

Annual, Progress and Final Reports

Part 1 - Summary Details

REPORTS

| Please use your TAB key to complete Parts 1 & 2. | |
|------------------------------------------------------|-------------------------------------------------------------------|
| CRDC Project Number: | US65C |
| Annual Report: | Due 30-September |
| Progress Report: | Due 31-January |
| Final Report: | Due 30-September |
| | (or within 3 months of completion of project) |
| Project Title: Diversity of VAM fungi in soil health | |
| Project Commencement Da | te: 1/1/2004 Project Completion Date: 31/12/2005 |
| Research Program: | 4 Farming Systems |
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Part 3.2 - Annual Reports (due end of September)

(Maximum four pages)

1. What were your major project objectives, milestones and performance indicators for the past year? (Please list these and any project results).

Objectives of 2003/4 were:

- a. To develop and refine DNA extraction techniques for soil and plant material
- b. To complete the development and testing of primers to identify VAM fungi.
- c. To isolate further reference VAM fungi to pot culture and obtain further sequence data.
- d. To collect field material from cotton cropping soil for experimental work.
- e. To set up growth experiments to answer the question: Does crop rotation change the diversity of VAM fungi in cotton soils?

2. Which of these have been achieved?

- a. DNA extraction techniques have been refined for soil and plant material.
- b. The primers from the UK that were to be used in laboratory were found to be unsuitable for detecting VAM fungi in cotton soils. New primer pairs were developed in Japan, and were found to detect mycorrhizal fungi in Australian cotton soil. Preliminary tests suggest that it will be suitable to complete the field studies. Analysis of field material is now underway.
- c. Expansion of the current reference culture collection is underway. Sequence data for each species will be gathered when purity of the pot cultures is confirmed.
- d. Cotton cropping field soil has been used to set up AMF trap cultures and in a growth experiment. The material is currently being processed. In addition, some of the field fungi have been placed in *in vitro* culture to overcome problems of contamination of fungi in soil.
- e. Growth experiments will commence when VAM fungi are determined to be in pure culture.
- 3. Which were not achieved and why? (Please provide detail of any problems you have had during the year and how you plan to address these problems).
- Full analysis of field material using molecular probes has not been completed but it anticipated that the work should be complete by the end of 2004. The molecular probes that were to be used were found to be unsuitable for detecting Australian AMF. Necessary changes have been made in the past few months. In addition, some fungi in pot culture appear to be contaminated, and this is being dealt with by placing these species in *in vitro* culture.
- 4. Are there any aspects of your research project do you envisage having problems with in the coming year and what is your contingency plan?

No problems are anticipated.

- 5. What are your specific project objectives, milestones and performance indicators for the coming financial year? Have any of these changed?
 - a. To process material from trap cultures.
 - b. To develop the *in vitro* culture collection of VAM fungi from Australian cotton cropping soils.
 - c. To complete molecular analysis of VAM fungal communities colonising cotton grown in field soil.
 - d. To complete growth experiments to determine whether AMF in cotton cropping soils are functionally diverse, ie: do they vary in their ability to promote growth in cotton.
- 6. Are changes to the Intellectual Property Register required? (You may also submit a separate confidential report of information, which should be included in the report but which you reasonably consider is confidential information).

No

7. How do you plan to demonstrate that your research is addressing the Corporation's three outputs - Economic, Environmental and Social?

Experiments will address the impact of the cultivation of cotton soils on VAM fungi, a crucial component of the soil biota. Thus the outcomes will enable a more reasoned approach to managing the sustainable production of cotton.

8. To what extent have your research results to date been disseminated to other researchers, growers or the industry? Please provide details and list any publications.

Ms Loke has been involved in discussions within the Molecular Biology and Soil Ecology groups of the cotton industry. She has also made contact with researchers in similar fields around the world and can now discuss specific issues with appropriate individuals.

Poster presentation at the 10th International Symposium on Microbial Ecology, Cancun, Mexico (August 22-29th, 2004). Poster title: Molecular community analysis of Arbuscular mycorrhizal fungi in cotton cropping soils.

Attended the postgraduate "On call Cotton tour" in Narrabri, April 29th and 30th, 2004. Met with other Cotton Research and Development Corporation funded postgraduates and discussed research topics.

9. How do you intend to communicate the results or findings of your research to other researchers /growers /industry in the next year? What assistance will you need?

We are already part of the Soil Ecology group, which has close links to the extension program of the Industry. The outcomes will also be published in scientific and trade journals.

10. Were there major highlights in your work over the last twelve months? Please give a brief outline.

Development of adequate probes to detect VAM fungi of cotton soils has now been largely achieved, enabling the analysis of important experiments. Good progress has been made in developing *in vitro* cultures of VAM fungi. A new technique has been developed to establish VAM fungi in clear culture with root tissue on agar. The process is being refined to culture VAM fungi from Australian cotton soils. These cultures provide pure fungi for molecular and growth experiments in the coming year.

Part 3.2.1 - Annual Supervisor Report (Scholarships Only)

This has been a challenging period for Stella. Attempts to gain funding to visit the molecular biology/ecology laboratory at York University, UK, were unsuccessful. Thus Stella has had to learn a number of new techniques without the support of a team experienced in the use of techniques necessary for this research. However, the probes used at York were found to be inadequate for detecting VAM fungi in soil at Narrabri, and those isolated to pot culture by me from cotton soils. Stella dealt with the issue by sequencing the fungi we had and then checking the sequences with other published probes. This resulted in finding probes developed by a Japanese team. This part of the project is progressing satisfactorily.

Some VAM fungi in pot culture were found with unexpected sequences and thus thought to be potentially contaminated. Odd sequences within the fungi may also be due to the presence of unusual DNA within the VAM fungi from cotton soils. To verify the source of sequence variation Stella has commenced attempts to place the VAM fungi in pure dual cultures with transformed roots. This has been an extremely difficult process and Stella has achieved some very promising results. The data from her work will have much greater validity because of her extra caution.

These two major outcomes now allow Stella to complete the core of her research project. The project is developing logically and I expect the outcomes to be of enormous value to the industry. Stella will have no difficulty in completing the research requirements of her postgraduate program.

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TRAVEL REPORTS

1. A brief description of the purpose of the travel.

To attend the 10th International Symposium on Microbial Ecology, Cancun, Mexico

2. What were the:

- a) major findings and outcomes
 - i. Research links were established with important research groups from Europe and America.
 - ii. Valuable advice was obtained during the conference regarding methodology and research limitations.
 - iii. We are now in electronic communication with other research groups investigating VAM fungi in agricultural soils.
- b) other highlights
- 3. Detail the persons and institutions visited, giving full title, position details, location, duration of visit and purpose of visit to these people/places. (Note please provide full names of institutions, not just acronyms.)

Conference attended: The 10th International Symposium on Microbial Ecology, Cancun, Mexico. August 22- 27th, 2004

4. a) Are there any potential areas worth following up as a result of the travel?

There has been some interest in the techniques we are using to study VAM fungal diversity in soil. In particular, the molecular probes we are using. We are in contact with labs in the US and Germany who are interesting in sharing their findings if the techniques can be used in their research.

b) Any relevance or possible impact on the Australian Cotton Industry?

I am now more confident that I can produce results that will have important ramifications for the management of Australian soils used to grow cotton.

5. How do you intend to share the knowledge you have gained with other people in the cotton industry?

I intend to present a poster at the next cotton conference