaci* (silverleaf whitefly). Each track, represents of a single 0.75 whitefly

Results

Results of the whitefly survey on cotton, 1995 – 2001, are shown in Figs 2, 3 and 4. Numbers of silverleaf whitefly, native *B. tabaci* and greenhouse whitefly are plotted for eachin each each cotton growing district. Silverleaf whitefly (Fig 2) was found on cotton, albeit in low numbers, as early as 1995, with numbers progressively increasing each year. Silverleaf whitefly, were found on cotton in all districts. Increasing numbers were particularly evident in Emerald, south-east Queensland, the Macintyre and the Macquarie Valley and these areas were identified as of particular concern with respect to potential whitefly problems. The Silverleaf whitefly comprised the vast majority of whiteflies found on cotton, with only few native *B. tabaci* or greenhouse whitefly identified. (Figs. 3 and 4).

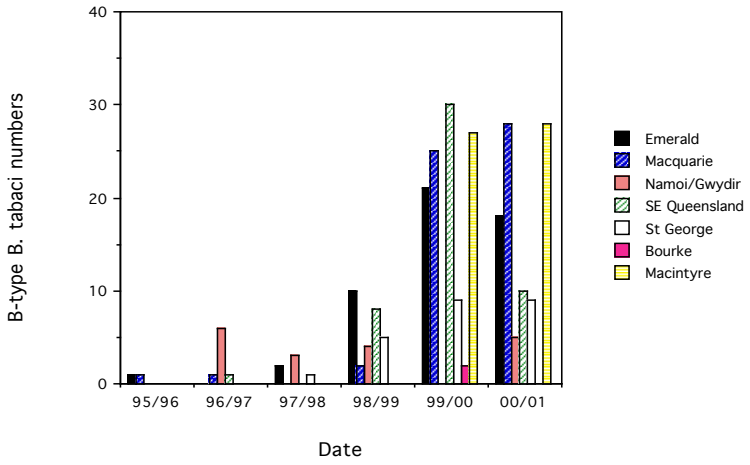


Figure 2 Distribution of ilverleaf whitefly on cotton in Australia 1995 -2001

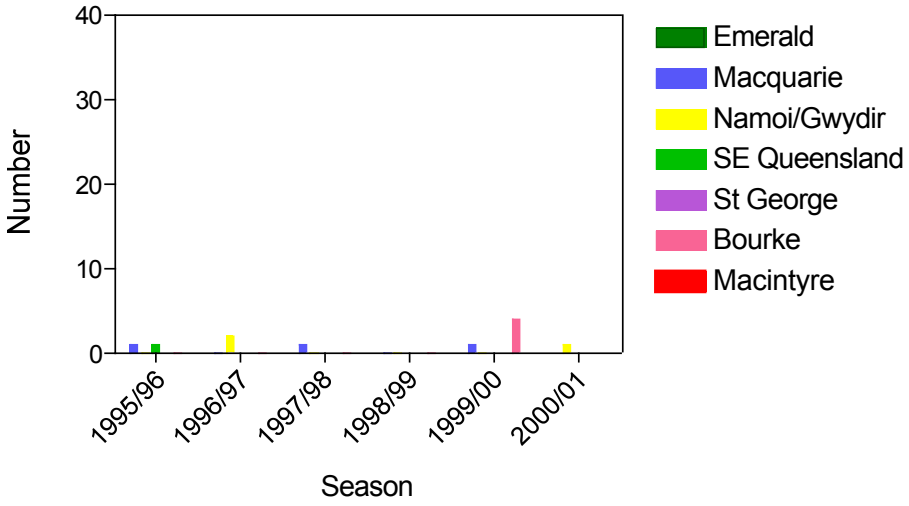


Figure 3. Distribution of greenhouse whitefly on cotton 1995 –2001

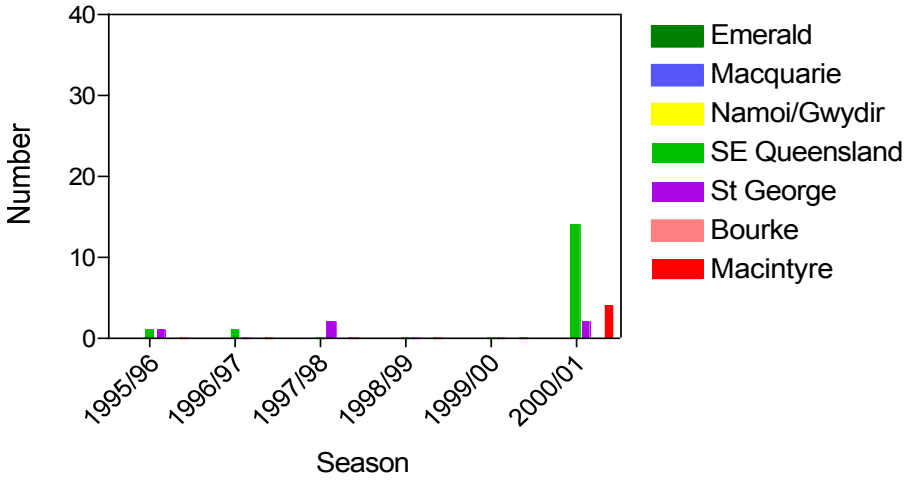


Figure 4. Distribution of native *B. tabaci* on cotton 1995 - 2001

In December 2001, our predictions of silverleaf whitefly problems on cotton were realised with a very rapid population build-up of silverleaf whitefly on cotton Emerald. A detailed survey of whitefly species and numbers on Australian cotton was imperative and all districts were urged to send in 200 randomly picked cotton 200 leaves from as many properties as possible, for whitefly counts. Results of theses surveys, of relative abundance of whitefly species and numbers are shown in Figs.5 and 6. On all, we made some 12,000 whitefly identifications.

While (with the exception of Emerald), numbers of whiteflies on cotton wer low, in early 2002, silverleaf whitefly were detected on cotton in all areas (Fig. 6). Species compoistion data (Fig. 5) showed that 100% of whiteflies on cotton in central Queensland (Emerald and Biloela/Theodore) were identified as silverleaf whitefly. Disturbingly, in other Queensland locations (Macintyre St George and Darling Downs), silverleaf whitefly comprised some 30 – 40% of the whitefly population on cotton while the remainder were largely native *B. tabaci*. In NSW, the whiteflies on cotton were largely greenhouse whitefly, but 5 – 15 % of the population were comprised of silverleaf whitefly.

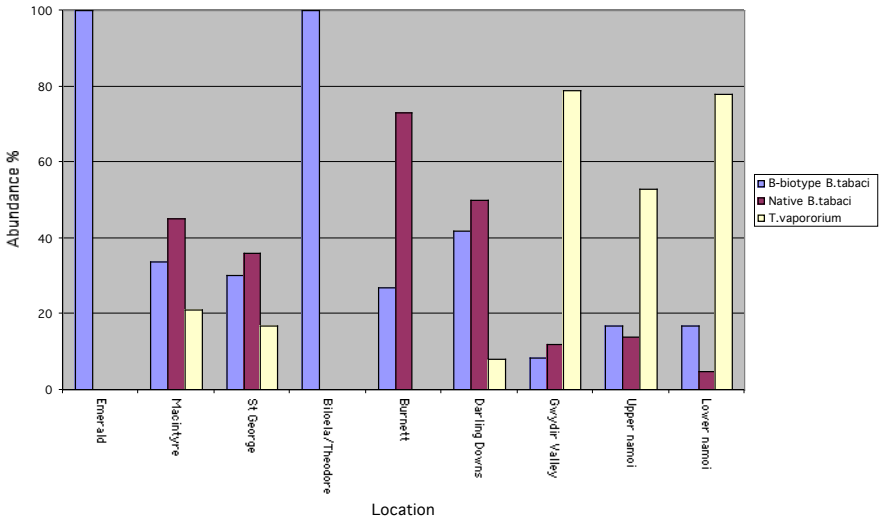


Figure 5. **Whitefly species on cotton Feb/March 2002**

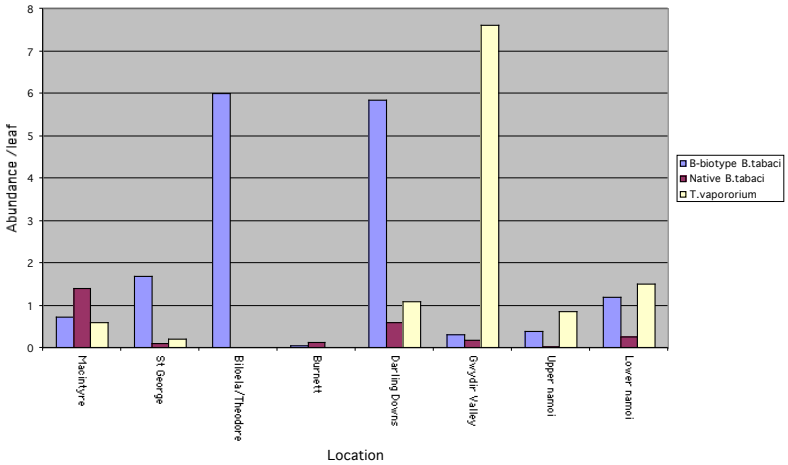


Figure 6. Numbers of whiteflies on cotton Feb/ March 2002

(b) Insecticide Resistance

Introduction

Our previous whitefly studies (DAN 106C) generated considerable baseline toxicity data on the insecticide resistance status of both native and silverleaf whitefly in Australia. While native *B. tabaci* are insecticide susceptible, silverleaf whitefly entered Australia with resistance to most pyrethroids, carbamates and organophosphates. Attempts at silverleaf whitefly control in horticulture in Queensland rapidly produced resistance to other insecticides to which the whiteflies were initially susceptible eg. (endosulfan, bifenthrin, amitraz, imidacloprid). Overseas, successful whitefly control has depended on using insect growth regulator to prevent population build-ups and is s a potential management tool for Australia. A major focus of this project was therefore to determine the likelihood of insect growth regulator resistance in Australian populations of silverleaf whitefly.

General methods.

A leaf dip bioassay method was used to test contact insecticides against *B. tabaci*. Cotton plants (Sicot 189) were grown in the glasshouse without any exposure to insecticides. Leaf discs were cut and dipped into aqueous solutions of insecticide containing 0.01% Agral® surfactant and allowed to dry at 25ºC. Control leaves were dipped in Agral® and distilled water only. Leaf squares were placed adaxial side down in a small petri dish on a bed of agar. Female adult whiteflies of required strains were captured using an aspirator, temporarily anaesthetised with carbon dioxide and placed on the cotton leaf discs. Twenty whiteflies were placed on each cotton leaf disc and sealed into petri dishes. The whiteflies were allowed to feed on the leaf squares and were assessed at maximum mortality (48 hours).

The bioassay technique for insect growth regulators on immature whiteflies was more complex. Silverleaf whiteflies were allowed to oviposit on young cotton plants. The cotton plants were then removed from the whitefly cages so that no more eggs would be laid, thus ensuring that test whitefly nymphs were at the same developmental stage. Immature *B. tabaci* on the leaves were counted and then were dipped into formulated insecticide and Agral® solutions (to ensure wetting). Mortality was assessed 20 days after oviposition, by counting the number of living nymphs.

Standard toxicological statistics were used for bioassay data. Bioassay data were analysed by Probit Analysis . Control mortality was corrected for using Abbott's formula. The computer Probit program was P-A Mod (A. Woods, C. Orton & C. Virgona, University of NSW, for Macintosh microcomputers). Probit analysis is a tranformation to facilitate computation, which converts the data to a straight line on probit graph paper. The method is to replace each percentage by its corresponding probit. The line which gives the best fit of the experimental data ($y = ax+b$) is computed from the transformed data, using a modified regression technique. In the equation $y = ax+b$, y represents the probit kill and x the log dosage. The calculations also give the slope of the line, 95% confidence limits for the estimated doses corresponding to percent mortality and a means for testing the homogeneity of the population used in the bioassay.

Laboratory selection of resistance to insect growth regulators in the silverleaf whitefly.

Introduction

Insect growth regulators such as buprofezin and pyriproxyfen have been used overseas (Arizona and Israel), with great success to limit early season populations of silverleaf whitefly and prevent later season, population outbreaks. There were expectations that a similar regime would be effective in Australian cotton. Buprofezin affects nymphal stages and embryogenesis through contact and vapour action, suppressing chitin formation, similarly to benzoylphenyl ureas. Buprofezin also reduces longevity, suppresses oviposition in adult whiteflies and causes some egg sterility, reducing hatch rate

Methods

B-biotype *B. tabaci* populations that had been held in glasshouse culture at Tamworth since 1995 were selected with buprofezin, by spraying whitefly host plants with 50ppm buprofezin (each alternate generation, three times in total. This concentration was selected as it fell in the higher range of field rates (buprofezin is used against *B. tabaci* at field rates between 100 and 250 mg/L (25ppm - 62.5ppm/L). The objective of these insecticide applications was to select for resistance by progressively decreasing the proportion of susceptible insects. A culture of the original unselected silverleaf culture was kept segregated from the selected strain. Bioassays were conducted after each buprofezin selection to determine the rate of resistance development.

Results and discussion

Data are shown in Table 2. Compared susceptible *B. tabaci*, the unselected laboratory population of silverleaf whitefly and the 1996 population had an pexisting, low-level, resistance to buprofezin. This strain had an LD_{50} of 3.0ppm and a slope of 0.76, indicating considerable heterogeneity of response in the population towards buprofezin. Compared to the susceptible strain, the resistance factor was 11 fold. Selection with buprofezin increased LD_{50}'s from 3 to 595 ppm, rapidly eliminating susceptibility, evidenced by increasing slope values of LDP lines (0.76 to 3.7). Buprofezin selected generations were 117 (F_2), 348 (F_4) and 2203 (F_6) fold resistant to b respectively.

Strain	LC50 (ppm)	Fiducial Limits	Slope	Resistance Factor
Native susceptible	0.3	(0.27 –0.34)	2.7	1
Silverleaf whitefly				
Buprofezin unselected	3.00	(1.8 - 5.0)	0.76	11
Selection1	31.60	(15 - 67)	1.40	117
Selection 2	94.00	(53 - 169)	1.40	348
Selection 3	595.00	(336 - 1054)	3.70	2203
Oz. B	1.50	(1.3 - 1.9)	1.60	5.5
1996 bioassay, M. Cahill and R. Gunning, at IACR-Rothamsted				

Table 2. Response of silverleaf whitefly populations to laboratory selection with buprofezin.

Buprofezin was not used in Australia until 2002, however, our bioassays (which confirmed suggestions of resistance in 1966 - "Oz B") Mathew Cahill and Robin Gunning at IACR-Rothamsted in 1996) showed that silverleaf whitefly were already resistant to buprofezin. Presumably, buprofezin resistance came into Australia with the silverleaf whitefly. Buprofezin tolerance and resistance has also been reported in *B. tabaci* populations from Israel, Europe and the USA. Findings of pre- existing resistance to buprofezin and of the ease of its selection should serve as a warning that overuse of buprofezin in Australia may result in rapid selection of resistance in the field. Strictest resistance management guidelines, involving non-consecutive buprofezin applications should be developed and adhered to.

Insecticide resistance at Emerald 2000/01

During early 2002 uncontrolled insecticide led to an extreme, silverleaf whitefly, insecticide resistance situation in the field at Emerald. During the cotton season, we collected silverleaf whiteflies from unsprayed populations and populations that had been sprayed in with insecticides. The silverleaf whitefly populations were bioassayed with specific whitefly control insecticides and other compounds, to which there had been exposure. Our objectives were to assess resistance levels, and the response of silverleaf whitefly populations to field selection with insecticides.

Results and discussion

Insecticide	Strain	Slope	LD_{50}ppm	RF	χ^2
amitraz	sus	4.4	2.0 (1.7-2.5)	1	5.7
amitraz	unsel	2.3	1.8 (0.9-3.7)	1	2.4
amitraz	field sel	0.97	8.4 (0.9-80)	4.2	2.3
Chlorpyrifos	Sus	2.9	0.48 (0.27-1.2)	1	4.8
chlorpyrifos	Field sel	2.7	0.55 (0.28-1.1)	1	5.9
Diafenthiuron	Sus	2.5	36 (25-52)	1	11.7
	field	1.4	19 (3.2-100)	1	
	Field sel	1.8	340 9250-470)	6.7	3.1
buprofezin	Sus	2.7	0.3 (0.27-0.34)	1	3.6
	field	1.0	3.87 (1-10)	12.9	40
	Field sel	0.37	50 (38-90)	167	12.6
pyriproxyfen	sus	2.8	0.11 (0.09-0.14)	1	3.8
	Field	0.92	0.43 (0.27 – 6.8)	4	6.3
	Field sel	1.3	4.3 (2.0 – 9.0)	40	12.2
Indoxacarb	field	1.6	1.3 (0.92-1.8)		1.7
Imidacloprid	Sus	2.6	20 (17-29)	1	3.9
	field sel	1.0	90 (40-170)	4.5	9.4
Ethion	Sus	3.1	0.51 (0.26-1.0)	1	3.4
	Field sel	2.9	0.55 (0.28-1.1)	1	5.9
Z-cypermethrin + ethin	Sus	2.0	0.22 (0.16-0.32)	1	2.9
	Field sel	1.9	0.23 (0.16-0.33)	1	2.5
Bifenthrin	Sus	3.5	0.084 (0.072-0.1)	1	10.2
	field	1.2	33 (20-50	392	1.6
	Field sel	1.3	190 (130-280)	2261	4.3
Z-cypermethrin	Sus	3.2	0.12 (0.10-0.14)	1	2.4
	Field sel	0.65	120 (60-230)	1000	10.9
Deltamethrin	Sus	2.7	0.13 (0.11-0.17)	1	1.6
	Field	0.4	20.1(0.6-8.0)	155	11.0
	Field sel	1.3	146 (88-267)	1121	5.2

Table 1. **Response of Emerald populations of silverleaf whitefly to insecticides Feb/March 2002**

During early 2002, a number of insecticides were used against the silverleaf whitefly at Emerald. Silverleaf whitefly was also exposed to other insecticides (used on *Helicoverpa*, aphids and mites). Insecticide use against silverleaf whitefly was uncontrolled and lead to a constantly evolving, serious, field insecticide resistance problem and there were situations where control failures had to be re-sprayed. Pyrethroids were probably the most commonly insecticide used; to control adult whiteflies to prevent honeydew damage to cotton lint. Insecticide bioassay results, are shown in Table1.

Compared to a susceptible, native strain of *B. tabaci*, unsprayed field populations of the silverleaf whitefly had considerable heterogeneity of response toward pyrethroids, indicating resistance to deltamethrin, bifenthrin and Z-cypermethrin. Field selection with pyrethroids further exacerbated the resistance problem (the resistance factor was in excess of 1000 fold for all pyrethroids tested) and resulted in pyrethroid failures in the field.

The silverleaf whitefly at Emerald was initially susceptible to amitraz and diafenthiuron, but field exposure rapidly produced resistance (4.2 and 6 fold respectively). Low slope values of dosage mortality curves in the field selected strains indicate heterogeneity of response toward these insecticides and the resistance factors at the LD_{99} levels were considerably higher than at LD_{50} levels.

While imidacloprid was not actively used to control silverleaf whitefly at Emerald, resistance was detected (4.5 fold) and it is likely that imidacloprid sprays on cotton aphids were at least partially responsible. The resistance situation is potentially serious because imidacloprid and other neonicotinoid insecticides are very useful for whitefly control and could easily be lost to resistance.

Perhaps the most disturbing feature of the silverleaf whitefly resistance situation at Emerald was the rapid selection of resistance to the insect growth regulators buprofezin and pyriproxyfen in field populations of. While unsprayed silverleaf whiteflies had a low level of IGR resistance (~10 fold) very limited selection in the field rapidly increased resistance to approximately 150 and 40 fold for buprofezin and pyriproxyfen respectively (and at the LC_{99} levels, the resistance factor was very considerably higher). Insect growth regulators buprofezin and pyriproxyfen have been used overseas (Arizona and Israel), with great success to limit early season populations of silverleaf whitefly and prevent later season explosive population outbreaks of silverleaf whitefly. Our findings of resistance and the rapidity of resistance selection in the field, at Emerald are most disturbing because resistance to insect growth regulators may jeopardise our chances of successfully controlling this whitefly. It is recommended that the most stringent steps to manage insect growth regulator resistance (one use per season) in the silverleaf whitefly be adopted.

While our bioassays showed that field resistance was rapidly selected for in most control chemicals, there was surprising susceptibility to two organophsphates, (chlorpyrifos and ethion). These data are in support of grower observations that both chlorpyrifos and Mustang® (a mixture of Z-cypermethrin and ethion) were effective in controlling silverleaf whitefly. Some organophosphate susceptibility is consistent with OP resistance patterns in other insects, as resistance does not usually develop to the entire class of organophosphates . Susceptibility is very encouraging and we should examine the range of organophosphates against silverleaf whitefly. If susceptibility were to be maintained, organophosphates it could be a very useful whitefly control agents in latter part of the cotton season.

Another possibility for control of the silverleaf whitefly is indoxacarb,as bioassay data showed quite good efficacy against silverleaf whitefly in the laboratory. Indoxacarb is an insecticide that requires bio-activation to a toxic metabolite by esterase enzymes (of which the silverleaf whitefly has an abundance). Further studies should investigate the potential of indoxacarb as whitefly control chemical.

Field selection of insect growth regulator resistance and potential cross-resistance

Introduction

Our bioassay data (Table 1) showed significant resistance in the field at Emerald to two insect growth regulators buprofezin and pyriproxyfen. Resistance mechanism studies of Emma Cottage suggested a cross-resistance between the two insect growth regulators. We investigated using field populations of silverleaf whitefly from Emerald in early 2002

Methods

We bioassayed unsprayed silverleaf whitefly populations (*field*) from Emerald and Emerald field populations that had survived field applications of buprofezin or pyriproxyfen (*field bup or pyri sel*).

Results

Results (shown in Table 3), indicated that, compared to unsprayed field populations, field exposure to pyriproxyfen increased resistance to buprofezin and that buprofezin exposure increased resistance to pyriproxyfen. These data are strongly suggestive of cross-resistance between pyriproxyfen and buprofezin but need confirmation by selection experiments under controlled laboratory conditions

Insecticide	Strain	Slope	LD_{50} ppm	RF	χ^2
buprofezin	Sus Field Field Pyri sel	2.7 1.0 0.6	0.3 (0.27-0.34) 3.87 (1 – 10) 16 (7 – 37)	1 12.8 53	3.6 4.7 16.7
pyriproxyfen	Sus Field FieldBup sel	2.8 0.93 0.49	0.11 (0.09-0.14) 0.43 (0.27 – 0.68) 1.8 (1.4 – 2.8)	1 4 16.3	3.8 6.3 15

Table 3. Response of populations of Emerald silverleaf whitefly to insect growth regulators. Unsprayed (*field*) after field selection with pyriproxyfen (*field pyri sel*) and to pyriproxyfen after field selection with buprofezin (*field bup sel*).

Improving the efficacy of piperonly butoxide as a pyrethroid synergist to control of silverleaf whitefly

Introduction

Piperonyl butoxide (PBO), has been used as a tank mix to improve the efficacy of pyrethroids against *H. armigera* on cotton since the early 1990's. Although effective control was initially achieved, control levels have been declining. In *H. armiger*, pyrethroid synergists such as PBO and ethion, work by inhibiting esterase enzymes responsible for resistance. However, pyrethroids bind to esterases much more rapidly than the synergists and effect and kill, it is necessary to apply the synergist some time prior to application of the insecticide.

Esterase metabolism is also responsible for pyrethroid resistance in the silverleaf whitefly and resistance is at such as high level that field control with pyrethroids is impossible. In light of the *H. armigera* experience, and in collaboration with Ms Susan Young, Imperial College, UK and Dr Graham Moores, Rothamsted-Research, UK) we set out to investigate whether PBO could acts as an esterase inhibitor in the silverleaf whitefly and whether pyrethroid synergism was possible.

Methods

In vivo and *in vitro* experiments were designed to test for esterase inhibition by PBO.

In vitro

Highly pyrethroid resistant (2000 fold), adult silverleaf whiteflies were homogenised in 0.02 M phosphaate buffer pH 7.0 containing 0.05% triton X-100 (20 μl/whitefly). PBO was added to aliquots, final concentrations were: 3, 1.5, 0.75, 0.38, 0.19, 0.09, 0.05 or 0.02 mM PBO, and the homogenates were incubated at 25^{o}C. Kinetic assays using 1-naphtholic esters were used to determine esterase activity. At least five replicates were carried out for each insect at each concentration of PBO.

In vivo

Cotton leaf discs (3 cm) were treated with PBO (EC) in water (1 %, v/v) using a leaf dip method (Cahill *et al*., 1995) and laid, adaxial surface down on agar beds (0.5 %), in ventilated Petri- dishes (3 cm x 1.5 cm). Control leaves were dipped in dH_20. All leaves were left to dry prior to whitefly introduction. Six to 10 adult *B. tabaci* (2 – 5 batches) were placed on the treated leaf discs and contained with a transparent, close fitting, ventilated lid, sealed with tape and left at 25 °C for the following time intervals; 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 30 h. As in Section 4.2.1.1 esterase activity was determined using kinetic assays and the results tested for significance using Students' one-tailed paired t-test.

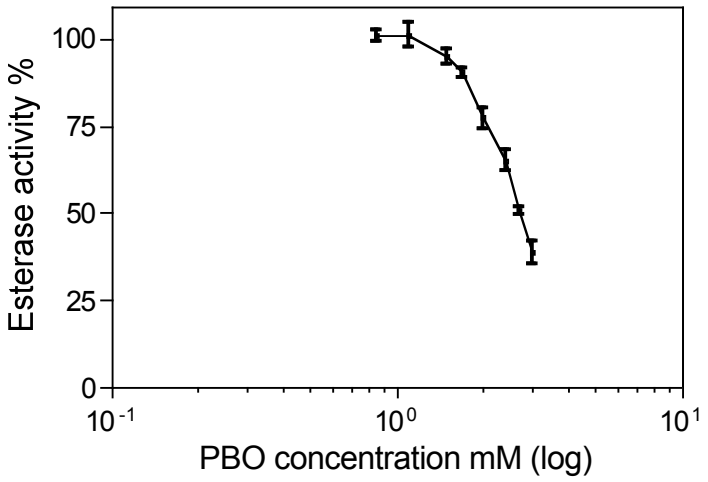
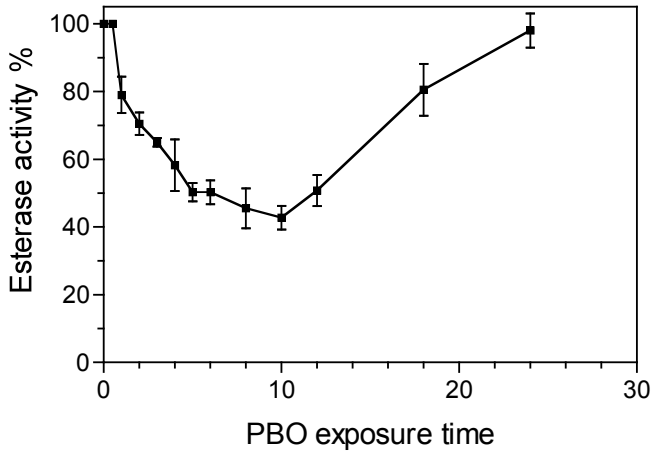


Figure 7. Mean *in vitro* silverleaf whitefly esterase activity plotted against log concentration PBO . Error bars represent 95% confidence limits.

In vitro data (Fig. 7), show that PBO inhibited esterases *in vitro* in pyrethroid resistant silverleaf whitefly. Esterase inhibition by PBO was not observed in insecticide susceptible, native, non-B biotype *B.* tabaci. While PBO inhibits esterases associated with pyrethroid resistance in the silverleaf whitefly, successful use of PBO as insecticide synergist, would depend on the speed and duration of esterase inhibition. This was tested by *in vivo* experiments (Fig 8).

Esterase assays confirmed that PBO inhibited B-type *B. tabaci* esterases *in vivo* and for some considerable period (Fig. 8). After 1h exposure to PBO, there was evidence of esterase inhibition and after 10 h, some 50% of esterase had been inhibited by PBO. Esterase activity was gradually restored thereafter.

Figure 8. ***In vivo* Esterase inhibition in silverleaf whitefly after exposure to piperonyl butoxide. (Error bars represent 95 confidence limits).**



Given that the time to maximal esterase inhibition was 5h – 10 at 25, we tested the hypothesis, both in the laboratory and in the field, that a lengthy delay between PBO application and pyrethroid application would increase pyrethroid efficacy against resistant silverleaf whitefly.

Lab Methods

We used leaf dip bioassays (as described else where in this report). Pyrethroids tested were lambdacyhalothrin and cypermethrin.

Field trials

Small plot replicated field trials (2 rows x 20 metres) were done on populations of silverleaf whitefly on Pima cotton at Emerald in March 2002. Treatments used were: untreated control, PBO sprayed control, pyrethroid alone, a pyrethroid plus PBO tank mix and a pre-treatment with PBO 5h prior to a pyrethroid application. Cotton plants were sprayed using a gas-hand operated boom sprayer (Hozelock), with 6 hollow coned nozzles and diaphragms (spacing 30 cm). The sprayer was run using a total water volume of 80 – 100L/ha at a pressure of 300 kpa. Insecticides were applied at the registered rate on cotton (Table 4). Initial spray application was carried out at 0800 – 0900 h with no cloud cover and the delayed pyrethroid application was at 1300 – 1400 h (10 % cloud cover). The maximum temperature was 29 °C with a RH of 20 %, and a wind speed of 5 – 10 km/h throughout.

Silverleaf whitefly were collected by placing a freezer bag over the top 4 terminals of 20 randomly chosen cotton plants per plot, and shaking vigorously to collect the adult whiteflies (via static electricity). The numbers of whiteflies were counted in the laboratory. Laboratory bioassays indicated that the test population was approximately 2000 fold resistant to pyrethroids.

Active ingredient	**Trade name**
250g /L Cypermethrin	Scud
800g/L Piperonyl butoxide	PBO (EC formulation)
100g/L Lambda-cyhalothrin	Karate Zeon

Table 4. Insecticides used in field trials

Results- lab bioassays

Data are shown in table 5. Under laboratory conditions ar 25^{o}C, similtaneously applied pyrethroid and PBO incompletely supressed pyrethroid resistance. However, a 5 hour pre-treatment with PBO, prior to application of pyrethroid, gave complete supression of resistance in the resistant whitefly population.

Insecticide	Strain	Slope	LD_{50} ppm	RF	χ^2
Lambdacyhalothrin	Sus Resistant	3.0 0.9	0.6 (0.5-0.73) 0.23 (0.16-0.33)	1 146	2.9 2.5
PBO pre-treat +Lambdacyhalothrin Lambdacyhalothrin + PBO in mix	Resistant resistant	2.9 0.89	0.58 (0.48 – 0.70) 31.1 (21 – 46)	1 52	3.4 12.1
cypermethrin	Sus Resistant	3.2 0.65	0.12 (0.10-0.14) 120 (60-230)	1 1000	2.4 10.9
PBO pre-treat +Cypermethrin Cypermethrin + PBO in mix	Resistant resistant	3.1 1.2	0.12 (0.10 – 0.15) 61 (35 – 231)	1 500	2.6 9.2

Table 5. Response of adult silverleaf whitefly populations to pyrethroids alone and to pyrethoids synergised by PBO (whiteflies were traeted with PBO 5 h proir to pyrethoid application).

Results – field trial

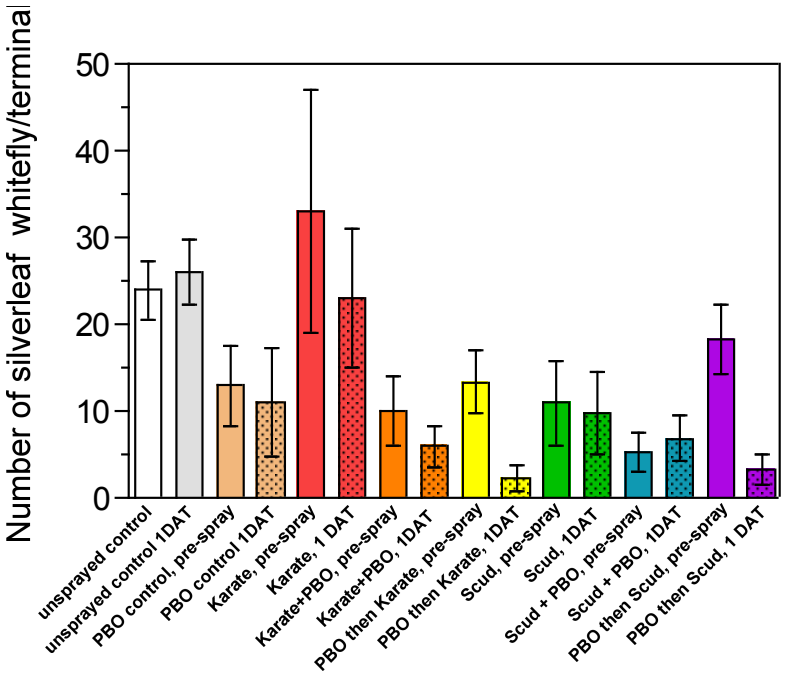


Figure 9. Response of Emerald, adult, silverleaf whitefly populations to pyrethroids, PBO + pyrethroid tank mix and PBO then pyrethroid 5 h later. (P = 0.05)

Results of the field trial (Fig 9), showed that no significant control (P= 0.05) of highly pyrethroid resistant silverleaf whitefly with pyrethroid sprays alone or with pyrethroid + PBO tank mixes. However, after a 5 h pre-treatment with PBO prior to pyrethroid application, there was a highly significant control of highly pyrethroid resistant silverleaf whitefly (p=0.05).

(c) Mechanisms of Resistance to Insect Growth Regulators in the Silverleaf Whitefly

Introduction

Our finding of resistance to insect growth regulators in the silverleaf whitefly gave rise to some insecticide resistance mechanism studies on which Emma Cottage recently completed a Doctor of Philosophy. It is not the intention of this report to provide more than a summary of this work, as full details are to be found Dr Emma Cottage's thesis, which has been provided to the CRDC.

The PhD project particularly concentrated on the potential role of esterases in silverleaf whitefly to the insect growth regulator buprofezin. Esterases in B-bioytpe *B. tabaci*, are over-expressed and we have found them to mediate a number of insecticide resistances. A summary of important findings are as follows:

Result and conclusions

Compared to buprofezin susceptible silverleaf whitefly, buprofezin resistant silverleaf whitefly had additional, inherited strongly staining esterase bands in both adult and nymphal lifestages ($E_{0.14\text{-}0.29}$). Total esterase titre was approximately 3-fold higher in the buprofezin resistant strain. These extra esterase bands could form the basis of rapid diagnosis of buprofezin resistant silverleaf whitefly.

.In vitro incubation ofinsect growth regulators buprofezin or pyriproxyfen with *B. tabaci* esterase showed a very marked inhibition of esterase activity in the buprofezin resistant silverleaf whitefly. After an incubation of one hour, approximately 50 - 60% esterase activity was inhibited by buprofezin concentrations as low as 10^{-9}M. Results from *in vivo* studies with buprofezin treated whiteflies also showed esterase inhibition in resistant silverleaf whitefly. Approximately 12 hours after exposure to buprofezin there was 85% enzyme inhibition. Esterase activity took some 50 hours to recover to the level of the uninhibited control.

The finding that two insect growth regulators, with differing modes of action, could bind to resistant silverleaf whitefly esterase adds weight to the possibility of cross-resistance between buprofezin and pyriproxyfen discussed in this report. Buprofezin is not an ester insecticide, therefore esterase/buprofezin binding cannot involve hydrolysis on the catalytic site of the enzyme. Esterases, however, are adhesive molecules and are well known for the ability to sequester toxins. Therefore, it is likely that esterases in buprofezin sequester buprofezin and other insect growth regulators, giving rise to a common resistance mechanism.

The PhD project also discovered that insect growth regulators acted as inhibitors of the neuro transmitter enzyme acetylcholinesterase (AChE) in the silverleaf whitefly. The buprofenzin resistant silverleaf whitefly population had evolved a mutant form of acetylcholinesterase (AChE) insensitive to attack by buprofezin. While the modes of action of insect growth regulators such buprofezin, are not well understood, sub-lethal inhibition of AChE would certainly contribute to general toxic effects on the silverleaf whitefly. AChE inhibition is known to slow insect growth and development and the evolution of AChE form insensitive to buprofezin, implies that AChE attack is an important mode of action of the insect growth regulators. The insensitive form of AChE in the silverleaf whitefly is unrelated to other mutant forms of AChE which are insensitive to organophosphates and carbamates in *B. tabaci*.

4. Conclusions

The outcomes of this research project have fully met the stated project objectives: To monitor the spread of the silverleaf whitefly onto cotton crops. To monitor and investigate insecticide resistance. To provide resistance information necessary for management of silverleaf whitefly in the field.

Accurate monitoring of whitefly populations on cotton since 1995 enabled us to forecast the development of whitefly problems on cotton, some years before the onset in central Queensland. Given that silverleaf whitefly numbers also increased in all other cotton areas of Queensland and NSW, it is only a matter of time and circumstance before whitefly population problems arise in other cotton areas. Our method of using the cotton leaves, collected for *Helicoverpa* resistance monitoring purposes proved an excellent and cost efficient means (for a very low budget project), of randomly sampling large numbers of cotton leaves for whiteflies. Our whitefly survey succeeded in detecting and accurately identifying silverleaf whitefly on cotton, where other more targeted surveys (at Emerald), failed to detect the silverleaf whitefly or its population build-up until it was too late.

Insecticide resistance studies on the silverleaf whitefly, funded by the CRDC, provided a valuable database on the resistance status of this pest. When faced with the silverleaf whitefly outbreak in central Queensland, we knew what insecticides would be effective for control and what were the potential field resistance problems. The serious resistance problems in Emerald, which we now face, due to application of insecticides in 2002 without any thought to resistance management, could have been avoided.

Without doubt, the greatest problem, which has come from the Emerald 2002 experience, is rapid field selection of resistance to the insect growth regulators, buprofezin and pyriproxyfen. Overseas, in the USA and Israel, silverleaf whitefly has been controlled due to effective use of insect growth regulators to prevent early season population build-up on cotton. Our findings that silverleaf whitefly had a pre-existing resistance to buprofezin, that the resistance is rapidly selected for, and that there may be cross resistance between buprofezin and pyriproxyfen, places doubt on whether reliance on insect growth regulators will work in Australia.

The outcomes of this project have been enhanced by the insect growth regulator, resistance mechanism studies by Emma Cottage. These data have provided knowledge of two resistance mechanisms and a mechanism for the suspected cross-resistance between buprofezin and pyriproxyfen.

The insecticide resistance project outcomes have been used as the basis for the central Queensland, silverleaf whitefly resistance management strategy.

Another major benefit to the industry from this project has been the development of a strategy for the effective use of the insecticide synergist, piperonyl butoxide as an economic means to control highly resistant silverleaf in the field. The discovery has been further developed with the lodging of a patent, in collaboration with Rothamsted-Research, UK Patent Application 0309773.0, to apply insecticide synergists with microencapsulated insecticides with the express purpose of controlling resistant insects by allowing prior synergist action in a tank mix with insecticide. Such novel insecticide application technology has the potential to provide control of many resistant insect pests in addition to the silverleaf whitefly.

While the development of suitable, delayed release microencapsulated insecticides for tank mixing with insecticide synergists may be some time in development, we have taken steps to ensure that the basis of this technology, has been made available to the cotton industry. In collaboration with NuFarm, a permit was obtained to allow the separate application of PBO and pyrethroid on cotton, "Ennervate Technolgy ™" and we are currently seeking registrations.

Clearly, there are many issues arising from this project that require follow-up and these are currently being addressed in CRDC project DAN 162C. While there is still much to learn about insecticide resistance in the silverleaf whitefly, results of this project have provided a scientific base to manage silverleaf whitefly on cotton in Australia.

5. Corporation's three Outputs - Economic, Environmental and Social

This project has addressed the three outputs of the CRDC by providing a proper knowledge and understanding of whitefly distribution, insecticide resistance. Without our data, effective resistance management could not have been planned. Uncontrollable populations of resistant whiteflies will limit the economic production of cotton, lead to excessive insecticide use with the consequent adverse environmental and social impacts.

6. Technical advances

A major output of this project has been the development of a strategy for the effective use of the insecticide synergist, piperonyl butoxide to economically control highly resistant silverleaf whitefly in the field. This research was further developed by lodgement of a patent (UK Patent Application 0309773.0), to apply insecticide synergists with microencapsulated insecticides with the express purpose of controlling resistant insects. This novel insecticide application technology is a weapon that has the potential to provide control of many resistant insect pests in addition to the silverleaf whitefly.

7. Plan to to further develop or to exploit the project technology

To exploit these discoveries, International patents will be filed and collaborations with agricultural chemical companies who have the potential to provide burst release microencapsulation technology, will be sought.

8. Presentation and dissemination of the project outcomes.

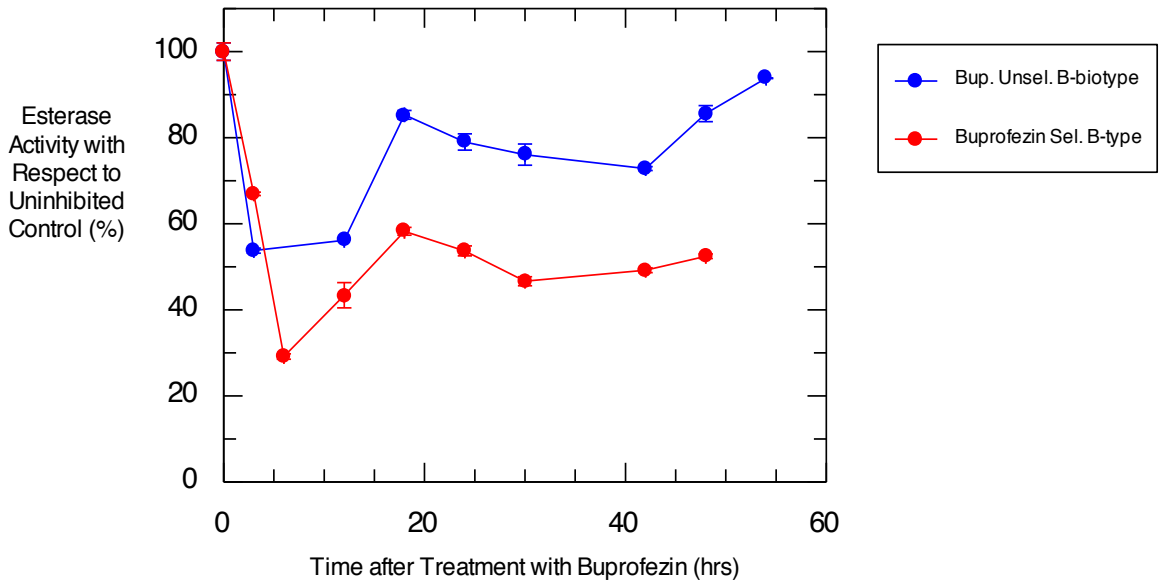
Results have been disseminated to researchers, growers and cotton consultants via the annual resistance tour, the CCA Annual General Meeting

9. Future research.

Issues arising from this project are being addressed in current CRDC project DAN 162C

10. Publications

We are currently preparing a number of papers for submission to scientific journals.


Esterase Activity with Respect to Uninhibited Control (%)
100
80
60
40
20
0
0
20
40
60
Time after Treatment with Buprofezin (hrs)
Bup. Unsel. B-biotype
Buprofezin Sel. B-type