FINREP93

Cotton ***Research and Development Corporation***

FINAL REPORT

"F.J. Byrne - Travel, whitefly paper World Cotton Research Conference"

DAN 84C

February 1993 to March 1993

Dr R.V. Gunning, Agricultural Research Centre, Tamworth (067-63 1128)
Dr F.J. Byrne, Rothamsted Experimental Station, UK.

NSW Agriculture

Cotton *Research and Development Corporation*

Project Title : **F.J. Byrne - Travel, whitefly paper World Cotton Research Conference**

Project Number: DAN 84C

Research Organisation: NSW Agriculture

Principal Researchers:

Dr R.V. Gunning
Senior Research Scientist
NSW Agriculture
Agricultural Research Centre
RMB 944, TAMWORTH, NSW 2340
Phone (067) 63-1128
Fax (067) 63 1222

Dr F.J. Byrne
Higher Scientific Officer
Department of Biological and Ecological Chemistry
Rothamsted Experimental Station
Harpenden, Herts., AL5 2JQ
United Kingdom

Project Supervisor:

Mr A. J. Shaw
Program Leader (Cotton)
NSW Agriculture
Narrabri Agricultural Research Station
PMB Narrabri, NSW 2390
Phone (067) 99 -1500

A final report prepared for the Cotton Research and Development Corporation

SUMMARY

1. INTRODUCTION

Whiteflies represent a major potential threat to Australian cotton. Whiteflies damage cotton directly feeding throughout the season and by gumming up the lint with honey dew in the late season. In Australia two species are reported to occur on cotton, the green house whitefly *Trialeurodes vaporarium* and *Bemisia tabaci* (the cotton whitefly). The cotton whitefly has a major pest status in cotton overseas (Africa, Middle east, Asia and America) which is exacerbated by insecticide resistance to pyrethroids and organophosphates. While whiteflies do not cause any problems on cotton in Australia at present, they are becoming more common on the crop. However, whitefly species, or insecticide resistance status on Australian cotton are not known

2. OBJECTIVES

The objectives of Dr Byrne's visit to Australia were:

1. For Dr Byrne to present a paper to the special whitefly workshop at the World Cotton Research Conference in Brisbane, February 1994 on "Resistance biochemistry of the cotton whitefly *Bemisia tabaci*".

2. To assist Dr Gunning to initiate some baseline toxicological and biochemical studies to assess the resistance potential of *B. tabaci* on Australian cotton crops.

3. To discuss colaborative research on *Helicoverpa* resistance biochemistry between Dr Gunning and the Rothamsted Insecticide Resistance Group.

3. RESULTS AND DISCUSSION

Whiteflies, collected from cotton in NSW and Queensland were all identified as the green house whitefly *Trialeurodes vaporarium*. Biochemical resistance studies showed no organophosphate resistance. However, whiteflies from sprayed cotton location had elevated esterase levels, compared to an unsprayed sites. This increased enzyme activity confers tolerance to pyrethroids.

4. CONCLUSION AND RECOMMENDATIONS

The greenhouse whiteflies found on cotton this year, have the biochemical capacity to develop pyrethroid resistance. While we presently do not appear to have *B. tabaci* on cotton in Australia, the cotton industry is constantly at risk of its accidental introduction. *B. tabaci* already occurrs in northern Australia and these whiteflies may be of the very troublesome insecticide resistant B-Types. Clearly, further research into both whitefly species is required.

We intend to continue the survey of insecticide resistance in the green house whitefly *Trialeurodes vaporarium* on cotton and to examine the biochemical

resistance profile of the *B. tabaci* from northern Australia. Funding for this project will be sought from the CRDC in the future

5. COMMUNICATION OF RESULTS

The results of this project were communicated to a meeting of the Cotton Pest Management Group at Narrabri in May. Their assistance was requested to help with the collection of whiteflies from cotton in Australia.

6. APPENDIX

Budget
Total funds contributed to the Cotton Research and Development Corporation were $3,890

7. SPECIAL CONSIDERATIONS

M.E. Balfe, N.A. Coleman and B.C. Crasswell (all of NSW Agriculture) provided technical support to this project.

ADDENDUM

Abstract

Whiteflies represent a major potential threat to Australian cotton. Whiteflies damage cotton directly feeding throughout the season and by gumming up the lint with honey dew late season. In Australia two species are reported to occur on cotton, the green house whitefly *Trialeurodes vaporarium* and *Bemisia tabaci*, the cotton whitefly. The cotton whitefly has a major pest status in cotton overseas (Africa, Middle east, Asia and America) which is exacerbated by insecticide resistance to pyrethroids and OP's. While whiteflies do not cause any problems on cotton in Australia at present, they are becoming more common on cotton. The species, or insecticide resistance status of whiteflies on Australian cotton was unknown

Dr Frank Byrne of Rothamsted Experimental Station, UK, is very experienced in the biochemical identification of whitefly species and resistance status. The CRDC funded Dr Byrne to attend a whitefly workshop at the World Cotton Conference in Brisbane and to collaborate with Dr Gunning (NSW Agriculture, Tamworth) to determine the species and resistance status of whiteflies on Australian cotton.

Whiteflies, collected from cotton in NSW and Queensland were all identified as the green house whitefly *Trialeurodes vaporarium*. Biochemical studies showed no evidence of any organophosphate resistance. However, whiteflies from sprayed cotton locations had elevated esterase levels, compared to unsprayed sites, which was expressed in pyrethroid tolerance.

Cotton *Research and Development Corporation*

Project Title : **F.J. Byrne- Travel, whitefly paper World Cotton Research Conference**

Project Number: DAN 84C

Research Organisation: NSW Agriculture

Principal Researchers:

Dr R.V. Gunning
Senior Research Scientist
NSW Agriculture
Agricultural Research Centre
RMB 944, TAMWORTH, NSW 2340
Phone (067) 63-1128
Fax (067) 63 1222

Dr F.J. Byrne
Higher Scientific Officer
Department of Biological and Ecological Chemistry
Rothamsted Experimental Station
Harpenden, Herts., AL5 2JQ
United Kingdom

Project Supervisor:

Mr A. J. Shaw
Program Leader (Cotton)
NSW Agriculture
Narrabri Agricultural Research Station
PMB Narrabri, NSW 2390
Phone (067) 99 -1500

A final report prepared for the Cotton Research and Development Corporation

INTRODUCTION

Whiteflies represent a major potential threat to Australian cotton. Whiteflies damage cotton directly feeding thoughout the season and by gumimg up the lint with honey dew in the late season. In Australia two species are reported to occurr on cotton, the green house whitefly *Trialeurodes vaaporarium* and *Bemisia tabaci*, the cotton whitefly. The cotton whitefly has a major pest status in cotton overseas (Africa, Middle east, Asia and America) which is exacerbated by insecticide resistance to pyrethroids and OP's. While at present, whiteflies do not cause any problems on cotton in Australia, they are becoming more common on the crop. However, the species and insecticide susceptibility of whiteflies on cotton in Australia are unknown. Clearly, this work is a high priority.

At Rothamsted Experimental Station in the UK, resistance researchers are very experienced in the biochemical identifcation of whitefly species and the resistance status. One of them, Dr Frank Byrne, agreed to assist Dr Gunning (NSW Agriculture) initaite a whitefly speciation and resistance monitoring program. Dr Byrne came to Australia to attend a whitefly research workshop at the World Cotton Research Conference in Brisbane. Dr Byrne is also involved in colaborative research on *Helicoverpa* resistance biochemistry between Dr Gunning and the Rothamsted resistance group. CRDC funded Dr Byrne's travel to Australia for the whitefly project and to allow him to present a paper at the World Cotton Conference in Brisbane.

2. OBJECTIVES

The objectives of Dr Byrne's visit to Australia were:

1. For Dr Byrne to present a paper to the special whitefly workshop at the World Cotton Research Conference in Brisbane, February 1994 on "Resistance biochemistry of the cotton whitefly *Bemisia tabaci*".

2. To assist Dr Gunning to initiate some baseline toxicological and biochemical studies to assess the resistance potential of *B. tabaci* on Australian cotton crops.

3. To discuss colaborative research on *Helicoverpa* resistance biochemistry between Dr Gunning and the Rothamsted Insecticide Resistance Group.

3. RESULTS AND DISCUSSION

Prior to Dr Byrne's arrival in Australia, Dr Gunning collected whiteflies from sprayed cotton crops throughout NSW (Macquarie Valley, the Liverpool Plains and the Namoi Valley) and from unsprayed sunflower crops. The whiteflies were taken back to Tamworth and stored, frozen at -20°C.

All whiteflies were identified biochemically as the green house whitefly, *Trialeurodes vaporarium*. The esterase enzyme system was used for identification of species. We did not find any *B. tabaci* and this was very encouraging. The pyrethroid and organophosphate insecticide resistance susceptibility of the whiteflies were

determined biochemically using assays for esterase and acetylcholine esterase enzyme activity respectively.

Esterases

Individual adult were homogenised in 5µl with 1.6 % Triton X-100 (100µl) containing 10% sucrose and a few grains of bromocresol purple and a further 15 µl of triton/sucrose mix was added. An aliquot, 15 µl was loaded directly from the microtitre plate onto a polyacryamide gel. Specially designed gel combs cast wells in the stacking gel which allowed the samples to be directly loaded from from the microtitre plate using a multichannel pippette. Gels were run at 250V for for 40 min at 5°C. Gels were stained for esterase activity with 0.1 mM 1-napthyl acetate and 0.2% Fast Blue RR salt in phosphate buffer (pH 6.0).

Results showed that some whiteflies from sprayed cotton locations had elevated esterase levels compared with unsprayed whiteflies (Fig.1). The elevated esterase levels allowed more pyrethroid metabolism in the whiteflies and this was expressed in pyrethroid tolerance.

Acetylcholine esterases

100 adults each from sprayed sites, were homogenised in 1ml of 0.1M phoshate buffer, pH 7.5, cotaining 0.1% Triton-X. The homogenates were tranfered to a 1.5 ml microcentrifuge tubes, spun for 5 min at 5000 rpm in a bench centrifuge and 100 µl aliquots from the supernatant diluted 10-fold with the same buffer. This was used as the enzyme source for the inhibition experiments.

A stock solution of the methyl paraoxon was freshly prepared in acetone. Aliquots were blown dry, and the insecticide was redissolved in a solution of ATChI/DTNB (0.75mM/0.075) in buffer. Sprayed and unsprayed whitefly strains were were tested simultaneously with the OP. This was done by adding 100 µl aliquots from each enzyme preparation (equivilent to a single insect) to adjacent wells of a microplate. ATChI/DTNB with inhibitor was added to the wells using an eight channel multipipette, giving an increasing inhibitor concentration down the plate and a final ATChI and DTNB concentrations of 0.5 and 0.05 mM respectively. A column of wells containing 100µl of buffer in place of homogenate served as a control for non-enzymic hydrolysis. Assays were run for a maximum of 30 min and the plots were assessed visually to determine any variation between strains to the inhibitors

Individual whitefly adults were similtaneously homogenised in 5 µl of 0.1M phosphate buffer, containing 0.1% Triton X-100, in seperate wells of a microplate, using a multiple homogeniser. The volumes in each well were immediately adjusted to 250 µl with the same buffer, remixed and then left at 4° C for approximately one hour to ensure efficient tissue solubilisation. Two 75 µl aliquots from each homogenate were then added to adjacent wells of a new microplate and their volumes further adjusted to 100 µl. After equibiration at room temperature, a solution of ATChI/DTNB, with or without 30 µM methyl-paraoxon was added (200 µl) to each of column of wells using eight channed multipipettes, to give an uninhibited control synchronised with the enzyme reaction. Reactions were monitored for 30 min in a microplate reader and a linear regression fitted by kinetic software to the data from each well.

We found no evidence of any organophosphate insensitive acetylcholine esterases in sprayed or unsprayed whitefly populations (Fig. 2), therefore organophosphate resistance in these greenhouse whitefly populations is vey unlikley.

4. CONCLUSION AND RECOMMENDATIONS

The greenhouse whiteflies found on cotton this year, had some biochemical capacity to develop pyrethroid resistance. Organphosphate resistance in the whiteflies is not likley to develop. While we presently do not appear to have *B. tabaci* on cotton in Australia, the cotton industry is constantly at risk of its accidental introduction. *B. tabaci* already occurs in northern Australia and these whiteflies may be of the very troublesome insecticide resistant B-Types. Clearly, further research into both whitefly species is required.

We intend to continue to survey insecticide resistance in the green house whitefly *Trialeurodes vaporarium* on cotton and to examine the biochemical resistance profile of the *B. tabaci* from northern Australia. Funding will be sought from the CRDC for this project in the future

5. COMMUNICATION OF RESULTS

The results of this project have been communicated to a meeting of the Cotton Pest Management Group at Narrabri in May. Their assistance has been requested with collection of whiteflies from cotton in Australia.

6. APPENDIX

Budget

Total funds contributed by the Cotton Research and Development Corporation were $3,890

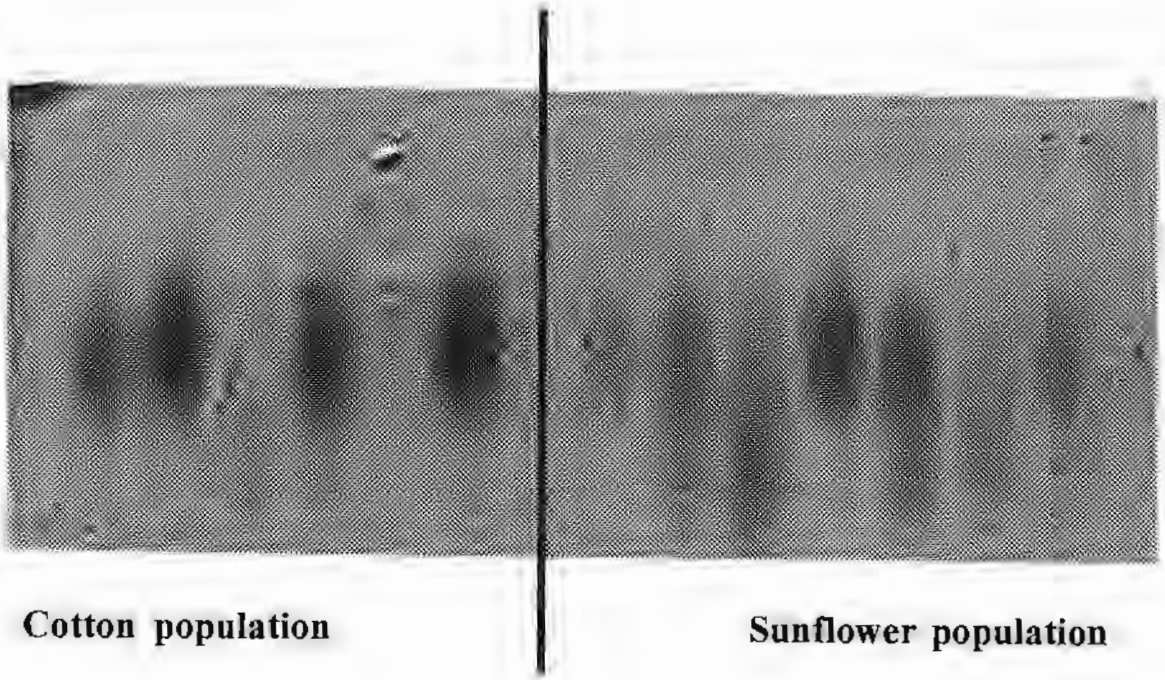
7. SPECIAL CONSIDERATIONS

M.E. Balfe, N.A. Coleman and B.C. Crasswell (all of NSW Agriculture) provided technical support to this project.

ITINERARY FOR DR BYRNE

February 9 1994	Depart London
February 10	Arrive Sydney
February 12	Arrive Brisbane
February 13 - 17	World Cotton Research Conference University of Queensland, Brisbane, Q
February 18 - 25	Tamworth Agricultural Research Centre - whitefly resistance work with Dr Gunning. Inspection of cotton crops in the Macquarie Valley.
February 26 -28	Discussions with resistance researchers at Narrabri
March 1 - March 24	Recreation leave
March 24	Depart Sydney
March 25	Arrive London

Figure 1
Polyacrylamide gels of individual green houseflies (*T. vaporaruim*) collected off cotton and unsprayed sunflowers. Gels were stained for esterase activity with α-napthyl acetate. Each track contains the eqivalent of 0.75 of an adult whitefly. The sprayed cotton populations contained many individuals with increased esterase activity.



Cotton population **Sunflower population**

Figure 2
Inhibition of acetylcholine esterase activity in greenhouse whitefly adults after treatement with methyl paraoxon. Whiteflies were collected from sprayed cotton or unsparayed sunflowers. There were no differences between the strains.