COTTON RESEARCH & DEVELOPMENT CORPORATION

FINAL REPORT

BIOLOGICAL CONTROL OF VERTICILLIUM WILT AND SEEDLING DISEASES OF COTTON

DAN 70C
1992 - 1995

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NSW AGRICULTURE

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SUMMARY

Virtually all of the cotton seed planted in Australia is treated with chemical fungicides to control seedling diseases. The control of Verticillium wilt was dependent on cultural practices until the recent introduction of resistant cultivars . The stability of this resistance is still being tested. Black root rot, Fusarium wilt and bacterial stunt have been recognised as important soil-borne diseases of cotton in the last few years. All of the pathogens that cause these soil-borne diseases are difficult to control because of their ability to survive and be dispersed in soil. Biocontrol methods are recognised as being more environmentally acceptable than chemical control measures.

A large collection of microorganisms with over 2000 isolates has been established at the Australian Cotton Research Institute for the purpose of identifying potential bio-control agents and developing biological control methods for seedling diseases and Verticillium wilt. These isolates have been collected from within, on and around the roots of cotton plants collected from commercial crops in different cotton growing areas of New South Wales. The collection is constantly being expanded.

About 29% of the isolates in this culture collection suppressed the growth of one or more major cotton pathogens when tested in the laboratory. About 300 of these isolates were further tested for disease control in pot experiments. Some microorganisms were highly effective in controlling seedling diseases and Verticillium wilt.

Biological control of seedling diseases was also evident under field conditions. Treatment of cotton seed with bacteria at planting resulted in increased plant stand. The biological control activity of the microorganisms need to be enhanced through further reasearch in order to make biocontrol methods agronomically feasible. There is also evidence of cotton growth-promoting effects produced by some isolates.

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Abstract

A large collection of microorganisms with over 2000 isolates has been established at the Australian Cotton Research Institute for the purpose of identifying potential bio-control agents and developing biological control methods for seedling diseases and Verticillium wilt. These isolates have been collected from within, on and around the roots of cotton plants collected from commercial crops in different cotton growing areas of New South Wales.

The culture collection has been screened for *in vitro* antagonism against the pathogens that cause seedling diseases (*Pythium* sp., *Rhizoctonia* sp.), Verticillium wilt (*Verticillium dahliae*), black root rot (*Thielaviopsis basicola*), and bacterial stunt (*Pseudomonas* sp.) of cotton. About 29% of the culture collection inhibited the growth of one or more pathogens.

A part of the culture collection was screened in *in vivo* glasshouse and growth chamber experiments for activity against the seedling disease pathogens and *Verticillium dahliae*. Several bacterial strains were found to be effective. Cotton seed inoculation with bacterial strains increased seedling survival by up to 80% (compared to the non-treated control). Some bacterial strains significantly reduced the incidence of Verticillium wilt. The proportion of plants with vascular symptoms ranged from 11-30% compared to 64-78% in the non-treated controls.

Some bacterial strains which were effective in pot experiments significantly increased seedling survival in field experiments. The addition of nutrients to the inoculum and the use of combinations of bacterial strains as a means of enhancing biocontrol activity need to be investigated.

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A final report prepared for the Cotton Research and Development Corporation

1. Introduction

Verticillium wilt is now recognised as the most important disease of cotton in Australia. As a result of its soil-borne habit, wide host range and its ability to survive for long periods, the pathogen that causes Verticillium wilt is difficult to control by conventional methods. Until the recent introduction of resistant cultivars control was dependent on cultural practices. The stability of cultivar resistance to the pathogen is still being tested.

Seedling diseases are always present and effective control is important so that the costs and problems associated with replanting can be avoided. Virtually all of the cotton seed planted in Australia is treated with chemical fungicides to control seedling diseases.

Black root rot, Fusarium wilt and bacterial stunt have been recognised as important soil-borne diseases of cotton in the last few years.

All of the pathogens that cause these soil-borne diseases are difficult to control because of their ability to survive and be dispersed in soil. There is increasing public concern about the use of pesticides in the cotton industry and biocontrol methods are recognised as being more environmentally acceptable than chemical control measures.

In recent years three biological products have been registered for the control of seedling diseases of cotton in the USA and there has been some progress in the development of biocontrol methods for verticillium wilt of cotton.

2. Objectives

(i) To evaluate potential biocontrol agents for seedling disease control.

(ii) To develop and compare strategies for the biocontrol of verticillium wilt.

(iii) To identify and evaluate potential biocontrol agents for the control of verticillium wilt of cotton.

3. Results and Discussion

Culture Collection

A large collection of microorganisms with over 2000 isolates has been established at the Australian Cotton Research Institute for the purpose of identifying potential bio-control agents and developing biological control methods for seedling diseases and Verticillium wilt. These isolates have been collected from within, on and around the roots of cotton plants collected from commercial crops in different cotton growing areas of New South Wales. The collection, which consists predominantly of bacteria, is constantly being expanded and maintenance of this collection has required regular subculturing.

Screening for *in vitro* antagonism

All of the culture collection has been screened for *in vitro* antagonism against *Pythium ultimum*, *Rhizoctonia solani*, *Verticillium dahliae*, *Theilaviopsis basicola*, and a fluorescent Pseudomonad (which causes bacterial stunt), all of which are pathogenic to cotton. About 29% of the culture collection inhibited the growth of one or more pathogens.

Inhibition of more than one pathogen by a bacterial strain may be the result of production of an antifungal compound which inhibits the growth of more than one pathogen, or production of more than one antifungal compound by the strain. Bacterial strains which inhibit multiple pathogens are attractive candidates for the control of the seedling disease complex whicb is caused by several pathogens.

In vivo screening in the glasshouse or growth chamber

Approximately 300 cultures have so far been screened *in vivo* against seedling disease pathogens (*R. solani* and *Pythium ultimum*) and/or *Verticillium dahliae*. The screening protocols developed for the bioassays and pot experiments mimicked field conditions as close as possible. Naturally infested soils were used in pot experiments.

Several bacterial strains that were effective against seedling disease pathogens were identified through the *in vivo* screening procedures. Results from two representative pot experiments are presented in Figure 1. A field soil naturally infested with seedling pathogens was used in these experiments. A large proportion of the strains that were tested increased seedling survival. The increase in seedling survival, compared to the untreated control, ranged from 16-64% in the first experiment (Figure 1A) and from 13-80% in the second experiment (Figure 1B).

Those bacterial strains which controlled seedling disease pathogens as effectively as, or better than, the standard fungicide seed treatment were subsequently tested in field experiments (see Tables 2 and 3).

Several bacterial strains provided good control of *Verticillium dahliae* in pot experiments. The proportion of plants showing vascular symptoms ranged from 11-86% compared to 64-78% for the non-treated control (Figure 2). Most of the promising bacterial strains have been evaluated in field experiments.

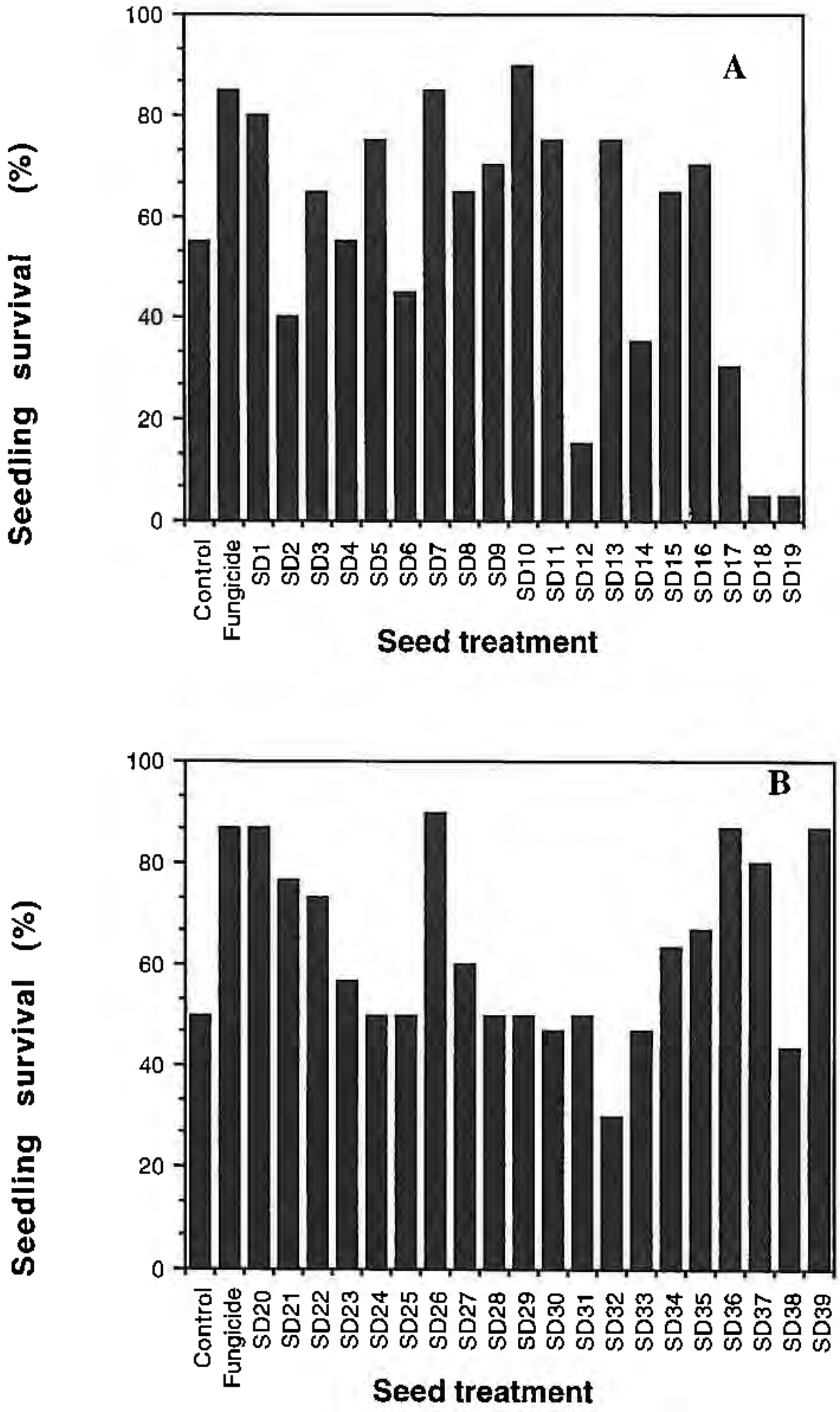

Figure 1. Effect of cotton seed treatment with bacterial strains on seedling survival in pot experiments. A and B are separate experiments. Seedling survival represents number of seedlings established as a percentage of the number of seeds planted.

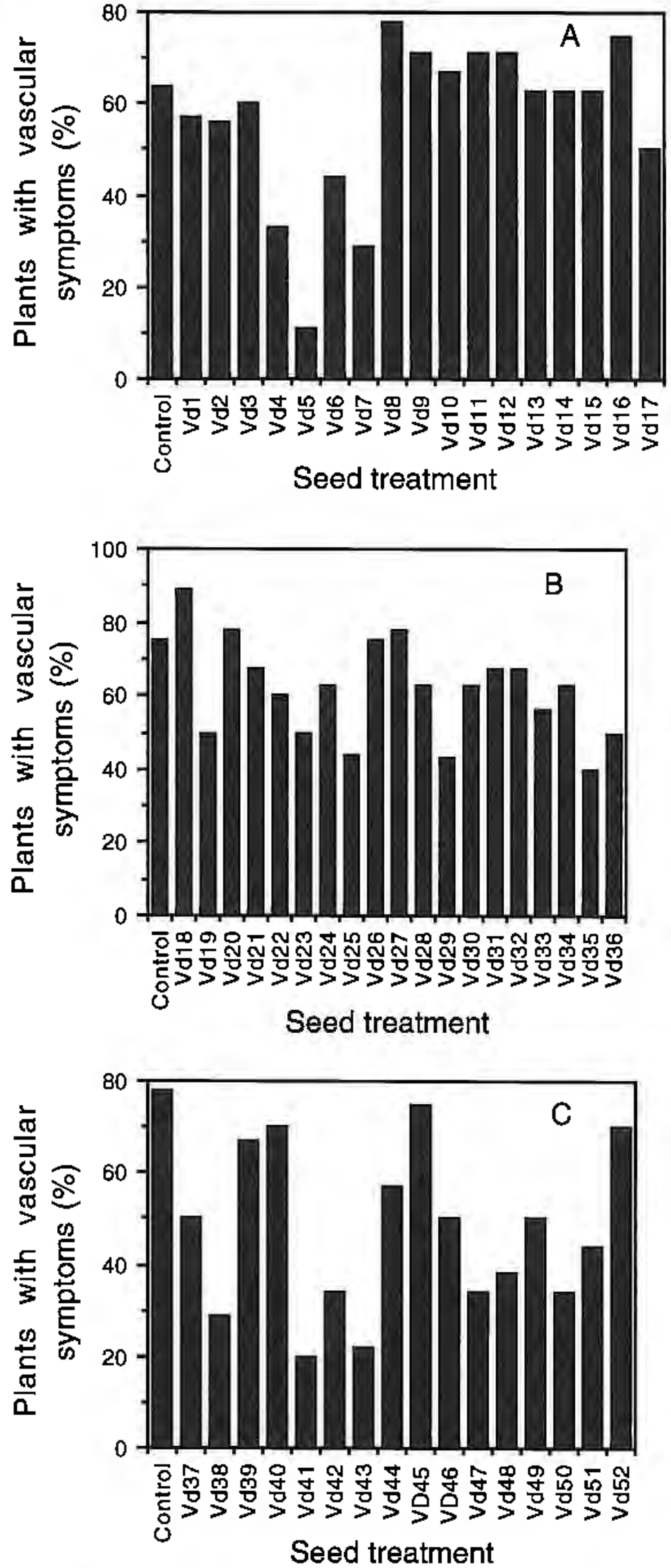

Figure 2. Effect of seed treatment with bacterial strains on Verticillium wilt of cotton in pot experiments. A, B and C are separate experiments

Field experiments

During the first cropping season of the project, potential biocontrol agents that were reported to have performed well elsewhere were tested in field experiments at Narrabri (Table 1). These treatments included a strain of *Bacillus subtilis* which was originally isolated in Sydney and then further developed and registered in the USA by Gustafson as Quantum 4000 for cotton seedling disease control and an isolate of *Pseudomonas fluorescens* resulting from extensive work at Texas A & M University. Other potential biocontrol agents resulting from work by a team from the University of Queensland were also included.

Seed treatment with bacteria resulted in only marginal increase in seedling survival. None of the strains in the ACRI culture collection had been evaluated under controlled conditions, and hence were not included in the early field experiments.

Table 1. Effect of seed treatment with bacterial strains on seedling survival in field experiments 1992-93.

Seed treatment#	Plant stand (%)§		Seed cotton yield (kg/ha)	
	Trial 1	Trial 2	Trial 1	Trial 2
Strain A	81	67	5606	5302
Strain A+Fungicide	87	79	5469	4975
Strain B	85	80	5665	5231
Strain B+Fungicide	81	81	5710	5318
Strain C+ Strain D	77	77	5531	4975
Strain A13	82	79	5534	5223
Strain Pf-5	74	78	5570	5183
Fungicide	84	78	5714	5123
Non-treated control	75	75	5460	5066
LSD	3.9	N.S.	N.S.	N.S.

All the bacterial strains were reported to have controlled seedling diseases and/or promoted plant growth elsewhere. Apron + Terraclor was used as the standard fungicide seed treatment.

§ Number of seedlings established as a percentage of the number of seeds planted.

N.S. - Statistically not significant

In the 1993/94 and 1994/95 seasons, 89 strains were tested in field experiments for seedling disease control. The selection of these strains was based on performance in pot experiments. However, the high degree of disease control achieved in the glasshouse did not translate into effective control in field tests. Seed treatment with bacterial strains resulted in only marginal increase in seedling survival (Tables 2 and 3). While this increase was statistically significant, it was agronomically not important. There was no significant increase in seed cotton yield during the 1994-95 season. In 1993-94, the experiment was severely damaged by a hail storm and hence not taken to maturity.

Table 2. Effect of seed treatment with bacterial strains on seedling survival in field experiments 1993-94

Seed treatment#	Plant stand (%)§		
	Trial 1	Trial 2	Trial 3
Strain SD1	58	74	NT
Strain SD5	55	74	64
Strain SD7	53	74	NT
Strain SD9	46	76	NT
Strain SD10	59	70	63
Strain SD11	54	73	50
Strain SD13	54	73	NT
Strain SD5+Fungicide	55	74	59
Strain SD10+Fungicide	NT	NT	63
Strain SD11+Fungicide	NT	NT	59
Fungicide	65	83	62
Non-treated control	57	75	56
LSD	9.1	N.S.	4.9

Apron + Terraclor was used as the standard fungicide seed treatment.
§ Number of seedlings established as a percentage of the number of seeds planted.
NT - Not tested N.S. - Statistically not significant

Table 3. Effect of seed treatment with bacterial strains on seedling survival in field experiments 1994-95

Seed treatment#	Plant stand (%)§		Seed cotton yield (kg/ha)	
	Trial 1	Trial 2	Trial 1	Trial 2
Strain SD20	73	49	5820	4283
Strain SD26	75	51	5540	4392
Strain SD20+SD26	71	NT	6150	NT
Strain SD36	NT	48	NT	4232
Strain SD39	NT	52	NT	4333
Fungicide	82	63	5750	4347
Non-treated control	69	50	5780	4333
LSD	4.2	6.8	N.S.	N.S.

Apron + Terraclor was used as the standard fungicide seed treatment.
§ Number of seedlings established as a percentage of the number of seeds planted.
NT - Not tested N.S. - Statistically not significant

Significant biological control of Verticillium wilt under field conditions was elusive. Sixty-three strains have so far been tested in the field for the control of Verticillium wilt. On one occasion, although seed treatment with bacterial strains did not result in disease reduction, it led to significant increase in seed cotton yield (up to 34%) compared to non-treated control (data not presented). These bacterial strains will be further investigated for plant growth-promotion effects.

4. Discussion with Reference to Objectives and Other Work

During the first field-season of the project, the culture collection was still in its inception. Potential biocontrol agents that were reported to have performed well elsewhere were obtained and tested in the field. Since then a large culture collection has been established at the Australian Cotton Research Institute. It has been necessary to develop bioassays and protocols for screening the culture collection.

Only a part of the culture collection has so far been screened under controlled conditions and tested in the field. The results indicate that some of the biological control agents can control seedling diseases in field. However, the activity of these biological control agents needs to be enhanced. The addition of nutrients to the inoculum and the use of combinations of strains needs to be investigated as possible means of enhancing the activity of biocontrol agents.

The bacterial strains tested in the field during the last two seasons were not effective in controlling Verticillium wilt. All of the field experiments were carried out in wilt-sick plots where the disease pressure was very high. Very few commercial farms would have such high disease pressure. Over the last year, several new bacterial strains with potential to control Verticllium wilt have been identified in the screening procedures. These strains will be field tested in the future.

The procedures used to select candidate biological control agents are important in finding field-effective strains. The bioassays and pot experiments were conducted under optimal disease conditions. However, microorganisms introduced into the field participate in a complex interaction with the host plant and the indigenous microflora. A more rigorous screening (eg. different soils, different pathogen strains) under controlled conditions would improve the ratio between 'glasshouse-successful' strains and 'field-effective' strains. Currently the screening of the culture collection is restricted to the cold part of the year due to the pathogen's requirement of cool temperatures for infection and disease development. Availability of a plant-growth room would greatly enhance the screening capacity.

Since the commencement of the project several other soil-borne diseases of cotton have attracted attention. Black root rot caused by *Thielaviopsis basicola*, Fusarium wilt caused by *Fusarium oxysporum* f.sp. *vasinfectum* and bacterial stunt caused by a *Pseudomonas* sp. are now considered to be significant. This project has increased relevance as a result of these new cotton disease situations. All of the pathogens that cause these soil-borne diseases are difficult to control with conventional methods because of their ability to survive and be dispersed in soil. Biological control methods which are based on the manipulation of the plant-micro-organism associations provide an ideal alternative.

5. Conclusions, Recommendations and Application to Industry

(i) Biological control agents developed elsewhere and in some cases registered for use on cotton in the USA did not give adequate control of seedling disease pathogens in field experiments at Narrabri.

(ii) A collection of microorganisms isolated from within, on and around the roots of cotton seedlings collected from commercial crops throughout NSW has been established at the ACRI, Narrabri and is continually being expanded. This collection of over 2000 isolates provides an invaluable resource for the development of biological control methods against all soil-borne diseases of cotton.

(iii) Approximately 29% of the isolates in the culture collection inhibited the *in vitro* growth of one or more of the cotton pathogens. Several bacterial strains gave significant control of seedling diseases and Verticillium wilt when tested in pots under glasshouse conditions with naturally infested soil.

(iv) Glasshouse and field screening of many isolates is still to be completed. The possibility of enhancing the activity of biological control agents by the addition of nutrients to the inoculum or the use of combinations of strains needs to be investigated.

(v) The approach considered, the methods employed and the resources of this project have direct application to other significant soil-borne diseases of cotton such as Fusarium wilt, black root rot and bacterial stunt.

6. Communication of Results

Contributions to Technical Conferences

Allen, S. J. (1992) Biocontrol of Verticillium wilt of cotton. *Proceedings of UNE's Third Graduate Seminar in Plant Pathology, 1992* .pp143-149.

Allen, S. J. (1994) Biological control of diseases of cotton. (Invited keynote address) *Proceedings of World Cotton Research Conference - 1.* Brisbane, Australia p 94.

Putcha, V. S., Dart, P. J. and Allen, S. J. (1994) Field application of biocontrol bacteria. *Proceedings of World Cotton Research Conference - 1.* Brisbane, Australia p 95.

Putcha, V. S., Dart, P. J. and Allen, S. J. (1994) Temperature as a factor in colonisation of cotton spermosphere and rhizosphere by *Pseudomonas cepacia*, and seedling disease control. p. 216 *In* Ryder, M. H., Stephens, P. M. and Bowen, G. D. (Editors) *Improving Plant Productivity with Rhizosphere Bacteria Proceedings of the Third International Workshop on Plant Growth-Promoting Rhizobacteria, Adelaide.*

Putcha, S. and Allen, S. J. (1994) The invisible 'goodies and baddies'. *Proceedings of the seventh Australian Cotton Conference*, Broadbeach, Qld. pp253-256.

Other Publications

Allen, S. J. and Putcha, V. S. (1993) Biological control of cotton diseases: Is it possible?. *The Australian Cotton Grower* **14**(6): 69-72.

7. Special Considerations

The maintenance and regular sub-culturing of a large culture collection with limited untrained assistance has been both difficult and time-consuming. NSW Agriculture has now purchased a freeze dryer which will eventually allow for long-term storage of cultures.

There has been an increased awareness by other researchers of the role and importance of soil micro-organisms and soil microbiology in nutrient cycling, trash management and soil structural studies. This has resulted in a major contribution on soil microbiology to the 1994 Annual CR&DC Soils Co-ordination Workshop and increased opportunities for involvement in other relevant projects.

The development of bio-control methods has potential for commercialisation and it has therefore been necessary to avoid giving details with regard to the identity of those organisms etc. that are being evaluated.

8. Appendix I - BUDGET

Item	1992/93	1993/94	1994/95
STAFF			
Salaries	$29,897	$32,492	$36.844
Leave loading	$ 449	$ 488	$ 553
Payroll tax	$ 2,093	$ 2,275	$ 2,579
Worker's insur	$ 747	$ 813	$ 921
Super. contrib.	$ 897	$ 1,625	$ 1,843
TOTAL STAFF COSTS	$34,083	$37,693	$42,740
TRAVEL			
Sustenance	$ 1,500	$ 1,500	$ 1,500
1994 ACGRA Conf.			$ 600
TOTAL TRAVEL	$ 1,500	$ 1,500	$ 2,100
OPERATING			
Lab. & G'house consum.	$ 1900	$ 1,900	$ 1,900
Automatic pipettes	$ 800	nil	nil
Vehicle running costs	nil	$ 900	$ 1,020
TOTAL OPERATING	$ 2,700	$ 2,800	$ 2,920
CAPITAL	$ 6,000	nil	nil
TOTAL REQUESTED	**$44,283**	**$41,993**	**$47,760**