Cotton Catchment Communities CRC

FINAL REPORT

(due within 3 months on completion of project)

Part 1 - Summary Details

Cotton CRC Project Number: 1.03.27

Project Title: Management of cotton rhizosphere microbial interactions in Australian soils

Project Commencement Date: 01/07/06 **Project Completion Date:** 15/08/08

Cotton CRC Program: The Farm

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Part 3 – Final Report Guide (due within 3 months on completion of project)

(The points below are to be used as a guideline when completing your final report.)

Background

1. Outline the background to the project.

It has been accepted for many years that there is an interaction between plants and the microbiological life that exists in soil. This interaction is particularly important in the rhizosphere (Hiltner, 1904), where plant exudates directly feed the microbial population, which in turn is responsible for nutrient cycling, production of growth promoters, and occasionally development of pathogenicity. These factors are important to plant health. However, the difficulties of studying such interactions in the soil and the inability to grow the majority of soil microorganisms in the laboratory have resulted in limited research in this area.

With the introduction of genetically modified (GM) crops into agricultural production systems public concern resulted in renewed interest and research into the possible environmental consequences of growing GM crops (Brookes and Barfoot, 2005). This included the potential impact of GM crops on soil microbiology. GM crops have the potential to influence soil microbiology through (i) the exudation of transgenic proteins from the root system, (ii) the release of transgenic proteins from broken and dying roots, (iii) the incorporation of above ground plant material into the soil, and (iv) differences in exudation chemistry (Gupta and Watson, 2004; Knox *et al.,* 2006; Saxena and Stotzky, 2001).

Between 2003 and 2006 we assessed the potential for GM cotton, expressing either insecticidal Bt-proteins, glyphosate tolerance or both traits, to influence the soil microbiota. The analysis of the rhizosphere microbiology showed some differences, but none that were specifically identified as being caused by the expression of the introduced transgenic material (Knox *et al.,* 2004). The results did, however, imply that cotton variety and family groupings were more likely to be associated with the observed differences in the rhizosphere microbiota.

In this project we investigated the impact and significance of variety driven alteration of rhizosphere microbiology for a number of Australian cotton varieties.

Objectives

2. List the project objectives and the extent to which these have been achieved.

2.1. To develop a better understanding of the biodiversity and function of life in cotton soils.

2.2. To identify some of the specific chemical ways in which cotton plants interact with the soil microflora and microfauna, and
2.3. To understand how these interactions can be exploited as a crop management strategy tools for increased and sustained cotton production (e.g. root disease suppression).

The extent to which these objectives were achieved is presented in the results section of this manuscript and is further reviewed in the discussion.

Researcher team:

Narrabri – Oliver G.G. Knox, and Kellie Gordon

Adelaide – Gupta V.V.S.R., Marcus Hicks, Paul Harvey, Richard Lardner (mainly for DNA analysis of microbial communities after January 2008 following the departure of Oliver Knox).

Methods

3. Detail the methodology and justify the methodology used. Include any discoveries in methods that may benefit other related research.

3.1. Rhizosphere microbiology - 2 years of field experiments
3.2. Laboratory experiments – root exudates
3.3. Other field experiments

3.1. Rhizosphere microbiology:

Field establishment:

Experimental plots for the 2006/07 and 2007/08 seasons were located at the NSW DPI, Myall Vale Research Station, Narrabri on fields B2 and A3, respectively. The 2006/07 study involved 15 varieties; Sicala 40, Sicala 40BR, Sicala 43, Siokra V16, Sicot 189, Auburn 623, DP 16, DP50, DP50B (Ingard), Coker 315, Sicala V2, Sicot 71B, Sicot 24B, Sicot 71 and Sicot 71BR. The experiment was planted over 16 rows of the field in 7 m length plots, each covering 4 rows and providing 4 replicated planting areas. In 2007/08 to enable increased measurements to be made, reflecting commercially available varieties, and to further interrogate the observations made in the previous year, varieties Sicot 189, Sicot 71, Sicot 71B, Sicot 71BR, Sicala 40, Sicala 40B, Sicala 40BR and Coker 315 were planted over 16 rows of the field in 20 m by 4 row plots in 4 replicate blocks. Planting was carried out in both years to a plan produced using DiGGER to reduce in-field variation.

Rhizosphere sampling:

Sampling of rhizosphere soil was undertaken using methods developed in the previous project (CRC 1.01.02). Briefly, the process involved lifting cotton plants with a garden fork to expose the roots to a depth of about 20 cm. Soil adhering to the root surface was recovered by hand and carefully placed into labelled plastic bags, which were stored in an Esky for return to the laboratory. In the laboratory, soil was stored at 4°C, and most experimental procedures were initiated within 48 h of sampling. When longer delays were experienced, soil samples were frozen at -20°C and thawed overnight prior to experimentation. In the 2006/07 season, rhizosphere sampling was conducted at 55, 124 and 168 days after sowing, which corresponded to 'first flower', 'cut out' and 'defoliation' of the majority of the varieties planted in the experiment. In the 2007/08 season sampling was conducted at 63, 107, 141 and 176 days after sowing.

Microbial biomass and Soil Respiration:

Soil microbial biomass was assessed from 1 g sub-samples of the rhizosphere soil collected from each experimental plot using an adaptation of the ninhydrin reactive N and chloroform fumigation method with extraction in 3 mL of 0.5M K2SO4 and soil removal with centrifugation at 3000 x g for 5 minutes (Sparling and Zhu 1993). For each sample a Kec value of 29.3 was used to calculate the microbial biomass (Sparling et al. 1993). Soil respiration was not assessed using the recovered rhizosphere soils as this had been found to be too variable during the previous project (CRC 1.01.02). Instead intact soil cores were removed from field plots around

the time of rhizosphere sampling, using a 2.5 cm diameter poly-pipe auger to a depth of 11 cm to provide a 50 cm^3 semi-intact core. All the cores were incubated for 7 days in the presence of a 0.5M NaOH trap at 25°C in sealed 0.6 l Kilner jars (Cospak Pty. Ltd., Brisbane) and CO_2 evolution was established using dual end point titration of the recovered NaOH trap with HCl (Gupta et al., 1994).

Composition of bacterial population – Diversity of bacteria and fungi:
The denaturant gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (tRFLP) techniques were used to analyse diversity of bacteria and fungal populations, respectively, in the soils collected at different times throughout the growing season. DNA was extracted from 0.2 g of each rhizosphere soil using the MoBio PowerSoil DNA extraction kit.

For analysis of bacterial communities, 16S rDNA sequences were amplified using primers F968-GC and R1401 as previously described (Duineveld et al., 1998). Each PCR reaction contained 1 unit of HotStar Taq DNA polymerase (Qiagen Inc.), 0.2 µM of each primer, 200 µM of each dNTP, 2.5 µl 10 x reaction buffer and 5 µl of DNA, in a total volume of 25 µl. PCR was conducted using an Eppendorf mastercycler gradient thermocycler, as follows: Initial denaturation for 5 min at 95°C was followed by 20 cycles of 60 s at 94°C, 60 s at 67°C and 60 s at 72°C, with the annealing temperature decreased by 0.5°C each cycle, followed by 20 cycles of 45 s at 94°C, 60 s at 57°C, 45 s at 72°C, with a final extension step of 10 min at 72°C. Confirmation of successful PCR amplification was achieved by electrophoresis of a 1.5 µl aliquot of each PCR reaction on a 1.5 % TAE agarose gel, staining with ethidium bromide (0.5 µg/mL) and viewing under ultra violet light. The remaining reaction mix was subjected to DGGE analysis using an IngenyPhor DGGE electrophoresis system. Polyacrylamide gels (8 % w/v acrylamide:bis-acylamide at 37.5:1) contained a linear formamide/urea gradient ranging from 45-70%, and were overlayed with a non-denaturing stacking gel. Electrophoresis was conducted at 110 V and 60°C for 16 h. DNA fragments were visualized by staining with SYBR gold (Molecular Probes Inc.) for 30 min followed by de-staining in water for 20 min, and gels were visualised on a Dark Reader (Clare Chemical Inc.). Images of stained gels were captured using an Olympus E500 SLR digital camera. DNA fragment position and intensity was determined using the GelQuant software.

tRFLP analysis of fungal communities was carried out using the same PCR conditions as above, except that primers ITS 1F and ITS 4 (Gardes and Bruns, 1993; Wakelin, 2007) were used to amplify a portion of the ITS region. The forward primer was labelled with FAM and the reverse primer with HEX. PCR Cycles consisted of 5 min at 95°C followed by 35 cycles of 60 s at 94°C, 60 s at 56°C and 90 s at 72°C, with a final extension of 10 min at 72°C. Following PCR amplification, products were cleaned up using the SureClean kit (Bioline Inc.) and DNA was re-suspended in 20 µl sterile water. 5 µl of cleaned PCR product was then digested using restriction enzyme CfoI for 3 h at 37°C, and the reaction stopped by heating to 65°C for 20 min. Terminal restriction fragments were separated using capillary separation (ABI 3730 DNA analyser, Australian Genome Research Facility, Adelaide), with a LIZ500 – 250 size marker (ABI). Size and intensity data were assessed using the GeneMarker analysis software (SoftGenetics Inc.), with a minimum cut off of 100 intensity units used to distinguish terminal restriction fragments from background noise.

DGGE and tRFLP fragment data were analysed using the Primer6 software package (Primer-E Ltd, Plymouth, U.K.). Abundance data were log(x+1) transformed and similarity matrices constructed using the Bray Curtis algorithm. Cluster analysis, followed by similarity profile testing was used to identify significant groupings of communities in response to cotton varieties. Relationships between samples were mapped in ordination plots using non-metric multidimensional scaling (MDS).

Populations of *Fusarium* spp. and *Trichoderma* were assessed in the 2006/07 season using selective media recovery. This involved preparing a 1 g of soil (DWE) dilution in ¼ Ringer's solution, spread plating 100 µl to dryness onto Trichoderma Selective Media (TSM) and PCNB agar plates, and incubating them at 25°C for 2 to 5 days to allow for colony development.

Samples from 2007/08 season were also analysed using a molecular based DNA technique that quantifies the levels of DNA present of populations of *Trichoderma* spp, a beneficial soil fungus. See http://www.sardi.sa.gov.au/pages/fieldcrops/csra/pathology_quarantine/diagnostics/diagnostics_services.htm:sectID=38&tempID=1 for further details and methodology outline.

Physiological profiling of microorganisms:
The respiratory response of rhizosphere microbial communities to 22 carbon and nitrogen based substrates was determined using a modified MicroResp® technique as described by Campbell et al. (2003) modified with specific substrates selected for Australian cotton soils (Gupta, VVSR, CSIRO unpublished; Gupta et al., 1998). Differences between varieties were analysed using canonical analysis (GenStat version 10.2.0.175, VSN International Ltd.)

Ammonium oxidizer populations:
The populations of ammonium oxidizing (AO) bacteria were determined from a soil dilution (1 g in 10 mL of ¼ Ringer's solution) using a 96 well plate assay, which allowed the establishment of 8 replicates of each rhizosphere soil. The inoculated plates were incubated in the dark for 21 days at 21°C before establishing the dilution point at which plated populations stopped oxidizing ammonium to nitrite (Weaver et al., 1994). Population numbers were estimated using a most probable number (MPN) assessment from each replicated dilution series.

Nitrification:
The ability of 0.5 g sub-samples of the recovered rhizosphere soils to produce nitrite both with and without the addition of ammonium sulphate was assessed using a soil incubation study. Levels of nitrite produced after 0, 5 and 24 h were determined colourimetrically following the addition of a nitrite reagent, from absorbances read at 520 nm, against a sodium nitrite standard series. Rates of nitrification were assessed following removal of the background levels of soil nitrification.

N fixation by free-living N fixing bacteria:
In order to assess the levels of potential N fixation by free-living N fixing bacteria rhizosphere soils under different cultivars collected at various times were analysed using the acetylene reduction method (indirect assay which provides an indication of N fixation level) based on modified method of Hardy et al. (1968). Ten gram of rhizosphere soil samples were weighed into 27 ml McCartney bottles and amended with water or carbon sources. Following the addition of water or C sources (@ 1 ml per sample) bottles were closed loosely and incubated overnight. The sample bottles were then sealed with a Suba seal (W.H. Freeman & Co., Barnsley, UK), 5 ml headspace was removed with a gastight syringe (SGE Pty Ltd, Melbourne, Australia) and replaced with 5 ml of C_2H_2 (20% vol/vol equivalent). All the bottles were incubated at 27°C in the dark. After 24 h, the production of C_2H_4 via nitrogenase activity was measured using a gas chromatograph fitted with a flame ionisation detector and a 2.5 m by 1.5 mm column packed with Poropak N, as described by Turner and Gibson (1980).

Statistical analysis:
All statistical analyses were done using Genstat (PC/Windows XP; Lawes Agricultural Trust, Rothamsted Experimental Station), ANOVA and regression coefficients were done as per the principals described by Snedecor and Cochran (1980). Multivariate analyses such as Principal Component Analysis (PCA) and Canonical Variate Analysis (CVA) were done using the carbon substrate utilization profile data as per details given in Harch et al. (1997).

3.2. Laboratory experiments – collection and interrogation of root exudates

Hydroponic and non-soil based growth of cotton seedlings
In both the hydroponic and ECAM (Wu *et al.,* 2000a; Wu *et al,.* 2000b) systems cotton seeds were sterilised by washing in a 50% ethanol (v/v) and 10% (v/v) household bleach solution for 3 minutes, before being recovered and rinsed three times in sterile distilled water. Seeds were then germinated on sterile filter paper disks, moistened with 2 ml of sterile distilled water in an inverted Petri dish at 25°C.

The hydroponic system consisted of a series of twelve 50 ml Nunc tubes, which had 2 mm diameter wire mesh disks suspended 3 cm form the top of the tube via pieces of aluminium wire. The Nunc tubes were filled with a 0.1 concentration of Hoagland's solution to just cover the wire mesh. Individual germinated seeds were placed onto the wire mesh and 0.25 μm filtered air was pumped to the bottom of the tubes using a fish tank air pump with a flow rate of 4 l/min in combination with sterile silicon tubing.

The ECAM method involved adding 30 ml of 0.3% water agar to the bottom of 250 ml sterile beakers. Five germinated seed of specific varieties were placed directly on to the agar surface of each beaker. In both systems the seedlings were allowed to develop within a laminar flow cabinet to maintain sterility.

3.3. Other field research

3.3.1 Colonization of cotton varieties by arbuscular mycorrhizae:

Cotton is an arbuscular mycorrhizal (AM) dependent plant and poor establishment of the symbiosis may have a significant impact on crop nutrition. AM fungi assist cotton with the acquisition of water, phosphorus (P), zinc (Zn) and several other trace nutrients**.** In response to the lack of scientific data (Walter, 2005) and concerns that GM crops may have negative impacts on mycorrhizal symbiosis (Davidson, 2005; Shaw, 2005) we conducted a study of AM colonisation of roots of several cultivars of cotton and demonstrated that colonisation was not affected by any of the transgenic modifications. This work was done in collaboration with David Nehl and his team in field based experimentation.

3.3.2. Nematodes in Australian cotton soils

In collaboration with other researchers at ACRI, the principle investigator has identified new associations between plant parasitic nematodes in Australian cotton soils**.**

3.3.3. Persistence of *Bt*-protein in Australian soils

There was a close collaboration between a project, funded by DEHWA, investigating the levels of bioactive Bt-proteins in Australian soils and the members of this project. Soil samples from field experiments conducted in this project were used in the DEHWA project while the findings about the persistence of bioactive Bt-proteins in cotton soils assisted with our understanding of the microbiology of cotton soils.

3.3.4. Association of soil organisms with *Helicoverpa armigera* in cotton soils

The principle investigator has made some preliminary observations on soil microorganisms, e.g. nematodes and fungi, interacting with the *H. armigera* larvae and pupae with potential to aid in the control of the insect pest.

Results

4. Detail and discuss the results for each objective including the statistical analysis of results.

4.1. Rhizosphere microbiology - 2 years of field experiments

Microbial biomass and soil respiration

Data on microbial biomass showed seasonal variation over the 2 years of this study. No significant differences were observed between the varieties at any sampling time (Figure 1). In general a high level of replicate variability was observed.

There were no significant and consistent differences in soil respiration between sampled varieties over the course of either season. However, in 2006/07 soil respiration at defoliation was roughly half that observed at first flower and cut out (Figure 1.). In 2007/08 the lowest periods of respiration were seen around cut out of the crop. Respiration significantly differed (P<0.05) between sampling times over each season, with varietal trends observed. In general, the use of a semi-intact core method reduced variability compared to the use of soil samples from the more destructive rhizosphere soil sampling.

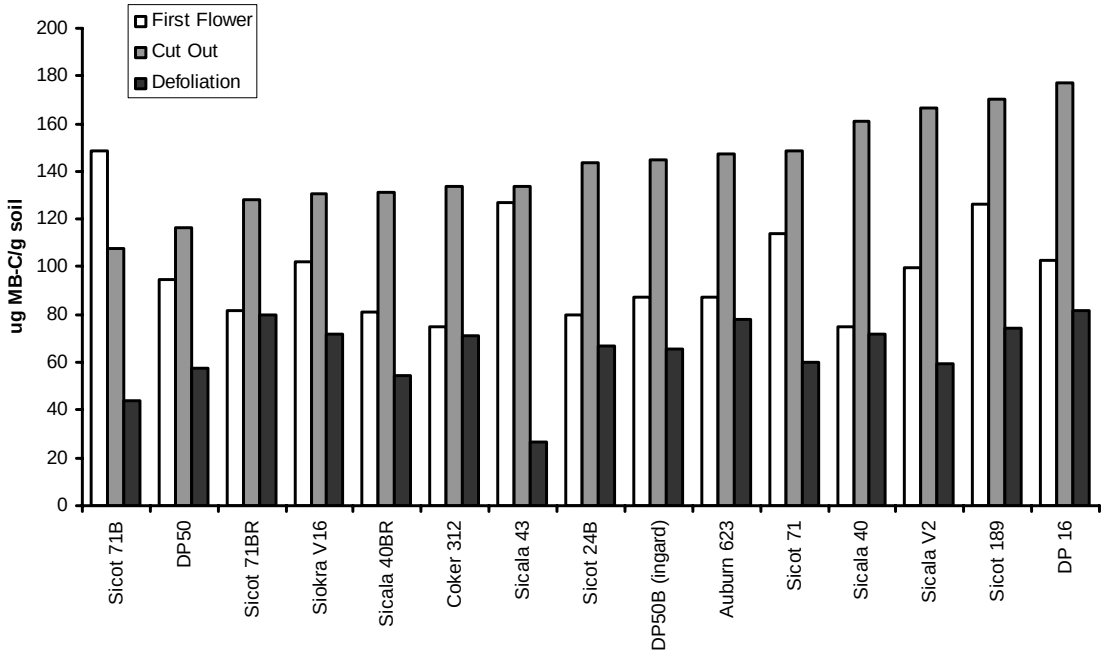


Figure 1. Ninhydrin reactive N assessed microbial biomass of rhizosphere soils sampled from various cotton varieties during the 2006/07 season.

Whilst respiration did not significantly different between varieties over both assessed seasons, a rough extrapolation of the average difference in respiration between soils collected from under Sicala 40BR (lowest levels) and either Sicot 71B or Sicot 189, with the highest levels of respiration recorded for 2006/07 and 2007/08, respectively, was around 6 µg CO_2 evolved per g soil per day. If we consider this could occur over a cotton season (180 days) for every hectare of cotton (10000 m^2, 7% rhizosphere, depth of 20 cm, bulk density of 1.3 g/cm^2) then we get a difference of 196 kg of CO_2. This evidence, although preliminary, indicates that there may be

potential for cultivar selection as part of systems designed to mitigate CO_2 emission in the cotton agricultural landscape.

Composition of bacterial population

An example for the population analysis based on DGGE banding patterns of 16SrDNA 2007/08 season is shown in Figure 3. Analysis of the banding patterns indicated that there was quite a strong variety and family based association of specific rhizosphere microbial populations early in the season (Figure 2). This familial and variety based association was not observed later in the season.

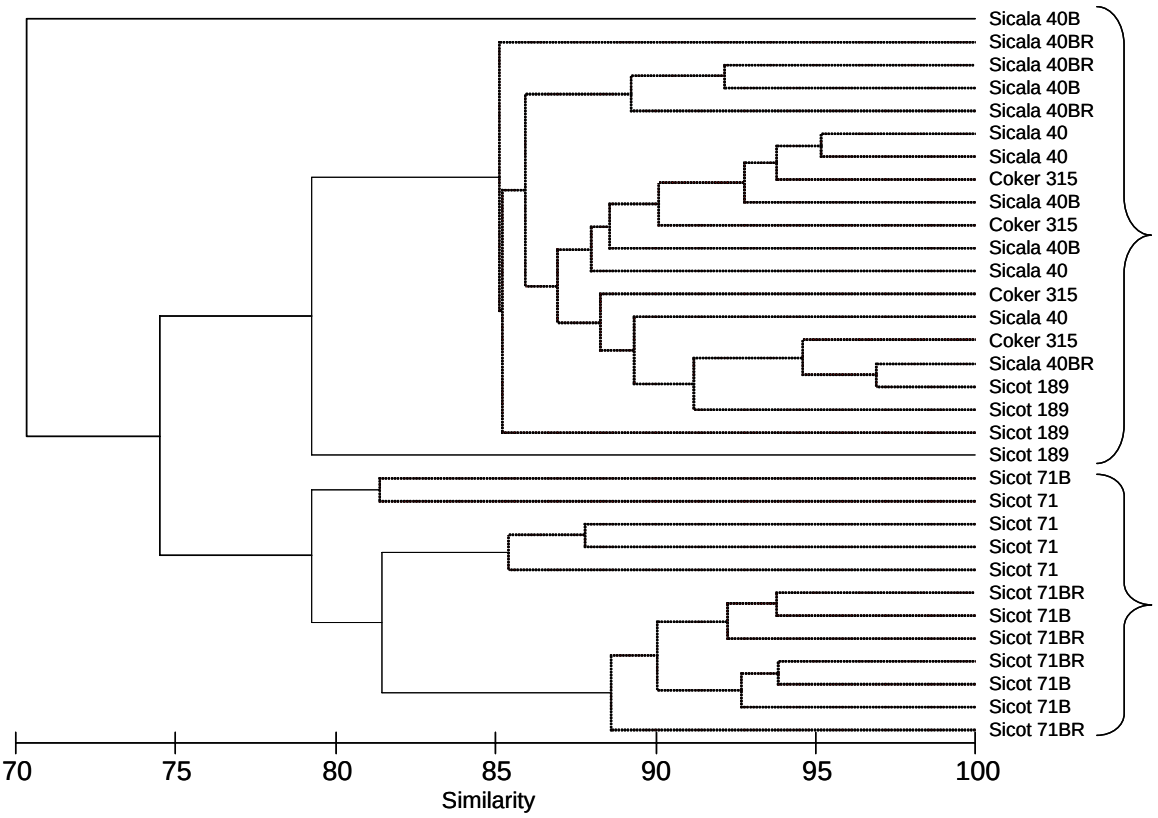


Figure 2. Cluster plot showing significant grouping of varieties by 16S DGGE analysis. Bracketed samples encompass groupings that are dissimilar (p<0.05); 2007/08 season.

Results from multivariate analysis of data for bacterial and fungal populations in rhizosphere samples from the two cotton season are given in Figures 4-7. Data shown in Figure 4 indicates that at the first sampling, during 2006/07 season, there was a significant separation of bacterial communities associated with Sicot 71 family of varieties from that for other varieties. A similar trend was observed in 2007/08 season (Figure 6). The varietal based clustering in terms of bacterial diversity was only observed during the first two samplings which disappeared in samples from later samplings (2nd and 3 sampling in 2006/07 season and 3rd and 4th sampling in 2007/08 seasons) i.e. 108 days after planting (Figures 4 and 6). A clear and separate clustering of data on bacterial populations associated with Sicot 71 family of varieties compared to other varieties can be seen at the first two samplings in 2007/08 season. During 2006/07 season, rhizosphere bacterial data for Coker 312 variety clustered with Sicot family but in 2007/08 season both Coker 312 and Sicot 189 varieties clustered with Sicala family varieties. The differences in the two

seasons are a reflection of the effect of environmental factors on cotton plant growth and associated microbial diversities.

Results of the analysis of data for bacterial diversity for the four parental varieties, i.e. without GM varieties, are shown in Figure 5. Results indicate evidence for varietal based differences in rhizosphere bacterial diversity. For example, a clear separation of banding pattern was seen (generally <60% similarity) at the first sampling during 2006/07 season). Sicala 40, Coker 312 and Sicot varieties clusters separately but the separation between the two Sicot varieties, i.e. Sicot 189 and Sicot 71, was not clear. Varietal differences were generally stronger with the data from earlier samplings which disappeared as the season progressed, a similar observation to that with all the data.

Rhizosphere samples from both seasons were also analysed for the diversity of fungal communities using TRFLP-ITS analysis. Multivariate analysis of the data showed very little or no clear and significant clustering of varieties in terms of their fungal community composition during both seasons (data not shown). Results from analysis of data for different sampling times also showed no clear separation in terms of rhizosphere fungal communities (Figure 7). In this analysis we only used the rhizosphere samples i.e. soil that is closely adhered to root and hence may not fully represent the hyphal networks that fungi can form away from roots. Results from the analysis of fungal communities using culture based methods also showed no clear variety based trends (discussed in a later section on page 20).

Figure 3. Photo of the DGGE gel for the 16S rDNA products from the rhizosphere samples collected at 1st sampling during 2007/08 field experiment. M – marker lane.

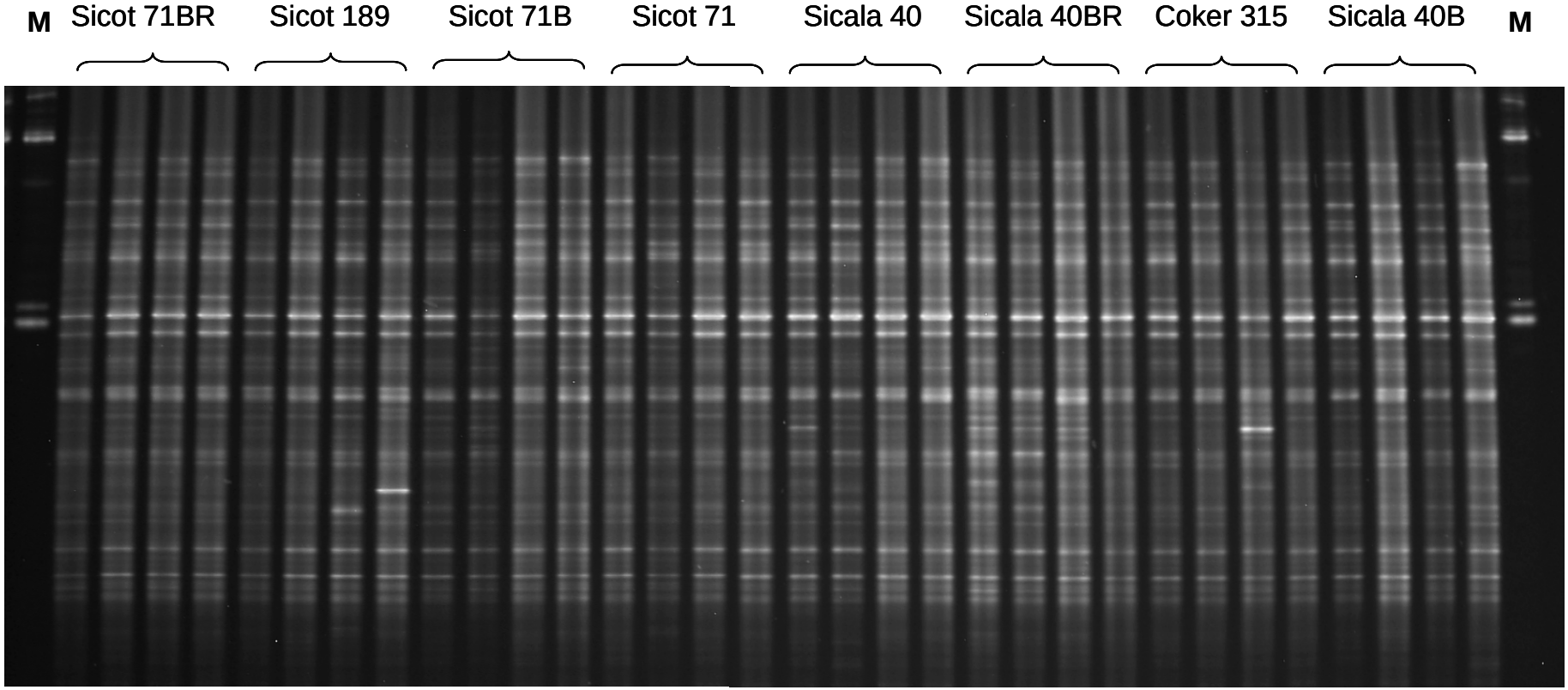


Figure 4. Dendograms and multi-dimensional scaling analysis of data on 16S rDNA-DGGE bacterial profiling for rhizosphere soils from 2006/07 field experiments. Circled groups of points represent significantly similar (P<0.05) groupings based on cluster analysis.

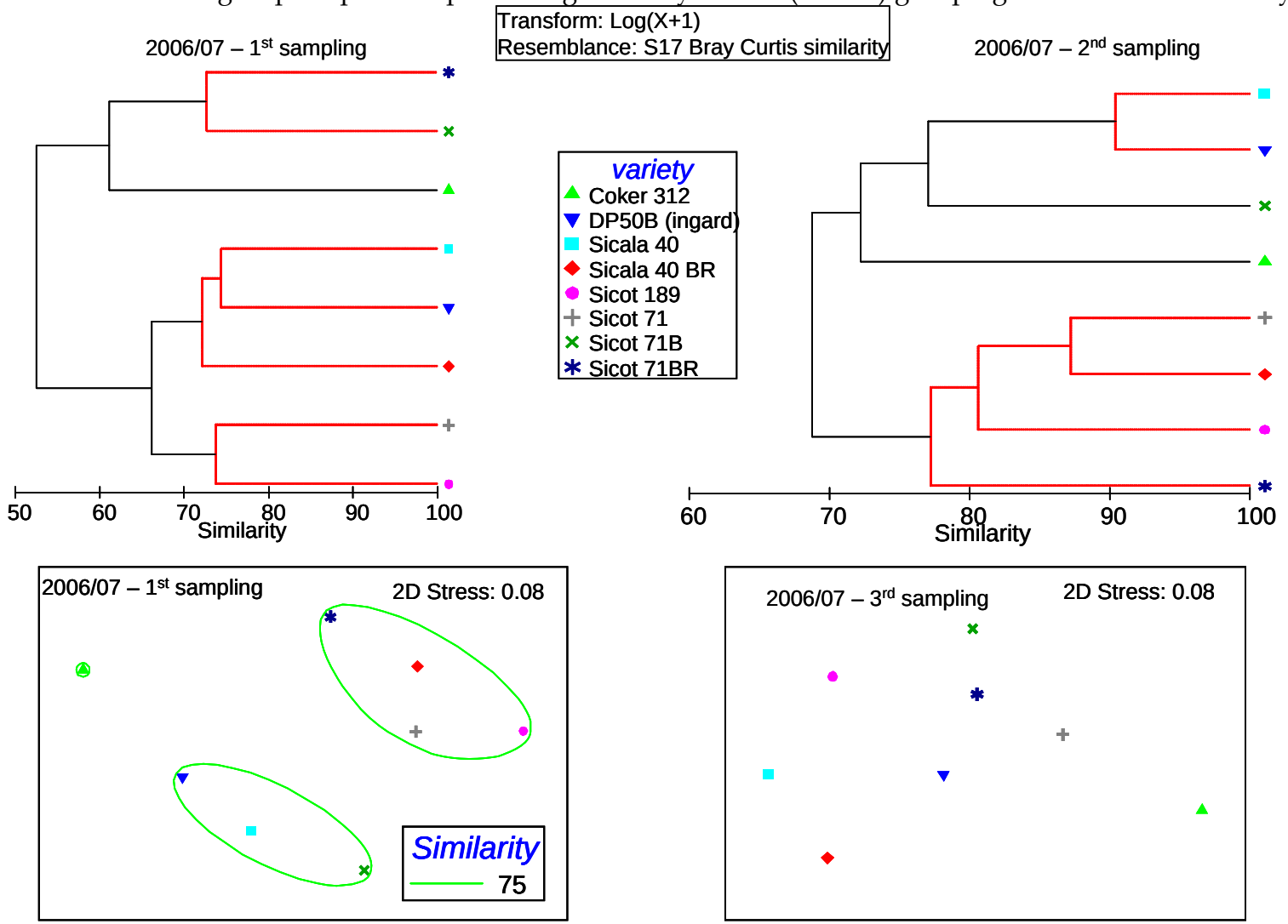


Figure 5. Dendograms for data on 16S rDNA-DGGE bacterial profiling of rhizosphere soils for different cotton parents from 2006/07 field experiments.

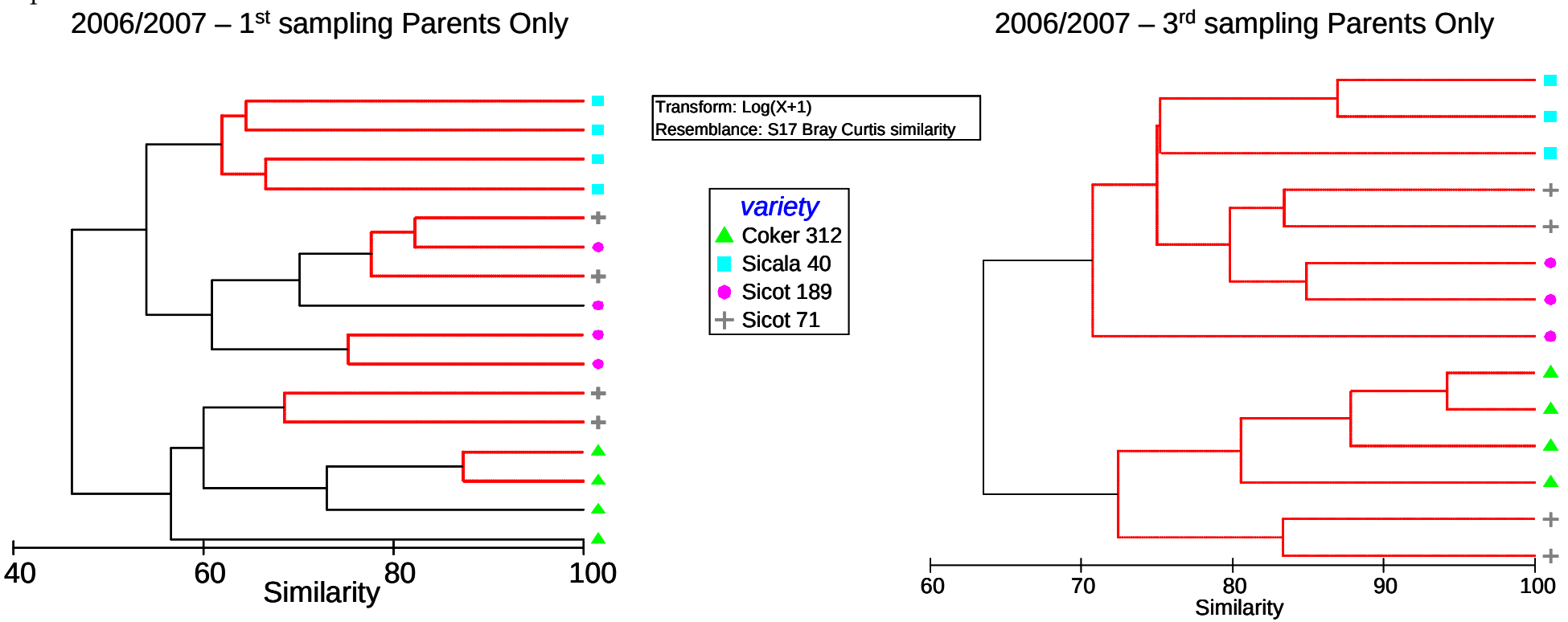


Figure 6. MDS analysis of data on 16S rDNA-DGGE bacterial profiling for rhizosphere soils from 2007/08 field experiments. Circled groups of points represent significantly similar (P<0.05) groupings based on cluster analysis.

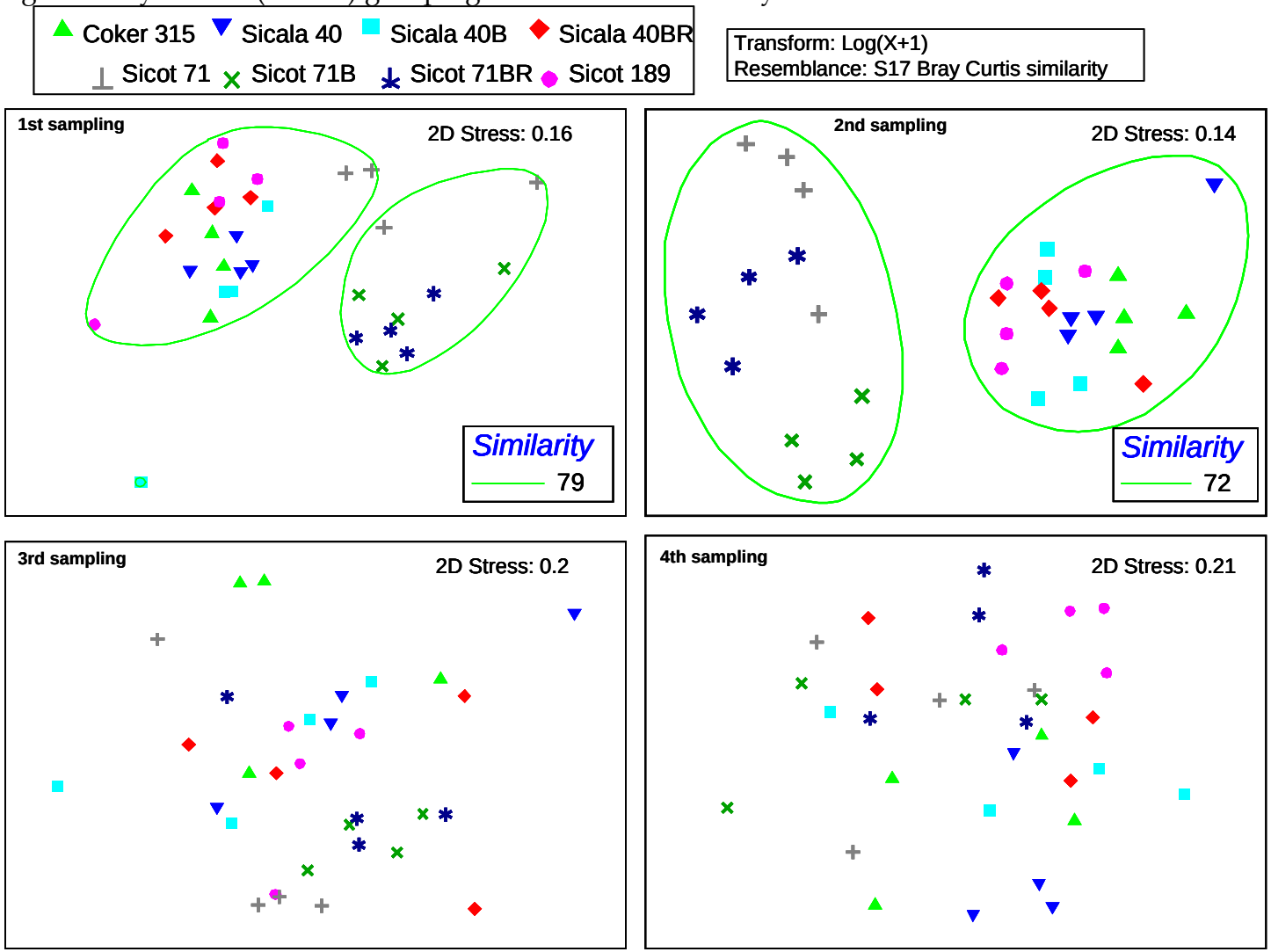
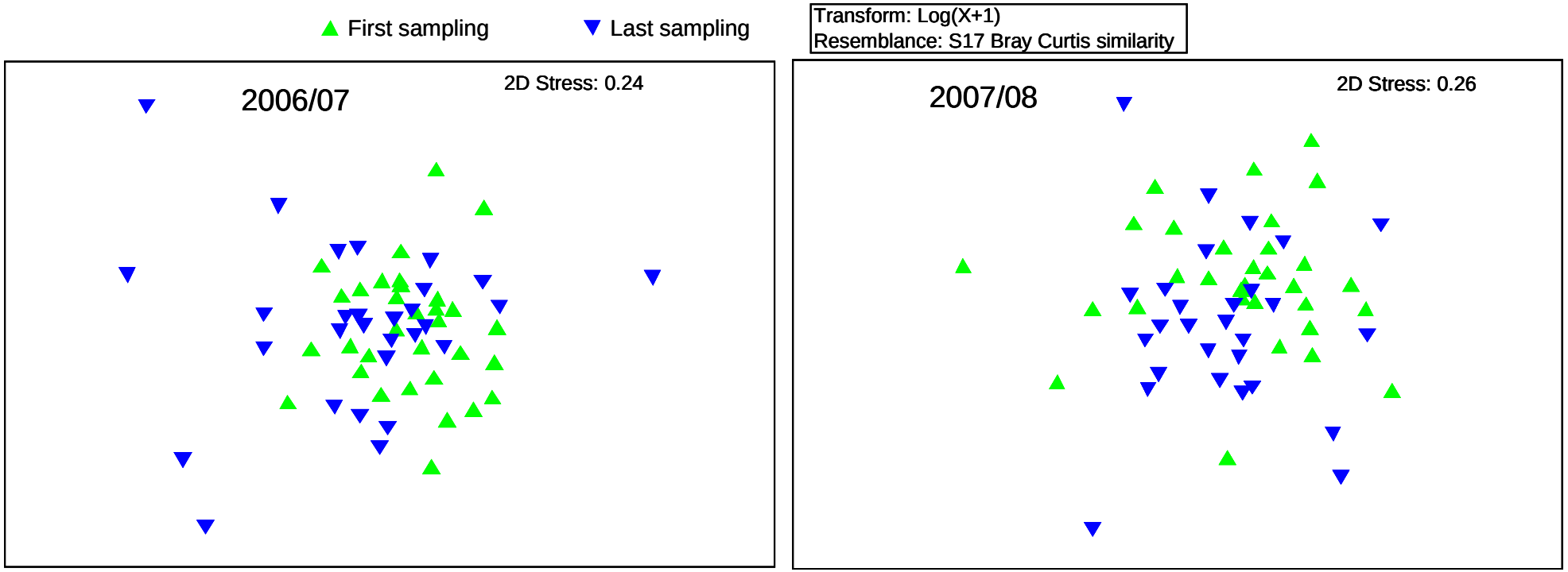


Figure 7. MDS plot of data for the rhizosphere fungal communities obtained using TRFLP-ITS analysis of soil samples from field experiments.



4 Physiological profiling

MicroResp® results over the two years showed clear varietal influences on the capability of the rhizosphere microbiology to utilize specific compounds. Canonical analysis of induced respiration indicated that certain hexose sugars (fructose, glucose and sucrose) and amino acids (asparagines, valine and aspartic) were preferentially used by some rhizosphere communities over others (Figure 8).

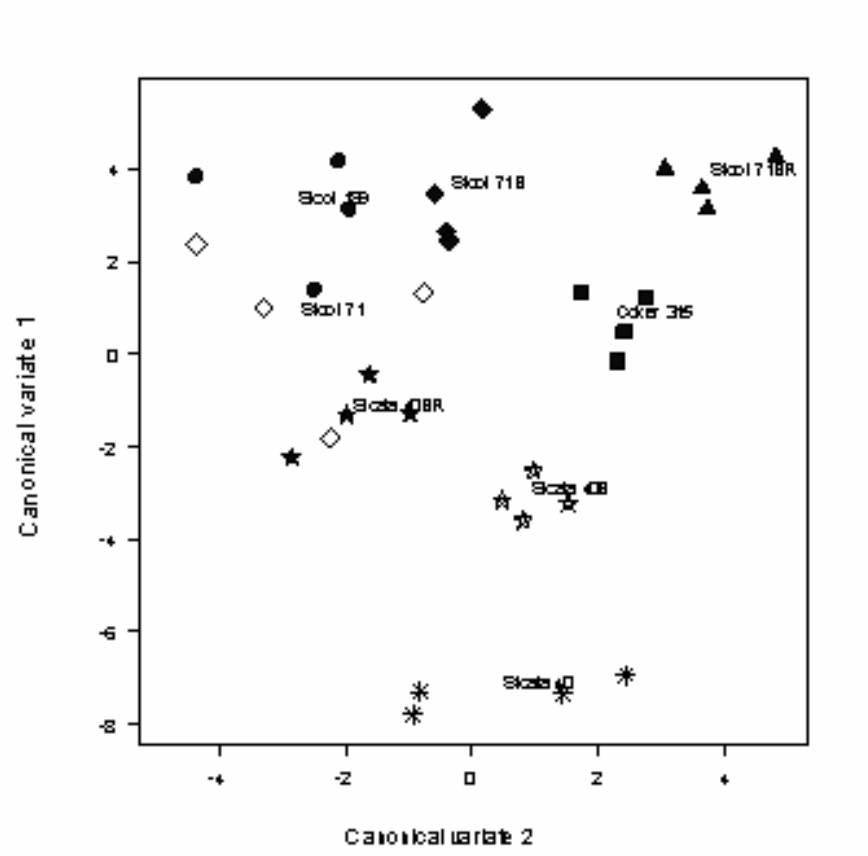


Figure 8. Canonical analysis of substrate induced respiration of rhizosphere microbiota (2006/07 season).

Ammonium oxidizer populations

As with the other microbial measurements, ammonia oxidizing bacterial populations fluctuated over the season, but often significantly differed between varieties. Varietal trends observed in the 2006/07 season are shown in Figure 9. Populations were lowest with the Sicala 40 group of varieties (<40000/g soil) whereas the Sicot 189 and 71 exhibited the highest populations (>60000/g soil). The general trend in the populations for different varieties remained the same at both sampling times (cutout and defoliation) i.e. Sicala varieties associated with lower populations where as the rhizosphere soil for Sicot 189 contained the highest populations. There was no clear trend between the populations associated with parental and GM varieties. Although, significant differences were seen in the 2007/08 season, trends did not remain the same for all varieties.

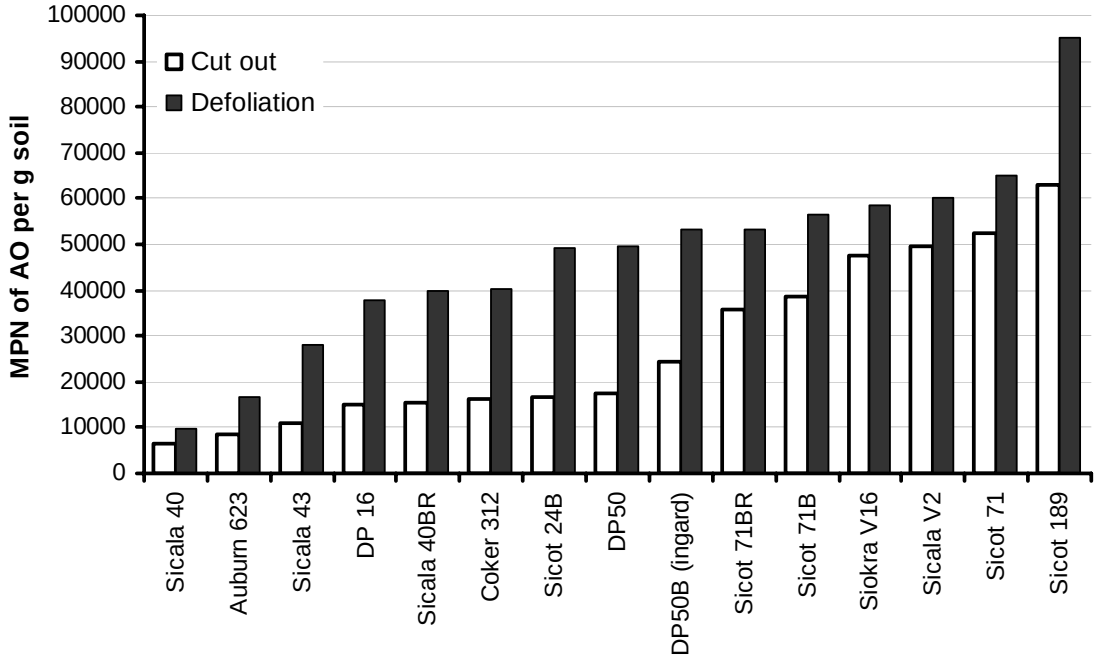


Figure 9. AO populations of rhizosphere soils from a number of difference cotton varieties sampled at cut out and defoliation in the 2006/07 season.

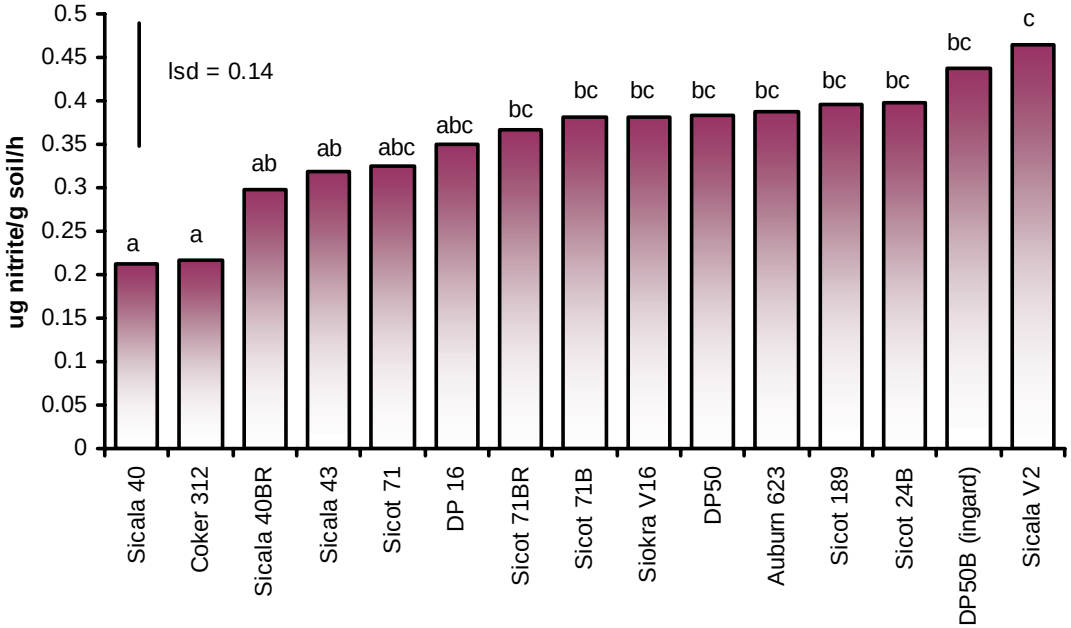


Figure 10. Mean rates of nitrification for rhizosphere soils from different cotton varieties (2006/07 season).

Nitrification

Nitrification rates could not be determined early in the season due to the high levels of nitrate present in the soil from crop fertilization. Significant differences in the rates of nitrification from varietal rhizosphere soils were observed only at some sampled time points throughout the season, although in 2006/07 there was evidence of a conserved trend existing between the varieties (figure 10). This trend was not

apparent in the 2007/08 data, but rates of nitrification were similar in both years. There was no consistent and significant relationship between the levels of AO populations and the recorded rates of nitrification in either of the assessed years.

N fixation by Free-Living N fixing bacteria:
There is capacity in most soils for the fixation of atmospheric nitrogen by non-symbiotic bacteria. The capacity for N fixation by these free living bacteria, such as *Azospirillum*, is greatly limited under conditions where N is readily available. However, when N is not inhibiting the capacity of these bacteria to fix atmospheric N can have significant implications for N input management of the system. Results presented in Figure 11 show significant varietal differences in the amount of acetylene reduced by the rhizosphere soil communities (3rd sampling during 2006/07 season), an indication of the capability of rhizosphere soils to carry out non-symbiotic N_2 fixation. Analysis of samples collected from the first two sampling indicated very low levels of acetylene reduction activity, probably due to the high levels of mineral N in the surface soils (Table 1.). Further work would be required to build this observation into potential management strategies, which aimed to capitalise on this process.

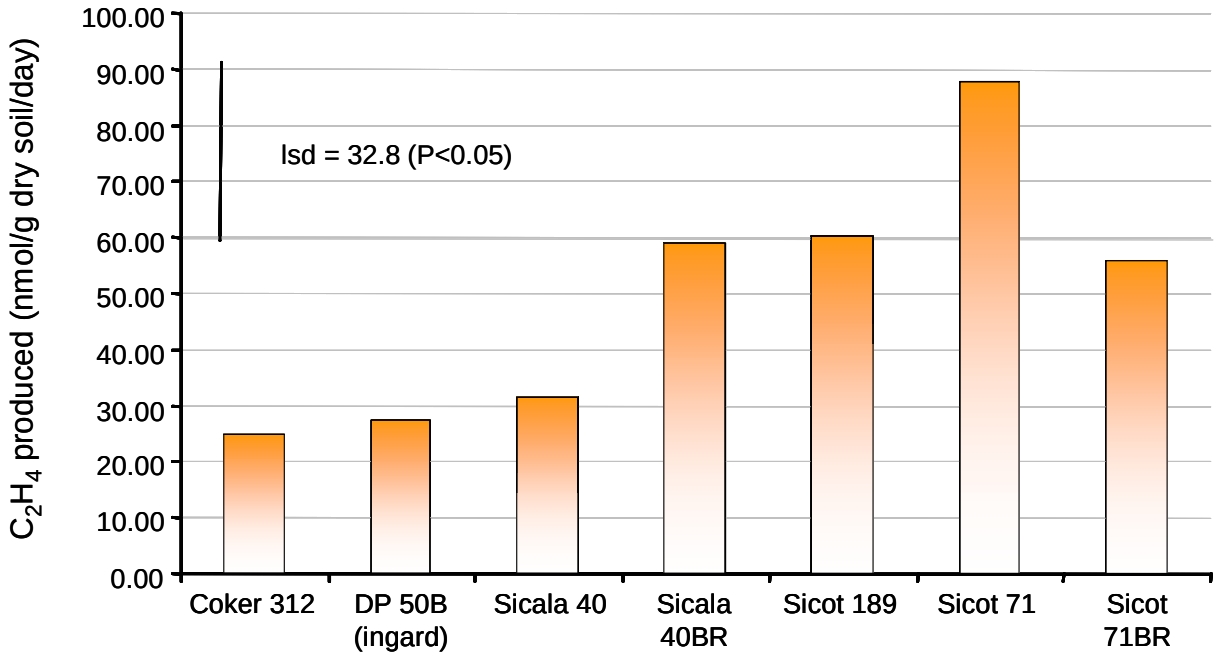


Figure 11. Average levels of acetylene reduction activity in rhizosphere soils from different cotton varieties (2006/07).

Over the both seasons we installed six Full Stop irrigation monitoring devices under Sicot 71BR. This allowed us to screen the bypass irrigation water for the presence of *Cry* proteins and the EPSPS gene product. With the exception of a few (believed to be false) positive reactions to the EPSPS gene product, no introduced expressed genetic material were detected. However, having collected the bypass irrigation water we were also able to assess the levels of nitrate, nitrite and conductivity of the soil water in the rooting zone at each irrigation or major rain event. These results,

presented in table 1, indicate the high levels of available N as nitrate early in the season, which would repress free N fixation.

06/07	sample date		29/12/2006	31/12/2006	09/01/2007	29/01/2007	08/02/2007	23/02/2007	09/03/2007
	40	Average of Nitrate	150.0	500.0	216.7	258.3	58.3	25.0	20.0
		Average of Nitrite	0.3	0.0	0.0	15.0	0.0	0.0	0.0
		Average of EC	1.3	2.1	1.2	1.0	0.7	1.2	1.3
	60	Average of Nitrate	200.0	250.0	266.7	203.3	29.0	15.0	20.0
		Average of Nitrite	0.0	0.0	2.0	13.3	0.0	0.0	0.0
		Average of EC	1.5	1.9	1.2	0.7	0.6	1.3	1.3
07/08	sample date		19/12/2007	07/02/2008	27/02/2008	11/03/2008			
	40	Average of Nitrate	333.3	20.0	20.0	0.0			
		Average of Nitrite	0.0	0.0	0.0	0.0			
		Average of EC	2.5	0.7	0.7	0.7			
	60	Average of Nitrate	333.3	28.3	20.0	0.0			
		Average of Nitrite	0.3	0.0	0.0	0.0			
		Average of EC	2.6	1.0	0.9	0.9			

Table 1. Averages of nitrate, nitrite and Ec values from bypass irrigation water collected from three full stop devices inserted at two depths under Sicot 71BR over the assessed seasons.

Populations of *Trichoderma* spp. and *Fusarium* spp

This was assessed in the 2006/07 season by combining the use of selective media for the recovery of specific fungal genera from dilution series of rhizosphere soil. The number of resulting fungal propagules on the selective media was recorded and analysed to assess if differences occurred under the various cultivars. Across each of the recoveries no significant difference was observed in either total fungi or the number of *Trichoderma* and *Fusarium* propagules recovered from the rhizosphere soils of the 15 varieties planted. Total fungal communities recovered on the selective media were observed to increase post defoliation in the rhizospheres of all tested varieties, but the amount of increase varied from slight to several orders of magnitude. Mean values for *Fusarium* rose from 589 to 1106 from cut out to defoliation, respectively, whilst mean *Trichoderma* recovery from the same period rose from 871 to 1426 propagules per g of soil. As well as no apparent trend or relationship between variety and the propagules of these fungi, no correlation was observed between F rank and either *Fusarium* or *Trichoderma* numbers (best R^2 of 0.1, n=11). Limited molecular and taxonomic identification was carried out on the selective media plates in an attempt to establish how effective they were at recovering fungi from the desired groups. Results from several of the plates examined in this manner indicated that the media chosen was not well suited to culturing just the desired fungal species from the mixed soil community. Additionally, the proportion of fungi identified as belonging to either *Trichoderma* or *Fusarium* from the background population was very different for each plate, making even a proportional representation impossible. In view of these difficulties this methodology was abandoned.

The soil fungus *Trichoderma* includes a number of species associated with a variety of beneficial functions. These free-living fungi have been recognised as plant symbionts, endophytes and parasites of other fungi, and are therefore recommended as biocontrol agents of plant pathogenic fungi. Some species also have the ability to promote plant growth (Harman et al., 2004). In a DNA based analysis undertaken in the 2007/08 season, the *Trichoderma* spp were grouped into two groups, e.g. *Trichoderma* A (*T. harzianum, T. aureoviridi, T. inhamatum*) and *Trichoderma* B group (*T.*

koningii, T. harzianum, T. viridi, T. hamatum). Our results indicated that, in general, the amount of DNA for *Trichoderma* group A was general higher (5 - 435 pg DNA/g soil) than that of *Trichoderma* group B species (0 - 21 pg DNA/g soil). There were no significant differences in the amount of *Trichoderma* group B in the rhizosphere soils for different cotton cultivars. The high variability between the replicates masked some of the trends observed with the amount of DNA of *Trichoderma* group A in the rhizosphere soils of different cotton varieties. In general, rhizosphere soils from Sicala 40BR contained the highest amount of *Trichoderma* group A – DNA where Sicot 71BR and Sicala 40 contained a lower amount (Figure 12). These results are from the analysis of one sampling time only and therefore require further analysis in order to draw any reliable conclusions.

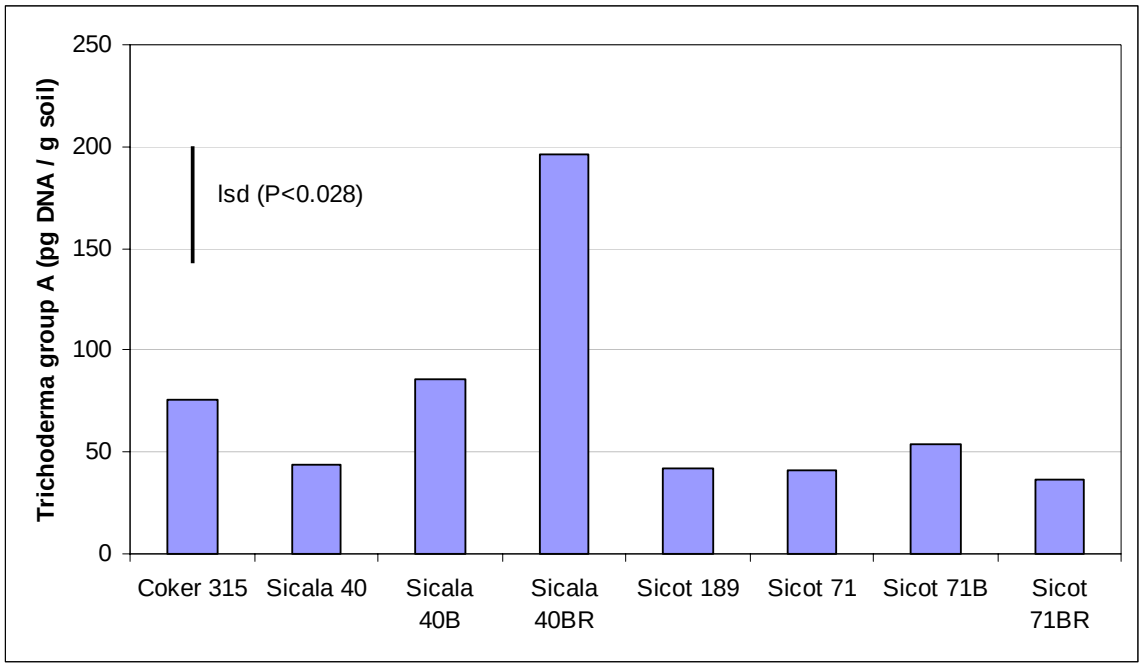


Figure 12. Amount of DNA for *Trichoderma* group A in the rhizosphere soils from 2007/08 season.

Yield:

Picking of the crop for analysis of yield was completed mid May in 2006/07 and 2007/08. Analysis of the machine picked yields indicated that there was a significant difference across the 15 planted varieties ($p<0.01$, lsd=402, n=60, figure 13.) for 2006/07. The Bollgard varieties had a higher yield, despite the field being managed for conventional cotton (Bt mean = 2098 kg/Ha, Conv mean = 1750, $p<0.01$, lsd=188).

In 2007/08 there were again significant differences in yield across the varieties (Figure 14), with the Sicot 71 family outperforming the Sicala 40 family and the conventional parent material represented by Sicot 189 and Coker 312. Transgenic varieties in each family outperformed their conventional parents, but not significantly.

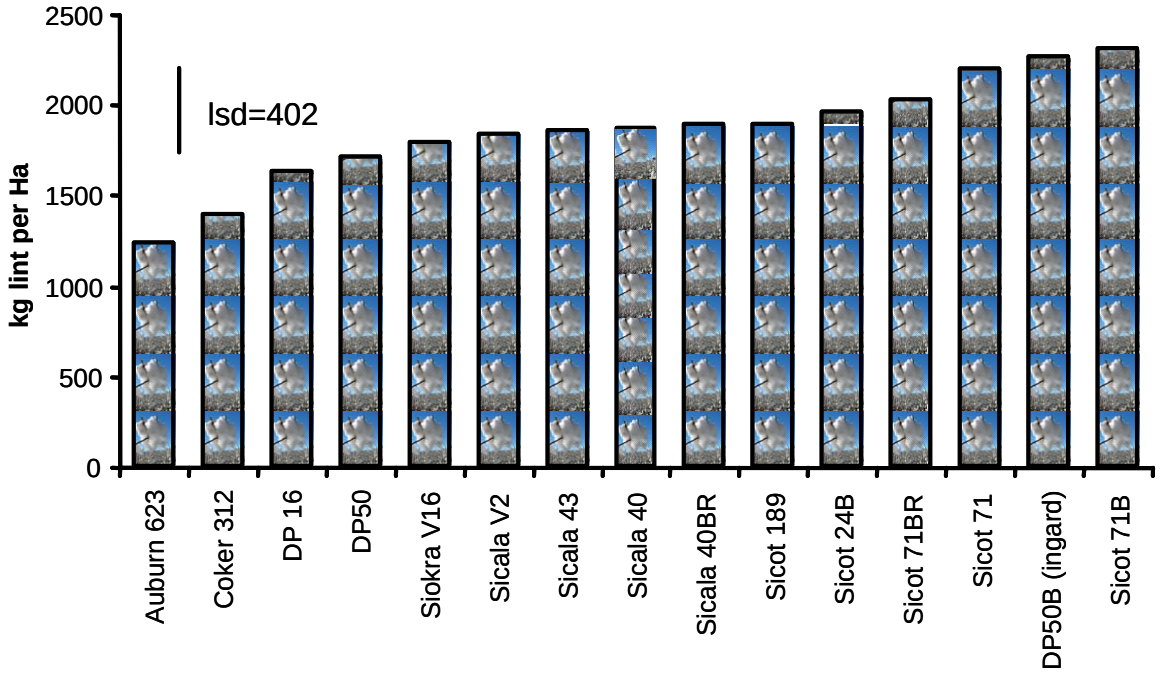


Figure 13. Mean yields of cotton lint from the 15 cultivars grown in the field trial in B2 during the 2006/07 season.

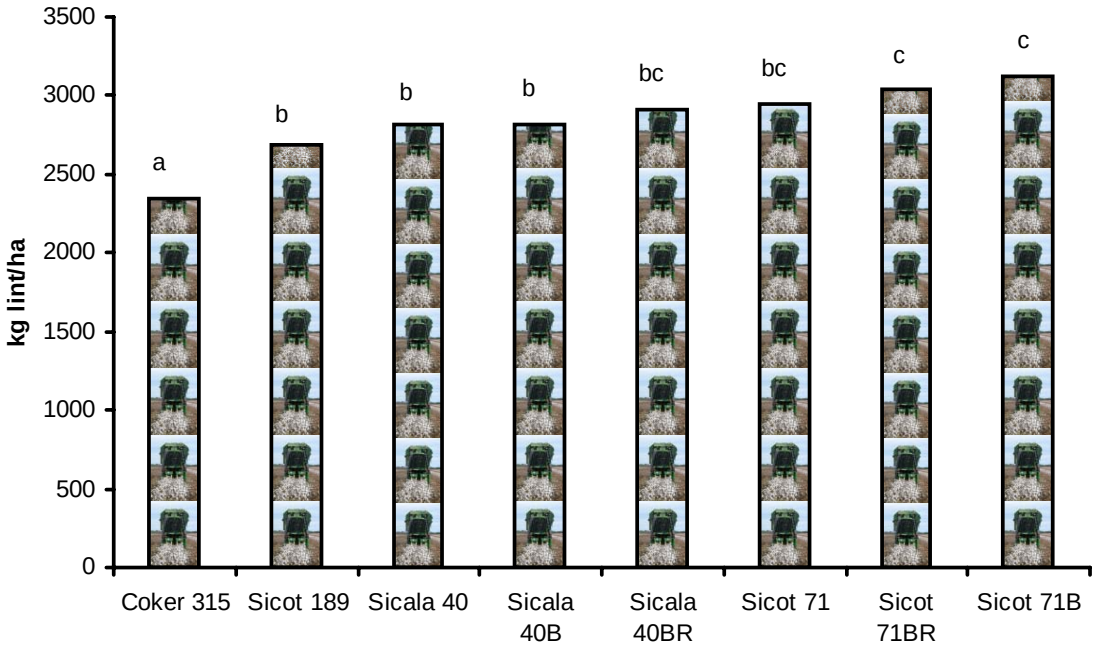


Figure 14. Mean yields of cotton lint from the 8 cultivars grown in A3 in 2007/08. Means represent the average based on four 20 m plots. Similar letters indicate similar means based on ANOVA assessment with $P<0.05$ and an lsd of 261.

4.2. Laboratory experiments – collection and interrogation of root exudates.

Hydroponic experiments

The hydroponics system failed to establish viable seedlings and constant air flow to each of the growth chambers was difficult to maintain with the pump and filtration levels applied.

The ECAM method allowed seedling development to occur and first leaf emergence was occasionally achieved. However, problems with this method were encountered. In several instances the roots of the developing seedlings became air borne and failed to grow into the water agar, prolonged use of the laminar flow cabinet became an issue of concern with colleagues that restricted continued use, and in some instances seedling and air borne contamination remained a problem. Consideration was given to other systems, such as that described by Hodge *et al.* (1996), but the early cessation of the project meant they were not developed further.

Despite the failure to establish and develop a hydroponic system, the results from the MicroResp® work (see start of section 4) have provided insight into what should be considered as initial target signal molecules. It is hoped that a future project would (i) establish that fructose, glucose, sucrose and asparagines, valine and aspartic are differentially produced, and (ii) exuded from cotton roots and (iii) can result in different functional expression when applied to soil in quantities similar to those provided by the plants.

4.3. Other field research

4.3.1 Colonization of new cotton varieties by AM fungi:

We compared mycorrhizal development in commercial cultivars of cotton expressing genes for insect resistance (Cry1Ac and Cry2Ab), glyphosate tolerance (EPSPS), or both, and their conventional parent line. AM development in cotton roots increased rapidly in the first three weeks after sowing, reaching a plateau level of around 70 to 80 % root length (Figure 15). This pattern of colonisation was virtually identical among both conventional and GM cultivars of cotton at each assessment, clearly indicating that colonisation by AM fungi was not affected by the expressed transgenic traits. Results of this work are published in a scientific paper (Knox et al., 2008) and a Cotton grower article (Knox et al., 2007).

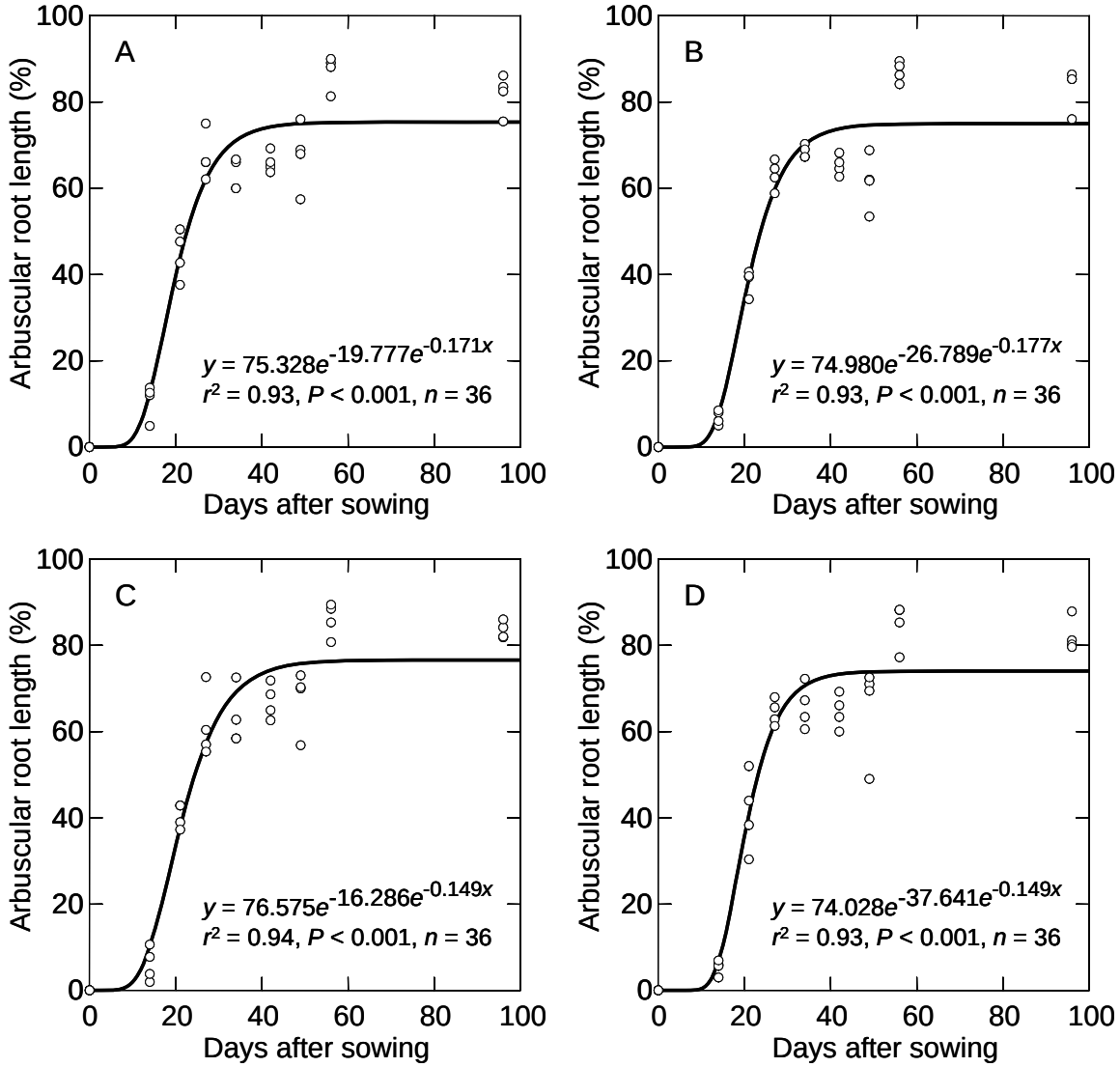


Figure 15. Similarity of AM development in roots of cotton cultivars; Sicot 189 (A), Sicot 189RR (B), Sicot 289B (C) and Sicot 289BR (D), sown at the Australian Cotton Research Institute in the 2004/05 season.

4.3.2 Identification Nematodes in cotton soils

As part of collaboration with other researchers at ACRI, the principle investigator has identified new associations between plant parasitic nematodes in Australian cotton soils, e.g. observation of *Tylenchorhynchus ewingi* in association with cotton soils in Australia (Knox et al., 2006).

Plant associated and plant parasitic nematodes are increasing in many arable soils around the world. Reasons for this remain unclear, but change and reduction in the chemistry used in the control of nematodes (e.g. aldicarb and methyl bromide) is likely to be of major importance. Currently no nematode problem, with the exception of some problems with *Pratylenchus* in the Burdekin, is associated in Australian cotton, but they are found as major pests in most other cotton producing parts of the world. With changing chemistry and the introduction of cotton to new parts of Australia the consideration for the potential of development and spread of nematodes pathogenic to cotton should be considered as an area of potential high risk to continued production.

3.3.3. Persistence of *Bt*-protein in Australian soils

Soil samples from field experiments conducted in this project were used in the DEHWA project while the findings about the persistence of bioactive Bt-proteins in cotton soils assisted with our understanding of the microbiology of cotton soils. A bioassay to determine the levels of Bt-protein in Australian cotton soils was standardized. Analysis of soils from field experiments indicated measurable levels of bioactivity from one season to the next, in particular in particulate organic matter fraction. The bioassay method was found to be suitable to determine, if any, the longer term persistence of Bt-proteins and the effect of environmental (soil type) and management (rotation) factors.

Gupta, Knox and Downes 2008, Levels and significance of *Bacillus thuringiensis* toxin in Australian soils during and after cultivation of genetically modified cotton, final report submitted to Department of the Environment, Water, Heritage and the Arts, Australia.

3.3.4. Association of soil organisms with *Helicoverpa armigera* in cotton

Preliminary observations with dying larvae and moth, pupae in cotton soils indicated the association of the fungi *Lecanicillium lecanii*, *Actinomucor elegans,* several *Aspergillus* species, and a *Rhabditoides* sp. of nematode with *H. armigera*. Results are presented in a manuscript for publication (Knox, Anderson, Tann and Gupta, Organisms with potential control *Helicoverpa armigera* in cotton, submitted)

Discussion

The sampling strategy used in these experiments was adequate to determine the varietal based differences in rhizosphere populations but could be enhanced if required. The number of field assessments was limited by the time taken to process the samples with the resources available, and occasionally by the weather. This meant that investigation of issues such as rhizosphere changes with depth and root physiological differences between varieties were not pursued within this project.

The observed fluctuations in microbial biomass values throughout the season were taken as an indication of the influence of changes in exudation, as plants directed resources according to physiological requirements. Biomass results in the 2007/08 season were on average much higher than those obtained in 2006/07 (data not shown); this was assumed to be due to differences in the field used and environmental conditions, i.e. soil moisture and temperature.

Previous research on microbial diversity in cotton soils, using culture based methods only, showed some differences due to management but sometimes masked by high variability and the inability of culture methods to detect more than 90% of microbial life in soils (Roper and Gupta, 2007). In this study we used both culture based and DNA methods to determine the diversity of soil microflora. Molecular interpretation of bacterial diversity in the rhizosphere clearly showed variety and familial grouping occurring early in both seasons (Figures 2-6). Such differences in rhizosphere microbial diversity previously have been attributed to differences in the quality and quantity of rhizodeposition (Bowen and Rovira, 1999). Variety based differences in rhizosphere bacterial diversity has been reported for other crops (Gupta et al., 2004; Grayston et al., 1996). However, in later samplings, e.g. in April 2007/08 (3rd sampling in 06/07and 4th sampling in 07/08), this relationship was no longer evident. To address why this relationship was seen to collapse it is worth considering the following points; (i) as the season progressed the vertosols that supported our experiments would have undergone periods of shrinking and swelling that would have moved soil and its associated microbiota. (ii) Work by Dr N. Hulugalle (NSW DPI) has demonstrated that root senescence can occur within the time frame in which we made measurements and at this stage the crop would have undergone significant physiological change. These factors are likely to have affected the quantity and quality of exudates, thus changing the associated bacterial community. (iii) Irrigation events throughout the season are likely to have had a significant effect on rhizosphere community structure as water would have carried bacteria through soil fissures and root channels between rows of varieties.

Unlike the bacterial diversity we did not find any clear cut differences in fungal communities between varieties. Soil fungi form hyphal networks which can extend long distances from rhizosphere to bulk soil and vice versa. Hence our analysis may not be adequate to distinguish hyphal networks from other structures etc. Our efforts to determine the populations of key groups such as *Fusarium* spp through a combination of culture based and DNA techniques encountered some methodological difficulties and did not show any trends. However, our observations on the amount of DNA for two *Trichoderma* groups indicated some trends with *Trichoderma* group A but require further research to draw any firm conclusions. The observation of higher amounts of DNA for *Trichoderma* group A compared to Group B is worthy of further investigation. As indicated before, the two DNA probes used in this study were designed to include specific species of Trichoderma in each group, e.g. *Trichoderma* group A - *T. harzianum, T. aureoviridi, T. inhamatum*) and *Trichoderma* B group – *T. koningii, T. harzianum, T. viridi, T. hamatum*. Liu et al. (2008) also suggested that the diversity of *Trichoderma* species may be influenced by a variety of factors including soil texture, crop type and sampling time along with management practices. A comparison of rhizosphere and bulk soils from conventional and no-till soils from rain fed broad acre agricultural soils in southern Australia indicated that in general, the DNA for *Trichoderma* Group A was higher in the rhizosphere soils, where as the DNA for *Trichoderma* Group B was higher in the non-crop soils with decomposing residues (Gupta., 2008). *Trichoderma* Group A includes a number of species associated with a variety of beneficial functions. It is well recognised that some species of this free-living fungi can be plant symbionts, endophytes and parasites of other fungi, and are therefore recommended as biocontrol agents of plant pathogenic fungi.

Altering our respiration assessment to a semi-intact core method reduced the variation we had encountered when analyzing loose rhizosphere soil, but did mean that we were likely to be analyzing soil that was not directly influenced by the plant. The use of the MicroResp® technique allowed us determine the composition of microbial community based on their ability to utilize different types of C and N substrates representing compounds that may be present in root exudates. Results showed a varietal based response of rhizosphere microbiota to specific hexose sugars and amino acids. We hypothesize from this that the rhizosphere microbial community of certain varieties had adapted to using specific compounds as a consequence of either increased quantity of these compounds in root exudates. Had the work continued, studies would have been undertaken to examine (i) differences

in composition of exudates between varieties and (ii) the impact of these compounds on soil functional capabilities.

Analysis of ammonia oxidizer bacterial populations and nitrification rates indicated that the availability of nitrate in the root environment varied between cotton varieties and so could determine physiological behavior. We also observed varietal differences in the capability of rhizosphere soils to carry out non-symbiotic N_2 fixation. Much of the work on these microbial populations and functions associated with nitrogen cycling was often complicated by high levels of freely available nitrate present in the soil, which we monitored in bypass rain and irrigation water with 'Full Stop' devices. The results do, however, suggest that in the development of a reduced and more efficient input cotton system, varietal choice should be a key consideration.

Environmental conditions during the two seasons were quite different and a seasonal based difference in the absolute numbers of populations or activities was observed e.g. level of soil respiration or CO_2 production, number of ammonia oxidizing bacteria per gram soil. However, the varietal based separation of rhizosphere microbial communities was evident during both seasons providing a clear evidence for the potential to modify rhizosphere microbial composition and functional capability, i.e. cotton plant-microorganism interactions, through varietal management. It has been estimated that up to 40% of photosynthetically fixed carbon can be released by the plant root and the chemical nature of rhizodeposits range from simple low molecular weight compounds (aminoacids, sugars) to complex macromolecules (e.g. polysachharides, proteins, antibiotics) (Cheng and Gershenson, 2007). In addition to the release of carbon and nitrogen sources for metabolism and growth, plant roots also are known to release a diverse range of molecules involved in biofilm formation, hormones, signal molecules that trigger specific inter- and intra cellular relationships including beneficial and pathogenic microflora. Overall our observation of a clear varietal based characterization of cotton plant-microbial interactions shows a great potential for a designer cotton rhizosphere in pursuit of a productive and environmentally sustainable cotton farming system in Australia. Finally, our work in this area was preliminary and further work is needed before advice on variety selection can be given to farmers.

Outcomes

5. Describe how the project's outputs will contribute to the planned outcomes identified in the project application. Describe the planned outcomes achieved to date.

In this project we established that rhizosphere plant-microbial interactions are influenced by variety type and the links may be linked to the quality of rhizodeposition. This is the first step in the effort to develop the concept of a 'designer rhizosphere' within the cotton system for the purposes of sustainable production at the field, farm and catchment levels. Additional, other outcomes of this research would be as promised in the PRP to CRDC.

5.1. Ability to target specific cotton cultivars, with particular root characteristics, to soils where they will be challenged by fungal diseases and invertebrate pests.

Preliminary results indicated no clear differences in general fungal communities for different cotton cultivars. However, our work to interrogate the response of key fungi to root exudates could not be completed due to the difficulties in establishing effective exudate collection techniques and time constraints.

5.2. Determine the influence of cotton cultivars on pathogenic and antagonistic functions and nutrient supply potential of bacteria and fungi in rhizosphere microbial communities.

Demonstration of cultivar based differences in the populations and functional capabilities of microbial communities involved in N cycling. The differences observed to date clearly indicate that there is a strong varietal influence on the rhizosphere soil microbiota associated with several of the key functional steps in a number of the major elemental cyclic pathways. The results looking at aspects associated with the N cycle indicate that cotton variety is involved in altering the microbiology of the soil in such a way that there are consequences in the provision of N to the plant e.g. fixation of N from the atmosphere, potential for losses of N to leaching and atmosphere as a greenhouse gas.

In addition, the demonstration of clear variety based differences during the early phase of cotton crop e.g. up to 100 days after sowing, suggests the potential to link these observations to plant performance and prospects for rhizosphere manipulation to achieve improved plant growth and nutrition.

The presence of high levels of mineral N in soil/water early in the season resulted in lack of measurable levels of acetylene reduction activity, as a surrogate for free-living N fixation, thus preventing further development of a low input system in which associated microbiological input can be properly investigated. Additional work to evaluate other methods to determine the level of N fixation is required.

5.3. Enhance BMP by providing information from rhizosphere functional group research to improve plant health and yield through managed plant-biota interactions.

Results from the 06/07 and 07/08 seasons have helped to establish the varietal based differences in key functional groups of microbial communities. Due to termination of the project at the end of June 2008, the development of these observations into a management tool will not be possible.

6. Please describe any:-

a) technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.);

N/A

b) other information developed from research (eg discoveries in methodology, equipment design, etc.); and

The use of the ECAM method in this project, in an attempt to grow and collect exudates from cotton seedlings, was the first time that this method has been applied to a dicot species. The results were favourable compared to the other hydroponic systems that were established and trialled, but still fell short of being entirely successful. Use of a system, similar to that described by Hodge *et al.* (1996), would facilitate establishing if differential exudate excretion is occurring.

c) required changes to the Intellectual Property register.

N/A

Conclusion

7. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. What are the take home messages?

We observed that different cotton varieties can cause shifts in their associated rhizosphere microbial populations and functions. The potential to capitalize on this within the cotton system requires further investigation, but could be profitable, especially if a move to lower input systems becomes desirable. With the current increases in fuel and fertilizer costs such systems are likely to be considered in the near future, but more work on cotton variety and soil biological interactions is needed to capitalize on the preliminary work reported here.

The take home messages from this project are:

- Farming causes a shift in the soil biology through its physical disturbance of the soil, the chemistry it applies and the particular plant varieties grown in the soil.
- Cotton cultivars are adapting their associated root microbiology.
    - This is particularly evident early in the season when the crops are establishing and developing factors associated with projected final yields.
- The functional capabilities, in terms of nutrient capture and provision, provided by these microbial communities is influenced by the cotton variety with which they are associated.
    - The development of cotton systems and plant varieties that can capitalize on these microbial functions (i.e. designer rhizospheres) is desirable given current fertilizer prices and the continued emphasis for improved stewardship of the land by those that practice agriculture upon it.
- Soil microbiology is exceptionally diverse, both in terms of species composition and functional capability, even in vertosols and cropping systems like cotton, which still rely on pesticide applications.
    - The only way to develop a better understanding of what is in these soils and the functional capabilities of the biota is to undertake investigations such as this.
- Nematodes are present in cotton soils, but currently do not appear to represent a problem.
    - Establishing the presence and distribution of nematode species that could present a threat to cotton production within Australia seems prudent. Without this sort of information it will never be possible to assess whether changes in farming practices or exploration of new areas could result in problems for cotton production.

Extension Opportunities

8. Detail a plan for the activities or other steps that may be taken:
(a) to further develop or to exploit the project technology.
(b) for the future presentation and dissemination of the project outcomes.
(c) for future research.

Principle investigator and Gupta Vadakattu have made presentations at industry organised meetings, national and international scientific meetings. Results and interpretations from the research (described in the final report) will be made readily available to all parties within the Australian cotton community through future presentations at CRDC, CCC CRC, CCA, CSD and ACGRA organized events, publication in the industry's literature, and extension with the support of the farming systems and environment teams.

Undertakings were made to ensure the project was extended at a number of opportunities despite early termination of the project and reduction in the farming system and environment teams. For further details of these realised extension opportunities please refer to section 9, 'Publications'. In addition to the manuscripts, talks, industrial publications and posters given here, the production of further scientific papers will be pursued by the research team following completion of the project.

Opportunities for future research have been highlighted in the results section of this document and these will be presented as potential project ideas through future funding opportunities.

Publications

9. A. List the publications arising from the research project and/or a publication plan.

Students (Principle investigator):

2006/07 Associate supervision of James Terry (CCC CRC) and Pippa Featherstone (CSIRO PI) summer scholarship.

2005/07 Associate supervision of Sam Alomari, (PhD candidate, Sydney University) investigating 'Molecular analysis of proteobacterial communities in soil under cotton'.

Papers:

Knox, O.G.G., D.B. Nehl, T. Mor, G.N. Roberts and V.V.S.R. Gupta (2008) Lack of effect of genetic modification of cotton on arbuscular mycorrhizal colonisation of roots. *Field Crops Research* in press

Knox, O.C.G., Gupta, V.V.S.R., Nehl, D.B. and Stiller, W.N. (2007) Constitutive expression of Cry proteins in roots and border cells of transgenic cotton. *Euphytica*. 154: 83-90.

Constable, G., Preston. C. and Gupta, V.V.S.R. (2007) The Genomic Age 3. GM cotton – benefits, risks and opportunities. *The Journal of the Australian Instititute of Agriculture Science and Technology*. 20: 28-32.

Knox, O.G.G., Anderson, C.M.T., Nehl, D.B., Gupta V.V.S.R. (2006) Observation of *Tylenchorhynchus ewingi* in association with cotton soils in Australia. *Australasian Plant Disease Notes*. 2006 (1): 47-48.

Refereed conference proceeding:

Luelf, N., Tan, D., Hulugalle, N., Knox, O., Weaver, T. and Field, D. (2006) 'Root turnover and microbial activity in cotton farming systems' 13th Australian Agronomy Conference.

James H Terry, Daniel KY Tan, Nilantha R Hulugalle, Damien J Field, Tim B Weaver, Oliver G Knox. (2008) 'Cotton yield and soil carbon under continuous cotton, cotton-corn, cotton-vetch-corn and cotton-wheat rotations'. 14th Australian Agronomy Conference.

Non refereed papers, presentations, articles and meeting proceedings:

Oliver G.G. Knox, Gupta VSR Vadakattu, Kellie Gordon, Richard Lardner and Marcus Hicks. 'Varietal differences in cotton – belowground' (September 2008) ACGRA 14th Australian Cotton Conference.

Gupta V.V.S.R. and Knox, O.G.G. (2008) How best can we design rhizosphere plant-microbe interactions for the benefit of plant growth? Oral presentation at the Rovira Rhizosphere Symposium held on August 15, 2008 in Adelaide, SA.

Seymour, N., Knox, O. and Gupta, V. (August 2006) 'Understanding Soil Biology'. ACGRA 13th Australian Cotton Conference.

Knox, O. and Gupta V. (August 2006) 'Variation in microbial community and function within cotton fields' ACGRA 13th Australian Cotton Conference.

Oliver Knox and Gupta Vadakattu. (2008) 'Nitrogen and Cotton – a microbial view'. *Cottongrower.* December 07-January 08 issue.

Oliver Knox, Chris Anderson, Gupta Vadakattu and Nikki Seymour. (February 2007) 'Tiny worms: Nematodes in Australian cotton'. *Cottongrower,* February 2007, issue.

Oliver Knox, David Nehl and Gupta Vadakattu. (2007) 'Spot the difference? Mycorrhiza in GM and non-GM cotton'. *Cottongrower,* February 2007 issue.

Oliver Knox, Ian Rochester, Gupta Vadakattu and Louise Lawrence. (August 2006) 'Composting in Australian cotton production'. *Cottongrower, August, 2006 issue.*

Knox, O.G.G. and Gupta, VVSR. CSIRO, Soil Root Discussion Group (2007) 'Cotton and Vertosols – adventures and opportunities'. December, 2007, Canberra, ACT.

Knox, OGG and Gupta, VVSR (July 2007) The Potential for 21st Century Development of Agricultural Crops to Alter Soil Microbial Diversity and Function - Cotton as a Case Study'. Abstracts of the Annual meetings of Australian Society of Microbiology held in Adelaide, during August 2007.

Knox, OGG and Gupta, VVSR (August 2007) Rhizosphere Biological Functions. Cotton CRC Review.

Knox, O.G.G. and Anderson, C.M.T. 'Nematodes in Australian Cotton', and Knox, O., Gupta, V. and Nehl, D. 'AM in GM and Conventional Cotton' (June 2007) FUSCOM PLUS.

Knox, O and Gupta, V. (April 2007) 'Herbicide and cotton variety effects on soil microbiota'. 2007 Landmark Agronomy Conference - Central and Southern Queensland and Northern NSW.

Knox, O.G.G. (2007) SAC, Crop and Soil Systems Research Group, Seminar series 'Introduction of GM technology – an environmental perspective.' (September 2007)

Posters Presentations:

Knox, O.G.G. and Gupta, V.V.S.R. (2008). Cultivar specific differences in N cycling functional groups in the cotton rhizosphere, Poster presentation at the 8th European Nitrogen Fixation Conference, September 2008 held at Aula Academinca of the Ghent University, Belgium.

Submitted:

Knox, O.G.G., Gupta, V.V.S.R., Roberts, G.N. and Downes, S.J. (2008) Improving environmental loading assessments of Cry protein from GM plants based on experimentation in cotton. The Open Agricultural Journal (submitted).

Knox, O.G.G., Anderson, C.M.R., Tann, C. and Gupta, V.V.S.R. Organisms with potential to control *Helicoverpa armigera* in cotton. In preparation.

B. Have you developed any online resources and what is the website address?

No

References:

Bowen, G.D. and Rovira, A.D. (1999) The rhizosphere and its management to improve plant growth, *Advances in Agronomy* 66, 1-102.

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Campbell, C.D., Chapman, S.J., Cameron, C.M., Davidson, M.S., Potts, J.M. (2003). A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. *Applied and Environmental Microbiology*, 69: 3593–3599.

Cheng, W. and Gershenson, A. 2007. Carbon fluxes in the rhizosphere. In: *The Rhizosphere: An Ecological Perspective*. Cardon, Z.G. and Whitbeck, J.L. (eds), Elsevier/Academic Press, New York, pp. 31-56.

Duineveld, B.M., Rosado, A.S., van Elsas, J.D., Van Veen, J.A. (1998). Analysis of the dynamics of bacterial communities in the rhizosphere of the chrysanthemum via denaturing gradient gel electrophoresis and substrate utilization patterns. *Applied and Environmental Microbiology* 64, 4950–4957.

Gupta, V.V.S.R. (2008). Sustaining soil biological functions in organic systems. Final report submitted to RIRDC, Australia.

Gupta V.V.S.R., Watson, S. (2004). Ecological impacts of GM cotton on soil biodiversity, Report to Australian Government Department of the Environment and Heritage, Canberra, ACT, Australia.

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Part 4 – Final Report Executive Summary

The presence of an active interaction between plants and microbiological life in soil has been accepted for many years. This interaction is particularly important in the rhizosphere, where plant exudates and other rhizodeposits directly feed the microbial population, which in turn is responsible for nutrient cycling, production of growth promoters, disease suppression, agrochemical degradation and occasionally development of pathogenicity. These factors are important to plant health and productivity. However, the difficulties of studying such interactions in the soil and the inability to grow the majority of soil microorganisms in the laboratory (for example, at present <10% of microbial life in soil is cultured in the laboratory) have resulted in limited research in this area. Up to 40% of photosynthetically fixed carbon is released by plant root.

With the introduction of genetically modified (GM) crops into agricultural production systems public concern resulted in renewed interest and research into the impacts of new varieties of cotton on plant growth, productivity and environmental health. (Gupta and Watson, 2004; Brookes and Barfoot, 2005). Results from the previous research indicated that although some differences exist between rhizosphere microbial communities of non-GM and GM crops, they were not specifically identified as being caused by the expression of the introduced transgenic material alone. The results did, however, imply that cotton variety groupings were more likely to be associated with differences in the rhizosphere microbiota. In this project, two years of field and laboratory trials were conducted to assess what level of influence cotton varieties have on their associated soil microbiota involved in key functions and if there might be potential to influence these as a management tool.

Results demonstrated that there is a very strong relationship between the bacterial communities that develop in the cotton rhizosphere from the start of the season. Although there were seasonal based differences in the populations of rhizosphere microbial communities, a clear varietal separation was observed. Differences in the genetic and catabolic diversity of microorganisms between varieties suggest that rhizosphere microbial communities may be adapted to the quantity and quality of root exudates from cotton plants. The released plant products act as selective carbon and nutrient sources enriching a select group of microbial communities. These diverse microbial communities demonstrated shifts in several functional capabilities, particularly relating to N cycling e.g. N mineralization, free-living nitrogen fixation. This could form the basis for development of lower input and more biologically orientated and efficient cotton farming systems. With the current increases in fuel and fertilizer costs such systems are likely to be beneficial in the near future, but more work would be required to capitalise fully on this potential.