COTTON RESEARCH AND DEVELOPMENT CORPORATION

FINAL REPORT

"Detection, distribution and control of early season growth disorder of cotton"

DAN100C

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DETECTION, DISTRIBUTION AND CONTROL OF EARLY SEASON GROWTH DISORDER OF COTTON

CONTENTS

Summary

DETECTION, DISTRIBUTION AND CONTROL OF EARLY SEASON GROWTH DISORDER OF COTTON

In early season growth disorder, or 'bacterial stunt', bacteria infect the roots of cotton and inhibit plant growth and VAM development. The disease is most severe on heavy clay soils that are often high in nutrients. The fine roots of seedlings turn brown (not black) when infected. Bacterial stunt was detected in 17 of 43 fields examined. Maturity was often delayed and yield losses were occasionally as high as 50 %. A low level of bacterial stunt appears to be widespread. The pathogenic bacterium can be isolated using simple laboratory media and collaborators at the University of QLD have used DNA fingerprinting to confirm its identity.

None of the currently available varieties have resistance to the bacterial stunt pathogen and there is little potential for controlling bacterial stunt by chemical means. Large increases in early growth and boll production were obtained in fields with bacterial stunt by maintaining moisture in the topsoil, using mulches and supplementary irrigation. This enabled proliferation of cotton roots in the most fertile part of the soil.

Apart from selecting cultivars with good agronomic characteristics, manipulation of soil water content and maintenance of good soil structure using cover-crop mulches and modified irrigation are the best options for improving early season growth of cotton affected by bacterial stunt. It is anticipated that if cotton crops with a mulch cover are managed to prevent early cut out, then the increases in early season growth observed in this project can be converted to yield increases.

Full Report

DETECTION, DISTRIBUTION AND CONTROL OF EARLY SEASON GROWTH DISORDER OF COTTON

BACKGROUND

Early season growth disorder is a soilborne disease that occurs in cotton grown on fertile clay soils in NSW. The obvious symptoms of this disease are stunted growth during at least the first half of the season and reduced yield. The disease is also known locally as 'Galathera syndrome' due to its proximity to Galathera Creek.

Previous research on the early season growth disorder included CRDC projects DAN 47L (*High yield packages for cotton*, researched by Dr Greg Constable), DAN61C (*Involvement of phytotoxins, probably herbicides, in the Galathera syndrome*, researched by Dr Paul Milham) and UNE7C and UNE17C (*The influence of vesicular arbuscular mycorrhizas on growth, development and yield of cotton*, researched by David Nehl). Research in DAN 47L showed that although nutritional problems were present they were not solely responsible for the disorder and that stunted plants were poorly colonised by mycorrhizal fungi. Research in DAN61C showed that phytotoxins, apparently present in some soils, were not a cause of the disorder. Projects UNE7C and UNE17C concentrated on elucidating the role of biological and non-biological factors in the early season growth disorder, including mycorrhizal fungi, as follows:

Within individual fields, cotton growth is normal at some sites (*normal-crop* soil) and stunted at others (*stunted-crop* soil). VAM colonisation of cotton roots occurs more slowly in stunted-crop soils than in normal-crop soils, and stunted cotton has reduced uptake of P and Zn. Paradoxically, the stunted-crop soil should be the most fertile, having higher levels of P, Zn, K and Mg and a more favourable pH than normal-crop soil. Stunted-crop soils also have higher Mn, clay content and soil water holding capacity but, Mn toxicity and anaerobic conditions are not responsible for stunting. Cotton is highly dependent on VAM fungi for successful growth at normal-crop soils. A series of bioassays showed that ample numbers of VAM fungi were surviving in stunted-crop soil between cotton crops.

Soil sterilisation treatments (steaming or fumigation with MeBr) consistently increase cotton growth in stunted-crop soil, even though this eliminates vesicular arbuscular mycorrhizal (VAM) colonisation of the roots and dramatically reduces P and Zn uptake. Hence, although the plants in non-sterilised soil have some benefit from extra P and Zn supplied by VAM fungi, their growth is stunted by pathogens. The incidence of the fungal pathogens *Verticillium dahliae*, *Thielaviopsis basicola* and Chytridiomycetes in the field is lowest in stunted-crop soil and greatest in normal-crop soil, and no other fungal pathogens are visually obvious in the roots of stunted plants.

Sterilisation of stunted-crop soils consistently eliminates root browning, a symptom of the disorder, indicating that browning occurs in response to soilborne microorganisms. Soilborne bacteria were shown to be causal in the growth because (i) cotton growth was increased by the application of streptomycin and penicillin, (ii) bacteria were observed in, and streaming from, browned cotton roots and (iii) bioassays showed that a fluorescent *Pseudomonas* sp. present in cotton roots was pathogenic. Variation in cotton growth within fields resulted from interactions between non-biological and biological properties of the soil, including both mycorrhizal fungi and pathogenic rhizosphere bacteria. The disease caused by these bacteria is now referred to as 'bacterial stunt' of cotton.

OBJECTIVES

The aims of project DAN100C are (i) to develop and evaluate tests for identification and assessment of the severity of the disease that will be useful for both researchers and growers; (ii) to survey the distribution and severity of the disease in NSW and Queensland; (iii) to fill gaps in the present knowledge of the causes of the disease; (iv) to develop integrated disease management practices.

METHODOLOGY AND JUSTIFICATION

Detection

Procedures suitable for rapid, cost effective detection and monitoring of populations of pathogenic bacteria in cotton roots and soil are needed for research in bacterial stunt. Molecular techniques are suitable for confirming the presence of a species of pathogen, but in studies of whole populations of bacteria they would be time consuming and expensive for the purposes of this project. Fluorescent antibodies would be a cost effective marker, enabling quantification of the density of populations of pathogenic bacteria in roots and soil. The specificity of the antibodies will be checked using the existing collection of identified isolates from cotton roots. The antibodies will be used for staining of whole colonies of bacteria in soil dilution plates by the method of van Vuurde, (1990). If the technique can be successfully applied here, bacteria that give a positive stain reaction could be isolated and their pathogenicity assayed to confirm the accuracy of the antibody binding.

The feasibility of using microplate culture systems, such as Biolog®, to characterise microbial communities in soils with, and without, the early season growth disorder will be assessed. The Biolog® system, normally used to characterise single isolates of a microorganisms, has successfully been used to assess the functional diversity of microbial communities in terms of the substrates utilised by those communities (Garland & Mills, 1991; Zak et al., 1994). If its specificity is great enough, the Biolog® system will be useful in assessing whether control treatments can cause lasting shifts in the functional structure of microbial communities associated with the early season growth disorder. The Biolog® system can also be used in identification of soilborne bacteria that are pathogenic to cotton.

Key symptoms of the early season growth disorder will be selected on the basis of their suitability for extension to growers. User friendly protocols for detection the disorder will be formulated and extended to the cotton industry in collaboration with existing extension activities.

Distribution

Early season symptoms of the disease will be assessed in conjunction with disease surveys conducted by Dr Stephen Allen in NSW and Dr Joe Kochman in Queensland. Factors to be assessed include shoot growth, root browning and mycorrhizal and bacterial colonisation of roots. Soil from sites representative of a cross section of cotton growing regions will be tested for growth increases induced by soil pasteurisation (steaming). The growth response of cotton in steamed of soil will be compared to yield in crops at the same sites. Observations of crops early in the season will be compared to yield values. These experiments and observations will enable an assessment of the extent of yield losses in the cotton industry caused by bacterial stunt.

Causes of the disease

A greater understanding of the ecology of the disease will increase the potential for management of it. The following gaps in the knowledge of the causes of the disease need

to be addressed. First, it is possible that not all the species of pathogenic bacteria that colonise cotton roots have been identified. Further isolations of bacteria will be made from cotton roots. New isolates will be tested for pathogenicity to cotton and for potential interactions with existing pathogenic isolates using *in vitro* bioassays. Secondly, the effects of edaphic factors, such as organic matter, soil moisture, anaerobic conditions and nutrient availability, on the populations and/or activity of the pathogenic bacteria will be determined experimentally.

Control

Integrated management of plant diseases utilises biological, chemical and cultural methods. Control methods befitting the nature of the early season growth disorder will be selected for experimental evaluation. Cotton cultivars will be screened for resistance to the pathogenic bacteria using *in vitro* and *in vivo* bioassays. The potential of a range of chemical control procedures will be assessed. Recommendations for integrated management of the early season growth disorder will be based on the outcomes of this project and the basic knowledge provided by previous projects.

RESULTS AND DISCUSSION

DETECTION

Detection of the pathogen

Selective media

Bacteria were isolated from roots of cotton from a range of sites in NSW and QLD (see section on Distribution) using the simple laboratory medium King's B agar. Salmon-pink colony colour and fluorescence were used as key criteria for selecting isolates and their pathogenicity was determined using a laboratory bioassay. Pathogenic isolates were consistently obtained from cotton roots using the King's B agar method, irrespective of the location of sites. Growth on King's B agar and colony morphology thus provide a rapid cost-effective method for preliminary diagnosis of the presence of the pathogen in cotton roots and the rhizosphere, and for enumeration of the pathogen in field and glasshouse studies.

Polyclonal antibodies

Polyclonal fluorescent antibodies were produced on a contract basis at the South Australian Department of Primary Industries Veterinary Laboratories. The antibodies were conjugated with the fluorescent marker FITC. In an initial test of the whole colony technique, nutrient agar in 9 cm Petri plates was spot inoculated with six isolates of the pathogenic *Pseudomonas*, incubated at 27 °C for 24 hours and then dried for a 3 to 4 hours in an incubator set at 40 °C. The FITC conjugated antiserum was diluted (10^{-2}) with 0.01 M phosphate buffer saline solution (PBS). A 5 mL aliquot of the diluted antiserum was added to each Petri plate and 2 hours later the solution was poured off one plate. The plates were given two washes with PBS for two minutes each. Much of the upper part of the colonies was washed off. A slight fluorescence was observed on only one colony, of the isolate GS19, but it was very faint and therefore of dubious nature. Soaking the Petri plates in for a greater length of time did not result in visible fluorescence of colonies.

Isolates GS2, GS7, GS14 and GS30 were cultured as 'pour plates' by mixing 0.02 mL aliquots of cell suspensions with molten nutrient agar (45°C) in 9 cm Petri plates. When colonies were approximately 0.2 to 0.3 mm in diameter, plates were dried in the incubator set a t 40°C for 4 hours. Antiserum was diluted in PBS and added to the plates as before. Plates were incubated overnight at room temperature and given two 15 minute washes with 10 mL of PBS. Fluorescence was not observed in any of the colonies. The procedure was repeated with isolates GS2, GS7, GS11, GS14 and GS19, GS 21, GS30, GS39 and, for reference, two isolates kindly provided by Dr Subbu Putcha. The polyclonal antibodies again failed to show specificity for the pathogenic strains. Due to the impending development of PCR primers for the bacterial stunt pathogen, by collaborators at the University of Queensland, further development on polyclonal antibodies was not pursued.

Molecular techniques and taxonomy of the pathogen

In collaboration with this project, Ms Anshu Raghuwanshi, Dr Lindsay Sly, of the University of Queensland, and Dr Mark Fegan, of the CRC for Tropical Plant Pathology, have undertaken a taxonomic study of the pathogenic species of *Pseudomonas* that is most frequently isolated from roots of stunted cotton plants. Phenotypic characterisation of the bacterium showed it to be most closely related to *Pseudomonas corrugata* (Raghuwanshi et al., 1999 in press). Phylogenetic analysis of 16s rDNA sequences indicated that the bacterium belongs to a novel cluster within the genus Pseudomonas. This cluster is close to, but distinct from, *Pseudomonas marginalis*. PCR primers were

constructed and, in testing against 129 cultures of pseudomonads, were found to be specific for the pathogen (Raghuwanshi et al., 1999 in press).

Diagnosis of the disease

Characterisation of soils using the Biolog® system

In November 1995, three replicated samples of cotton root systems (ten seedlings in each replicate) were collected from sites with and without severe stunting (Table 1). Microorganisms were sampled from rhizosphere soil at these sites by shaking the intact root system of ten seedlings, with soil attached, in 20 mL of sterile distilled for 2 minutes, diluting the resulting suspension by 10^{-3} and inoculating the wells of Biolog® plates with 100 µL of the diluted suspension. Microorganisms were sampled from non-rhizosphere soil in the same manner as rhizosphere soil. Microorganisms were sampled from inside cotton roots after surface sterilising of the roots in bleach (1%) for 2 minutes, macerating the roots and then diluting (10^{-2}) the root macerate with sterile distilled water and inoculating each well of a gram negative and a gram positive Biolog® plate with 100 µL of the diluted suspension. Biolog® plates were incubated at 27°C in the dark and the resulting colour changes recorded electronically by scanning the base of the plates with a desktop scanner. Each well was then assessed visually and given a rating of 0 to 4 according to the intensity of the colour change. The substrate utilisation patterns formed by different soils were analysed by multidimensional scaling (MDS) in three dimensions with Guttman scaling of Euclidean distances using 128 wells (ie substrates) as cases.

Table 1. Sites used for characterisation of the functional diversity of the soil microorganisms at Auscott Narrabri using the Biolog® system. DAS = days after sowing.

	Field 20	Field 18
Normal crop growth	**Site 13** (19 DAS)	**Site 23** (25 DAS)
Stunted crop growth	**Site 2** (18 DAS)	**Site 35** (25 DAS)

Some clear differences in the patterns of substrate oxidation by microbial communities were observed using the Biolog® system (Figure 1). There was a distinct separation between communities from the rhizosphere and from within the root (Figure 1A). The root (endophyte) and non-rhizosphere communities also tended to separate according to the field from which the samples were sourced (Figure 1A, B, C). The separation of microbial communities between the two fields may reflect the age of the seedlings at sampling (Table 1) and hence a temporal succession in the mix of specie. This hypothesis, however, does not fit with the pattern for non-rhizosphere soil that also tended to cluster according to field (Figure 1C). Factors other than seedling age, such as the timing of irrigation after sowing, may be involved.

There was no clear separation of microbial communities according to the severity of stunting of cotton seedlings in the field, in either the rhizosphere or the root (Figure 1A & B). In non-rhizosphere soil in field 20, there was a separation between the stunted-cotton site (site 2) and the normal-cotton site (site 13) (Figure 1C). However, the same separation did not occur in field 18. When the procedure described above was repeated using samples from sites 2 and 23 there was no distinct separation of sites, in either the rhizosphere or root communities.

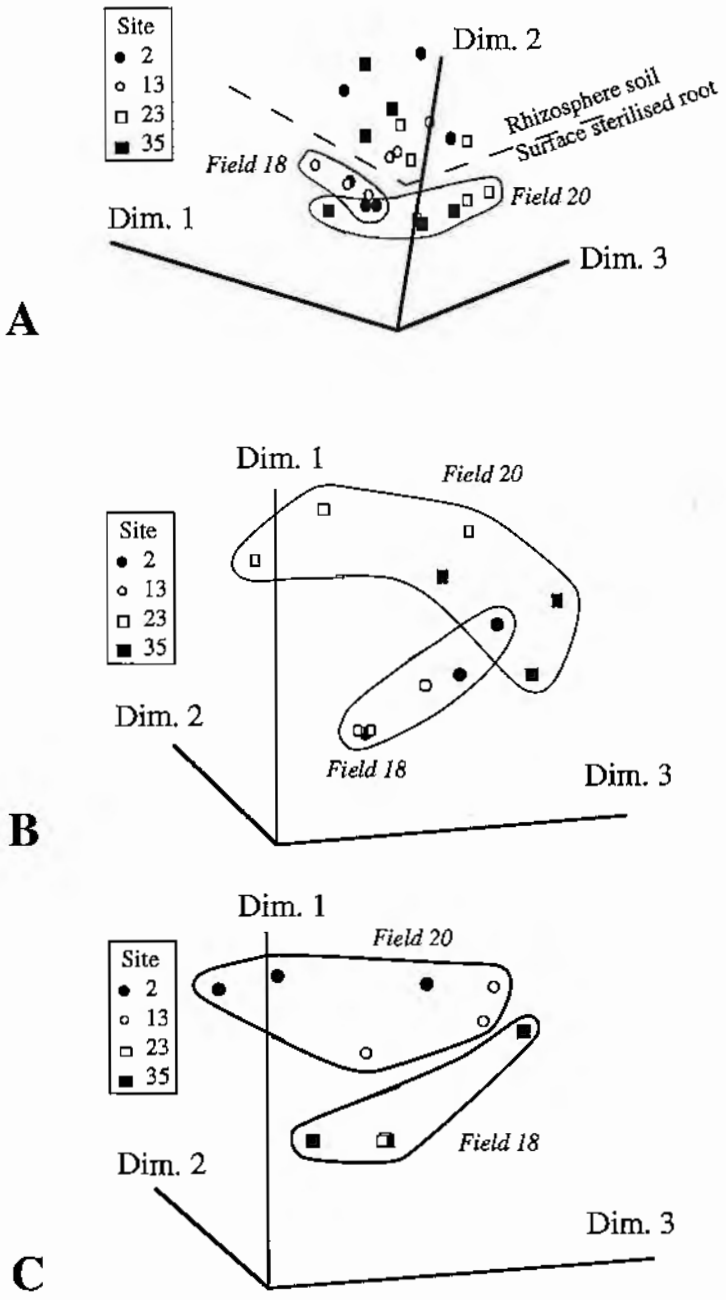

Figure 1. Multidimensional scaling of patterns of substrate utilisation by microbial communities from (A) both rhizosphere soil and surface sterilised roots, (B) surface sterilised roots only and (C) non-rhizosphere soil. Sites are as in Table 1.

Diagnosis of the disease by symptoms

Two Research Reviews are in preparation for publication by the Technology Resource Centre at the Cooperative Research Centre for Sustainable Cotton Production. The first review will describe mycorrhizal symbiosis in cotton and its management. The mycorrhizal review will complement the second review, on bacterial stunt in cotton. The bacterial stunt review will describe the key symptoms of the disease, including the growth and nutritional status of the plant, mycorrhizal colonisation and root browning, and the relationship between severe symptoms and soil properties (refer to Background above).

When bacterial stunt is severe, growers and consultants can achieve a preliminary diagnosis according to the following symptoms and circumstances:

* Areas of uniformly stunted plants early in the season, particularly in heavy grey clay soils. This is most easily discernible when areas with both heavy and lighter textured soils occur within the one field.

* Foliar symptoms of zinc deficiency in severely stunted plants. These symptoms include: yellowing between the main veins of the upper leaves, especially closer to the leaf margins; bronze to brown coloured spots developing after the yellowing; upward cupping of the leaves.

* Rapid development of brown (not black) discolouration of the fine roots. Browning becomes obvious by the time seedlings are two to three weeks old and should be gauged against healthy plants in lighter textured soils.

* Stunting occurs irrespective of cropping sequence and is hence not related to long fallow disorder.

* Unless stunting is very severe, the crop tends to grow rapidly after December and may yield normally but with delayed maturity.

* Diseases such as verticillium wilt and black root rot may be less severe in soils where bacterial stunt is greatest.

Plant pathologists can confirm the diagnosis of bacterial stunt according the above points as well as the following:

* Slow mycorrhizal colonisation in stunted plants. Colonisation approaches normal levels (50 to 70 %) by mid-season.

* Increased growth of cotton in steam sterilised soil and restoration of stunting in steamed soil by amendment with a small amount of unsteamed soil (refer to pot experiments in 'Distribution' section below).

* Bacterial streaming from brown roots and bacteria visible within cortical cells and root hairs.

* Presence of the pathogenic species of *Pseudomonas*.

* Lack of fungal pathogens associated with brown roots.

* Molecular techniques developed by researchers at the University of Queensland.

DISTRIBUTION

A survey for signs of bacterial stunt was conducted in most districts of NSW and three districts in QLD (Table 1). Bacteria were visible inside browned roots at numerous sites. The pathogenic species of *Pseudomonas* was isolated from most regions. Direct comparison of VAM colonisation between sites is not possible due to the variation in planting dates and sampling times. Invariably, however, when sites within the same field were compared, the smaller plants had a lower VAM colonisation in the roots (Table 1).

Early season cotton growth and seed cotton yield was measured at selected pairs of sites. Each pair included a site with early season stunting on heavier grey clay soil, and a site without stunting on lighter textured soil, within the same field. Thus, irrigation and other practices were effectively similar for both sites in the field. The larger the degree of stunting of seedlings, in comparison to plants at the other site in the pair, the larger the loss of yield, in comparison to the other site in the pair (Figure 2). Hence, irrespective of the absolute values of growth and yield, the greater the stunting, the greater the relative yield loss. Yield losses were sometimes as high as 50 % (Figure 2).

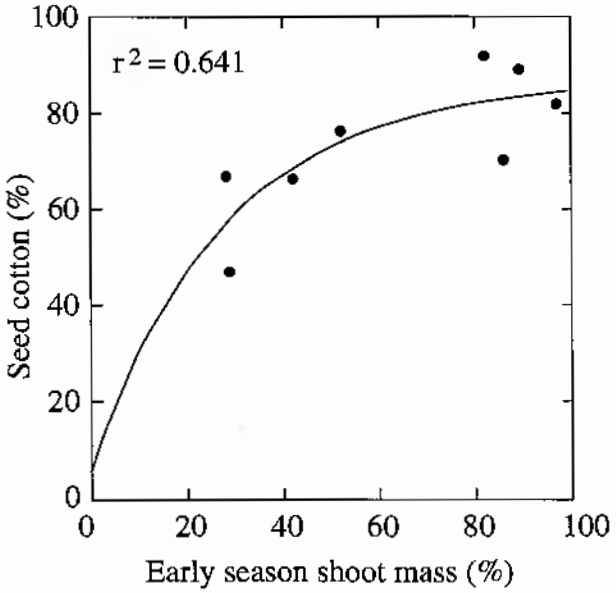

Figure 2. Relationship between relative stunting of cotton early in the season and subsequent relative yield in eight fields in the lower Namoi and Gwydir valleys in the 1995/96 season. Shoot and seed cotton mass are expressed as a percentage of the mass of plants healthy plants elsewhere in the same field.

The contribution of soilborne pathogens to stunting was examined in a pot experiment in which cotton was grown in steam sterilised soil, unsteamed (live) soil and steamed soil mixed with unsteamed soil (6:1). Steaming caused significant growth increases in 22 of the 39 soils examined (Table 3). Black root rot, caused by *Thielaviopsis basicola*, was present in four of these 22 soils and was probably the dominant pathogen. In the remaining 18 soils, however, there were no obvious fungal pathogens in the roots. In many cases, amendment of steamed soil with a small proportion of unsteamed soil partly or completely restored the growth depressing qualities of that soil (Table 3). Hence the growth increases were not attributable to release of nutrients by steaming (pots were all given N fertiliser during the experiment).

Table 2. Sites surveyed for bacterial stunt and VAM

Farm	via	Field	Site No.	Visible Bacteria in roots	VAM Roots (%)	Comments[x]
Colly Farm	Collarenebri	140	$S1_{large}$		23	Larger plants, following 2 year fallow
		140	$S1_{small}$		10	Smaller plants, following 2 year fallow
		126	S2	+	10	Black root rot
Iffley	Collarenebri	11north	S3	+	39	Following one year fallow
Dundee	Burren Junction	26	S4	+	5	
Waverley	Merah North	5	S5		31	
20 Stone	Warren	14	S6	+	32	
Buttabone	Warren	23	S7	+	30	
		20	S8	+	28	
Auscott	Warren	22	S9	+	28	Black root rot in 90 % of plants
		13	S10	+	44	Continuous cotton plot in CRC trial
Byron	Trangie	11	S11	+	34	
Toobaruma West	Warren	3	S12	+	35	
Riverview	Warren	4	S13	+	59	
Drayton	Breeza	8	S14			
		E	S15	+	7	Black root rot
Red Mill	Moree	8	S16	+	34	Stunted cotton
		8	S17	+	52	Larger cotton in the same rows as S16
Binneguy Station	Pallamallawa	1	S18	+	10	
Benwerrin	North Star	1	S19	+	23	Black root rot
Warendi South	Boggabilla	11	S20	+	18	
Cambooya	Boomi	1	S21	+	37	
Wyadrigah	Collarenebri	2	S22	+	36	
Retreat	Baan Baa	7	S23	+	41	Perennially smaller plants on heavy grey clay
		7	S24		68	Larger plants on lighter soil
Sunnyside North	Merah North	7	S25		9	Smaller plants
		7	S26		49	Larger plants
Beechworth	Merah North	B2	S27			Smaller plants
		B2	S28			Larger plants

Table 2. Continued

Farm	via	Field	Site No.	Visible Bacteria in roots	VAM Roots (%)	Comments
Athelstone	Wee Waa	6	S29	+	20	Smaller plants
		6	S30		52	Larger plants
Darling Farms	Bourke	1	S33	+	13	Smaller plants
		1	S34		26	Larger plants
		7	S35	+	38	
The Glen	Ashley	1	S36	+	10	Smaller plants
		1	S37		56	Larger plants
Topbox	Garah	45	S38	+	61	Small plants with Zn deficiency symptoms
Strathguyle	Garah	3	S39	+	44	
Drayton	Breeza	E	S40		36	Smaller plants
		E	S41		44	Larger plants
Warilea	Maules Creek	10	S42	+	52	Smaller plants, black root rot
		10	S43		38	Larger plants
Auscott	Narrabri	20	S44			Previously described in UNE7C
Coolibah	Emerald		S45	+	43	
Calrossie	Boggabri		S48		39	Smaller plants
			S49		78	Larger plants
			S50		63	Smaller plants, heavy grey clay
			$S51_{small}$		39	Smaller plants
			$S51_{large}$		78	Larger plants
Gunedra	Wee Waa	4	$S52_{small}$		17	Smaller plants
		4	$S52_{large}$		55	Larger plants
		4	S53			Smaller plants, heavy grey clay
		4	$S54_{small}$		25	Smaller plants
		4	$S54_{large}$		52	Larger plants
Farm 105	St. George	105	S55		19	Smaller plants, heavy grey clay, black root rot
		105	S56		25	Larger plants, lighter soil, black root rot
Currawildi (Clyde)	Dirranbandi	15b	S57		16	Very small plants, no VAM at 3 weeks, sodic soil (ESP=12%) in deep cut
					85	
		15b	S58		22	Small plants

Table 2. Continued.

Farm	via	Field	Site No.	Visible Bacteria in roots	VAM Roots (%)	Comments
		15b	S59		64	Larger plants in same rows as S58
		15b	S60		45	Very large plants
			S61		29	Larger plants near S58
		6	S62		29	Smaller plants, 10% VAM at 3 weeks, cut area
					52	
		6	S63		38	Larger plants, 16% VAM at 3 weeks, no cut
Auscott	Narrabri	20	S65			Previously described in UNE7C
		19	S66			
ACRI	Narrabri	4	S67			

[z] Reference to the size of plants is given only for comparison of sites within the same field.

Table 3. Increases in cotton growth at 25 days after sowing, due to elimination of soilborne pathogens by soil steaming. Mixed soil = steamed soil amended with 14 % unsteamed soil.

	Shoot growth (g plant^{-1})				
Site[z]	Live soil	Mixed soil	Steamed soil	Increase[x] with steaming (%)	Probability[y]
1996					
S1	0.24	0.25	0.27	11	NS
S2[w]	0.23b	0.32a	0.33a	**43**	p =0.002
S3	0.25b	0.34a	0.33a	**29**	p ≤0.035
S4	0.27	0.25	0.25	-10	NS
S5	0.29b	0.34b	0.38a	**30**	p =0.011
S6	0.34	0.35	0.40	17	NS
S7	0.29	0.33	0.30	6	NS
S8	0.33b	0.31b	0.39a	**19**	p ≤0.034
S9[w]	0.21b	0.28a	0.31a	**47**	p ≤0.003
S10[w]	0.26b	0.31b	0.40a	**55**	p ≤0.001
S11	0.29	0.30	0.30	5	NS
S12	0.32a	0.40b	0.34a	5	p ≤0.016
S13	0.30	0.35	0.33	8	NS
S14	0.31b	0.26b	0.39a	**26**	p ≤0.011
S15[w]	0.35b	0.43a	0.39ab	10	p =0.005
S16	0.29b	0.36a	0.38a	**29**	p ≤0.010
S18	0.32	0.33	0.38	17	NS
S19	0.24c	0.35b	0.45a	**86**	p ≤0.001
S20	0.32b	0.39a	0.38a	**19**	p ≤0.001
S21	0.32b	0.34b	0.37a	**16**	p =0.034
S22	0.38	0.37	0.39	3	NS
S23	0.29b	0.37a	0.37a	**28**	p =0.001
S25	0.30	0.36	0.35	17	NS
S29	0.24b	0.29a	0.32a	**34**	p ≤0.019
S31	0.33b	0.39a	0.37ab	13	p =0.012
S33	0.26b	0.34a	0.39a	**51**	p ≤0.004
S36	0.34b	0.38b	0.45a	**33**	p =0.007
S38	0.31	0.33	0.37	20	NS
S39	0.38	0.43	0.43	13	NS
S42[w]	0.34b	0.47a	0.48a	**41**	p ≤0.020
S44	0.29b	0.43a	0.44a	**53**	p ≤0.001
S45	0.32b	0.39a	0.35ab	8	p =0.015
1998					
S44	0.42b	0.84a	0.97a	**133**	p ≤0.001
S55	0.43b	0.84a	0.96a	**122**	p ≤0.001
S56	0.34b	0.46a	0.57a	**65**	p ≤0.035
S57	0.33	0.31	0.36	7	NS
S58	0.41	0.37	0.40	-2	NS
S60	0.42c	0.68b	0.72a	**72**	p ≤0.030
S66	0.40b	0.70a	0.68a	**70**	p ≤0.001
S67	0.47b	0.78a	0.80a	**70**	p ≤0.001

[z] Sites are described in Table 2.
[y] Means followed by the same letter are not significantly different at the stated probability level by Fisher's LSD test.
[x] Significant increases in bold.
[w] Black root rot present.

The relative increase in growth caused by steaming the soil was negatively proportional to the growth potential of the soil (Figure 3). In other words, the soils with the lowest potential for cotton growth, gained the most from steaming. Clearly, soilborne pathogens have a major influence on the potential for growth of cotton in some soils. While *Thielaviopsis basicola* was a factor, non-fungal pathogens, namely bacteria, are a major determinant of the relative fertility of cotton growing soils. The pathogenic strain of *Pseudomonas* was isolated from roots of cotton growing in most of these soils. Bioassays confirmed the pathogenicity of these isolates to cotton. The identity of these isolates is currently being confirmed with PCR primers by collaborators at the University of Queensland. This information will be compiled for publication of the survey results in scientific journals (see section on publications in preparation below).

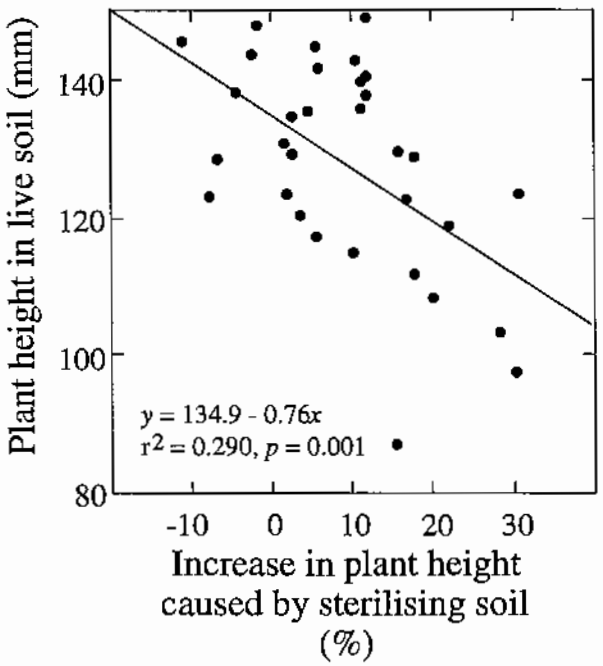

Figure 3. Relationship between relative increase in cotton growth due to steaming and the growth potential of cotton in unsteamed soil, using 32 soils in 1996 (see Table 3).

To gauge the relevance of the observations made in the pot experiment to field conditions, the increase in cotton growth caused by steaming soil was compared to absolute values of seed cotton yield in the 1995/96 crop (Figure 4). The sites used in this comparison included grey clays in the lower Namoi valley and the Gwydir valley, (ie with comparable climatic conditions), and excluded sites known to have black root rot. There was a strong negative relationship between growth increases caused by steaming soil and lint yield (Figure 4). Hence, the pot experiment gave a useful measure of the severity of bacterial stunt.

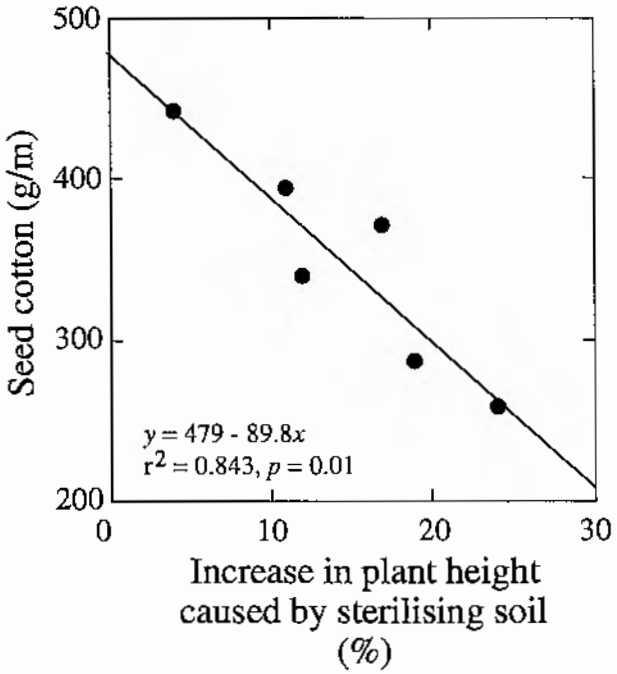

Figure 4. Relationship between relative increase in cotton growth due to steaming and the yield potential of cotton in the field, at sites in the lower Namoi Valley and Gwydir Valley in 1996.

CONTROL

SCREENING FOR RESISTANCE

CSIRO breeding lines and commercial cultivars were sown in stunted-crop soil at Field 19, Auscott Narrabri on 4 October 1996. Plant growth was assessed as shoot height on 1 November. Seedling growth was assessed in a range of lines selected to include high and low yielding lines, as determined by a previous trial at ACRI by Dr Greg Constable (Table 4). Some differences in shoot height were observed (Table 4) but they were not significant (p = 0.528). Early season growth, measured as shoot height, was not correlated with either previous or present yield (Table 4).

Table 4. Early season growth and yield of experimental cotton lines in Field 20 in 1996/97, compared to previous yield at ACRI.

Line	[z]Previous yield (kg lint ha^{-1})	Shoot height 96/97 (mm)	[z]Yield 1996/97 (kg lint ha^{-1})
V16*	-	-	1948
V16	-	-	1922
Sicala V2	-	95	1587
Sicala V2	-	99	1688
91208-41	1231	92	1195
91208-59	1348	108	773
91236-112	1878	104	1514
91236-316	1272	114	750
91236-407	2066	95	900
91236-423	1917	104	1222
91237-216	1946	107	1596
91237-748	1240	100	1224

[z] Data courtesy of G.A. Constable.

Commercial cultivars Siokra 1-4, Siokra L23, Sicala V2, Sicot 189, Siokra V15, DPL 90, and CSIRO breeding lines 91208-41, 91208-59, 91236-407, 91236-423, 91236-316, 91236-810, 91236-112, 91237-379, 91237-410, 91237-748, 91237-947, 91237-878, were inoculated in an *in vitro* bioassay with isolate S55-1. All these lines were highly susceptible, with growth of germinating seedlings reduced by more than two thirds and 60 to 70 % of the total seedling length (combined hypocotyl and radicle) showing brown discolouration due to infection by the bacterium.

CHEMICAL AND BIOLOGICAL CONTROL

Several chemical and biological control agents were evaluated in field experiments in a **commercial cotton field where early season stunting of cotton growth is severe, at** Galathera Creek.

In the first experiment various biocides and other chemicals were applied, as a soil drench (1.0 L) at sowing, to 6 replicated plots 1.5 m long in a completely randomised block design. The treatments included water, agrimycin, benomyl, metalaxyl, SM9, CGA245704, L-tryptophan 0.015, L-tryptophan 1.5, PGR-IV and 'K-Komplex'. Agrimycin contains the antibiotic streptomycin sulphate (17%). Benomyl and metalaxyl are fungicides. SM9 is an anionic surfactant. L-tryptophan is an amino acid that, applied as a soil drench at 10 DAS, increased the total mass of cotton in a sandy loam by up to 43 % and boll number by up to 22 % (Arshad et al., 1995). PGR-IV is a commercial plant growth regulator containing gibberellins. CGA 245704 is a chemical used to induce systemic resistance to pathogens in plants. K-Komplex is a commercial fertiliser produced using a fermentation process.

There were no significant differences in shoot fresh mass ($P = 0.263$) or dry mass ($P = 0.272$) between any of the treatments at 21 days after sowing (Table 5). CGA245704 was phytotoxic to cotton and omitted from the statistical analysis.

Table 5. Chemicals applied as soil drenches to control bacterial stunt of cotton.

Treatment	Rate	Shoot fresh mass (g plant^{-1})	Standard Error	Shoot dry mass (g plant^{-1})	Standard Error
Control (Water)	1.0 L	1.77	0.24	0.31	0.036
Agrimicyn	8.8 g L^{-1}	2.02	0.15	0.36	0.022
Benomyl	1.6 g L^{-1}	2.02	0.14	0.36	0.022
Metalaxyl^{x}	15 g	1.99	0.13	0.34	0.024
SM9	7.5 mL L^{-1}	2.08	0.08	0.36	0.015
CGA245704	0.48 g L^{-1}	0.47	-	0.09	-
L-tryptophan	0.015 g L^{-1}	1.87	0.21	0.33	0.031
L-tryptophan	1.5 g L^{-1}	1.86	0.20	0.33	0.027
PGR-IV	37.5 µL L^{-1}	1.82	0.12	0.34	0.013
K-complex	0.75 mL L^{-1}	2.41	0.20	0.41	0.025

^{x} Applied as dry granules and then drenched with 1.0 L of water.

In a second experiment, two commercially available biological control agents, Kodiak and *Trichoderma harzianum*, and a plant growth promotant, glycine betaine, were applied as a seed dressings on cotton sown in 4 replicate plots 10 m long in a completely randomised block design. Pairwise comparison of means with Fisher's LSD test showed that growth of the cultivar Sicala V2 at 21 days after sowing was significantly greater than that of the cultivar CS189 (Table 6). However, there were no significant effects of the treatments on cotton growth or emergence.

Table 6. Biological control agents applied to cotton seeds to control bacterial stunt.

	Rate	Emergence (%)	Std Error	Shoot fresh mass (g plant^{-1})	Std Error	Shoot dry mass (g plant^{-1})	Std Error
Sicala V2							
Control	0	40	52.0	1.95a	0.446	0.97a	0.218
T. harzianum	2g kg^{-1} seed	40	12.9	2.10a	0.121	1.05a	0.056
Kodiak	2.5g kg^{-1} seed	32	10.2	1.95a	0.301	1.00a	0.130
CS189							
Control		30	11.4	1.07b	0.196	0.53b	0.094
Glycine betaine	7.5% coat	25	16.6	1.33b	0.257	0.71ab	0.146
Overall probability		NS		$P = 0.016$		$P = 0.028$	

In collaboration with this project, Dr S. Putcha applied 56 biological control treatments in 10 m single row plots in a field at Galathera Creek. The soil was relatively dry at sowing and, although the plots were irrigated 4 days after sowing, the dry conditions were unfavourable for establishment of the biocontrol agents in the rhizosphere (S. Putcha, pers. comm.). Furthermore, hot (40°C) dry conditions were experienced after seedling emergence, causing the loss of some seedlings.

CULTURAL CONTROL

Research in project UNE7C indicated that maintaining soil water content at water holding capacity increased cotton growth in soils with bacterial stunt, provided that nitrogen supplies were adequate. In this project, a series of experiments that manipulated soil moisture using organic mulches, furrow irrigation and novel irrigation techniques were conducted to evaluate the potential for improving early season cotton growth. All these

experiments were conducted in irrigated fields at Auscott Narrabri or at ACRI, with 'double' beds (2 m wide) and no cultivation in plots with mulches.

Experiment 1

In June 1996, lucerne hay was laid at least 50 mm thick over plots 14 m long by 6 rows, in Auscott field 19. Mulching earthworms, being a mixture of 'tiger' (*Eisenia fetida*), 'red' (*Lumbricus rubellus*) and 'big blue' (*Perionyx excavatus*), were spread under the hay in the central 10 m of the middle bed in half of the hay plots. Throughout the 1996/97 season the earthworms multiplied and migrated throughout the 3 double beds within each plot, but did not spread to other plots with hay alone. Hay and earthworms were not applied to control plots. Soil water content at sowing was sufficient for a good stand establishment without irrigation.

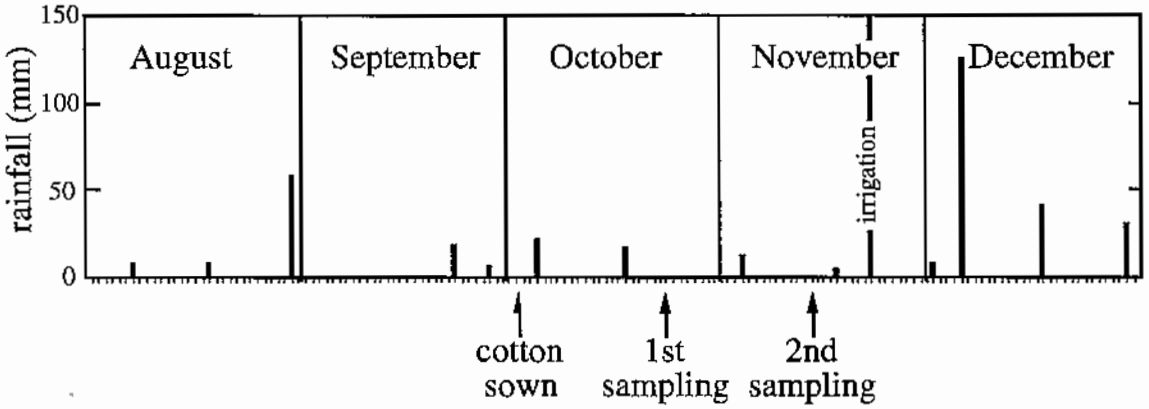

Figure 5. Precipitation, irrigation and sampling times in Experiment 1.

The hay treatments increased early season growth by 30 % and maintained a higher soil water content (Table 7). The mulching worms had no effect on cotton growth or soil water content. By mid-November the N content of shoots in the hay plots had fallen below that of cotton in the bare plots, which is consistent with the greater shoot mass and total above ground N in these treatments (Table 7). By mid-December the plants in the hay treatments were yellow, and their growth had slowed, in comparison to cotton in the bare plots (N content not determined). This suggests that, in comparison to the bare plots, the N supply was exhausted in the hay plots due to a combination of either (i) greater early season growth and hence N uptake, or (ii) wetter soil leading to greater denitrification, or both. Hence the hay did not result in greater seed cotton yield (Table 7).

Table 7. Effect of hay mulch and earthworms on cotton growth in Experiment 1.

	Control	Hay	Hay Worms	Probability	[z]Increase/decrease over control (%)
	23 October 1996				
Shoot height (mm)	42b	55a	54a	$p \leq 0.003$	31
Shoot dry mass (g plant^{-1})	0.13	0.15	0.15	NS	15
Soil water (% oven dry)	31b	35a	36a	$p \leq 0.002$	13
	14 November 1996				
Shoot dry mass (g plant^{-1})	0.37b	0.45a	0.48a	$p \leq 0.050$	28
Shoot N content (%)	4.8a	4.6a	4.5b	$p \leq 0.032$	-5.1
Total shoot N (mg plant^{-1})	18b	22a	21ab	$p = 0.018$	22
	28 April 1997				
Seed cotton (kg ha^{-1})	3713	3526	3550	NS	-4.7

[z] For mean of both treatments with hay.

Experiment 2

Experiment 2 was a repeat of Experiment 1 using the same plots in the 1997/98 season. Earthworms did not survive the winter period in 1997 and little hay remained by the start of Experiment 2. The earthworms treatment was substituted with extra N, applied in two applications of 600 g of 'nitram' fertiliser per plot, on 11 and 25 November, each equivalent to 25 kg of N ha^{-1}. N fertiliser had been previously applied by the grower to the whole site as ammonia gas prior to sowing. The hay mulch was applied at 24 days after sowing, when the cotton seedlings were well established, and the maturity of cotton was assessed by hand picking in March. (Figure 6).

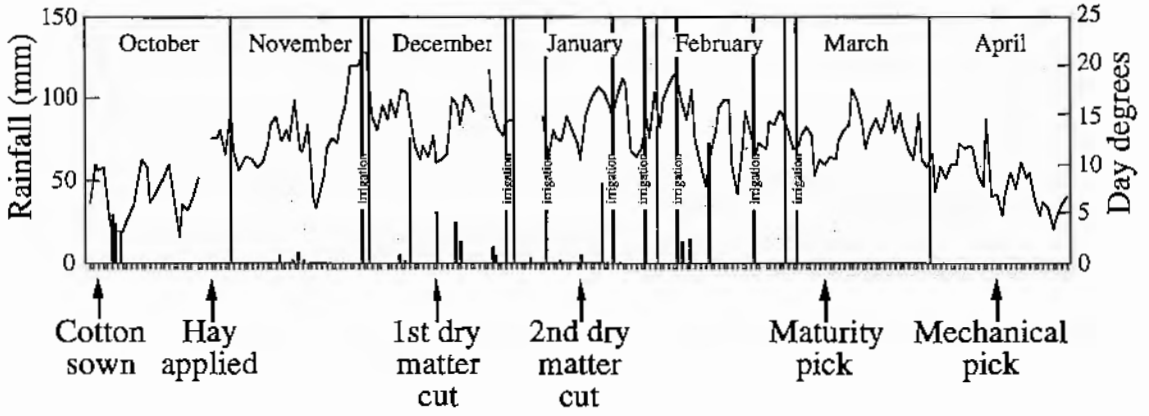

Figure 6. Precipitation, irrigation, day degrees and sampling times in Experiment 2.

In Experiment 2, the hay mulch resulted in large increases in early growth of cotton and this was reflected in greater boll mass in January (Table 7). However, shoot mass in the bare plots had caught up to that of cotton in the hay plots by January, suggesting that plants in the hay plots had 'cut out'. Later in the season there was no difference in cotton maturity and final yield between treatments (Table 7), as occurred in Experiment 1.

Table 7. Effect of hay mulch and extra N on cotton growth in Experiment 2.

	Control	Hay	Hay N		[z]Increase/decrease over control(%)
	15 December 1996				
Shoot fresh mass (g $plant^{-1}$)	17.9b	23.3a	23.9a	$p \leq 0.007$	32
Shoot dry mass (g $plant^{-1}$)	4.6b	7.5a	7.3a	$p \leq 0.004$	62
	15 January 1997				
Shoot fresh mass (kg m^{-1})	2.0	2.3	2.2	NS	14
Shoot dry mass (g m^{-1})	311	328	335	NS	7
Shoot N content (%)	6.7	6.9	6.9	NS	3
Boll number (m^{-1})	18b	28a	23ab	$p = 0.045$	43
Boll fresh mass (g m^{-1})	343b	569a	469ab	$p = 0.026$	51
Boll dry mass (g m^{-1})	50b	74a	65ab	$p = 0.058$[x]	40
Verticillium (% plants)	3.3	1.7	5.1	NS	
	9 March 1998				
Seed cotton (kg ha^{-1})	2620	3150	2820	NS	
	15 April 1998				
Total seed cotton (kg ha^{-1})	5239	4950	5099	NS	

[x] Blocks included as a cofactor in general linear model.

Root growth in the upper 10 cm of the soil profile was significantly ($p = 0.026$) increased by the hay mulch (Figure 7). In the hay mulch treatments, 70 % of the total root mass recovered was in the top 10 cm of soil, compared to 59 % in the bare treatment (Figure 7). The hay mulch maintained soil water content in the upper part of the bed and roots grew right to the surface. The extra soil water did not increase the incidence of verticillium wilt (Table 7).

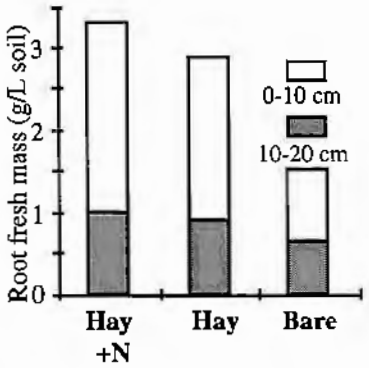

Figure 7. Mulching with lucerne hay 24 days after sowing in a furrow irrigated field increased root growth near the soil surface in January. Hay = hay mulch. +N = additional nitrogen (50 units ha^{-1}). Bare = untreated.

The Hay N treatment was included to account for any loss of N which may have occurred due to extra moisture in the soil, as was suspected in Experiment 1. Nitrogen uptake, measured in January, did not vary between treatments (Table 7). Hence N release from the hay, which was essentially dry during the first six weeks after mulching, was not an important factor. Initially there was no difference in cotton shoot growth between the Hay and Hay N treatments. Later in the season, cotton growth and boll production in the Hay N treatment fell behind that of the Hay treatment. This may partly reflect a greater allocation of carbon budget to root growth by cotton in the Hay N treatment, in comparison to the Hay treatment (Figure 7).

Experiment 3

In the 1996/97 season, supplementary water was applied to plots 3 m long by 2 rows wide in a furrow irrigated cotton field with double beds. The water was delivered by maintaining a constant water table (CWT) in a PVC channel (5 cm square by 3 m long) buried in the centre of the cotton beds. Water was fed into each PVC channel from a 50 L steel sealed drum. Atmospheric pressure held the water in the drum until the water table dropped below the outlet pipe, thus creating a self-regulated water supply. The CWT maintained soil moisture close to field capacity but without waterlogging. Drums were filled daily. In the CWT N treatment, nitram fertiliser was supplied in the reservoir drums at the rate of 50 ppm of N. The CWT plots were covered with a lucerne hay mulch to prevent excessive evaporation. Irrigation and rainfall events (Figure 8) resulted in adequate water supply to the whole site throughout the experiment.

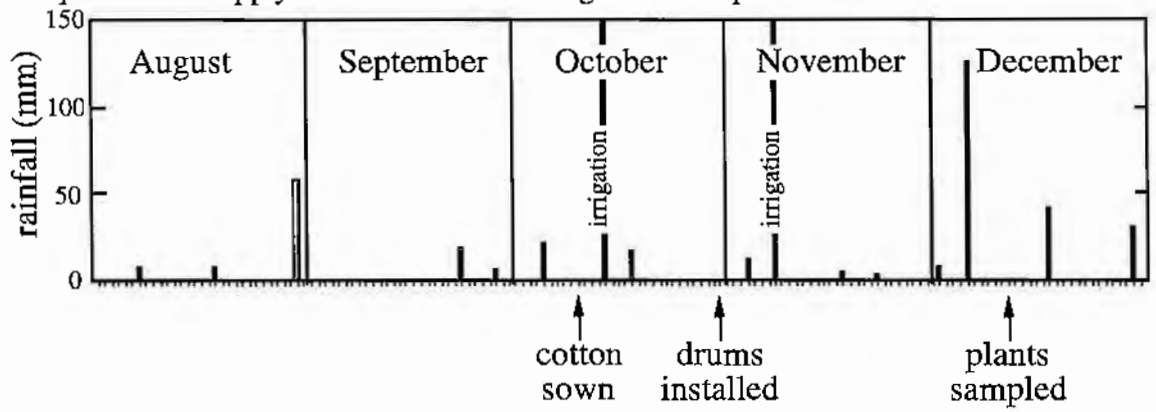

Figure 8. Precipitation, irrigation and sampling times in Experiment 3.

Large increases in early season growth were obtained by providing a constant water table (CWT) in the centre of double beds (Table 8). The gains in early season shoot growth in December were reflected by greater boll production in January. By January, however, shoot mass in the CWT plots was falling behind that of the CWT N plots and the plants looked yellow. Shoot N content in the CWT plots fell below that of the control plots (Table 8), confirming that the additional N supplied in the water was necessary to maintain N at a similar level to that of plants in the control plots.

Table 8. Effect of a constant water table (CWT) and extra N on cotton growth in Experiment 3.

	Control	CWT	CWT N		[z]Increase/decrease CWT N over control (%)
	12 December 1996				
Shoot height (mm)	149b	216a	206a	$p \leq 0.001$	38
Shoot fresh mass (g plant^{-1})	9.1b	15.3a	14.7a	$p \leq 0.001$	62
Shoot dry mass (g plant^{-1})	1.9b	2.8a	2.8a	$p \leq 0.001$	46
Root browning (%)	14b	25a	11b	$p \leq 0.013$	
VAM root (%)	29	26	28	NS	
	20 January 1997				
Shoot height (cm)	57b	58ab	64a	$p = 0.030$	14
Shoot dry mass (g m^{-1})[z]	246b	276ab	292a	$p = 0.009$	19
YFEL N content (%)	6.0a	5.1b	5.8a	$p \leq 0.033$	-
Boll number (m^{-1})[z]	6.3	9.8	10.3	$p \leq 0.037$	63
Boll fresh mass (g m^{-1})[z]	110	199	183	$p = 0.044$	66
Boll dry mass (g m^{-1})[z]	15b	26a	24a	$p = 0.036$	60
$Root_{0-10}$ dry mass (g L^{-1})	0.09b	0.34a	0.29a	$p < 0.001$	232
$Root_{10-20}$ dry mass (g L^{-1})	0.13b	0.19ab	0.24a	$p = 0.004$	85
$Root_{0-10}$ proportion (%)	41b	65a	55ab	$p = 0.004$	36
$Soil_{0-10}$ water content (%)	24b	27a	27ab	$p = 0.039$	12
$Soil_{10-20}$ water content (%)	29	33	30	NS	
$Root_{0-10}$ browning (%)	30	25	29	NS	
$Root_{10-20}$ browning (%)	31	21	23	NS	
VAM $root_{0-10}$ (%)	59	55	56	NS	
VAM $root_{10-20}$ (%)	58	54	51	NS	
Verticillium (% plants)	2.5	7.5	5.1	NS	

[z] One outlying value removed.

[x] Mean comparison by Fisher's LSD test

Total root growth was increased, and the proportion of root growth in the top 10 cm of the soil profile was increased, by provision of the CWT (Table 8). VAM colonisation was not affected by the treatments. Root browning, which is a symptom of bacterial stunt, was increased by the CWT treatment but reduced when N was added (Table 8). However, in all three treatments the values for root browning are much lower that previously measured at the same site (Nehl et al., 1996). Verticillium wilt was not increased significantly by the CWT treatments.

Experiment 4

Experiment 4 was a repeat of Experiment 3 in a different field in the 1997/98 season. There was a single CWT treatment with N supplied in the water as in Experiment 3. Hay mulch was used without a CWT to distinguish its relative contribution to effects on cotton growth. Otherwise the experiment was conducted as before. Rainfall events and irrigations ensured adequate water supply to the whole site (Figure 9).

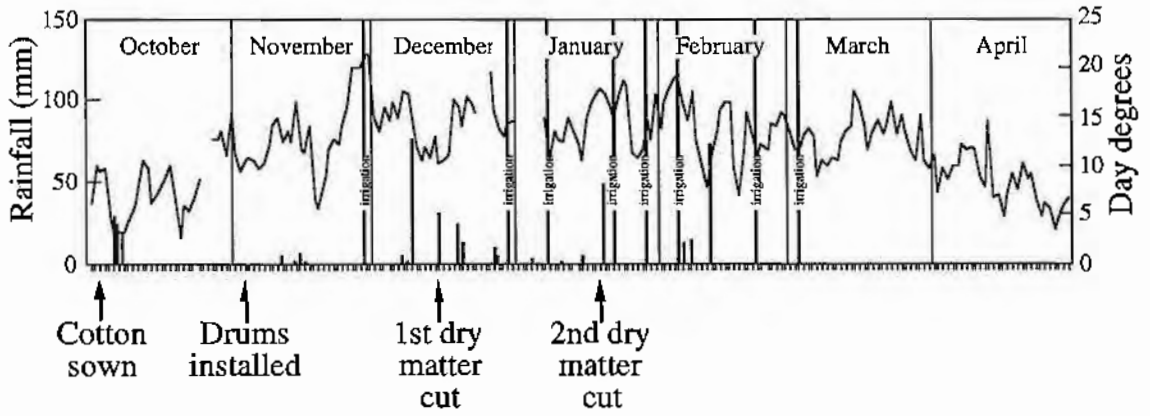

Figure 9. Precipitation, irrigation, day degrees and sampling times in Experiment 4.

Experiment 4 confirmed the observations of Experiment 3. The CWT increased shoot growth and subsequent boll mass (Table 8). This effect on growth was not due to the additional N fertiliser because shoot N content in the CWT treatment in December was lower than in the control (Table 8). The effect of the hay mulch alone on shoot growth (Table 8) was not as great as in Experiment 2, which was in the same field, reflecting the later starting date of this experiment (compare Figures 6 and 9). However, shoot growth tended to be higher in the hay treatment and root growth was significantly increased by the hay mulch (Table 8). In the hay treatment, the relative development of VAM colonisation was greater in the 10 to 20 cm section of the soil profile, with no obvious explanation. Root browning tended to follow the same pattern as VAM colonisation and verticillium incidence was not affected by the treatments.

Table 8. Effect of hay mulch (Hay) and hay mulch with a constant water table (CWT) on cotton growth in Experiment 4.

	Control	Hay	Hay CWT		Increase/decrease in CWT over control (%)
	15 December 1997				
Shoot height (mm)	81b	81b	88a	$p = 0.001$	9.2
Shoot fresh mass (g plant^{-1})	40b	47b	57a	$p \leq 0.013$	41
Shoot dry mass (g plant^{-1})	6.5b	7.8b	9.4a	$p \leq 0.023$	44
YFEL N content (%)	6.4a	5.9ab	5.7b	$p = 0.021$	-11
	19 January 1998				
Shoot height (cm)	81b	81b	90a	$p \leq 0.001$	11
Shoot fresh mass (kg m^{-1})	2.9b	3.1ab	3.8a	$p = 0.016$	32
Shoot dry mass (g m^{-1})	437b	451b	591a	$p \leq 0.007$	35
YFEL N content (%)	7.2	7.1	7.2	NS	
Boll number (m^{-1})	31	38	40	NS	
Boll dry mass (g m^{-1})	84b	106b	118a	$p = 0.037$[z]	42
$Root_{0-10}$ dry mass (g L^{-1})	0.08c	0.14b	0.32a	$p \leq 0.030$	278
$Root_{10-20}$ dry mass (g L^{-1})	0.13b	0.14b	0.21a	$p \leq 0.005$	53
$Root_{0-10}$ proportion (%)	38b	51a	61a	$p \leq 0.035$	58
$Root_{0-10}$ browning (%)	42ab	37b	48a	$p = 0.024$	16
$Root_{10-20}$ browning (%)	46	49	45	NS	
VAM $root_{0-10}$ (%)	67a	48b	76a	$p \leq 0.045$	14
VAM $root_{10-20}$ (%)	62ab	73a	54b	$p = 0.012$	-13
Verticillium (% plants)	4.2	9.2	4.2	NS	

[z] Comparison of Hay and CWT means to Control using Dunnett's test.

Experiment 5

In this experiment extra irrigations, over and above the normal schedule, were applied to plots 16 rows wide in a Field 19, Auscott. The field was sown on 10 October 1996. The soil was moist, such that the field would not normally have been watered after sowing. The experiment was a 2 x 2 factorial design with half the plots either irrigated or not, six days after sowing, and half of these plots either irrigated or not in mid November. All plots were irrigated in early December and all received scheduled irrigations thereafter (Figure 10).

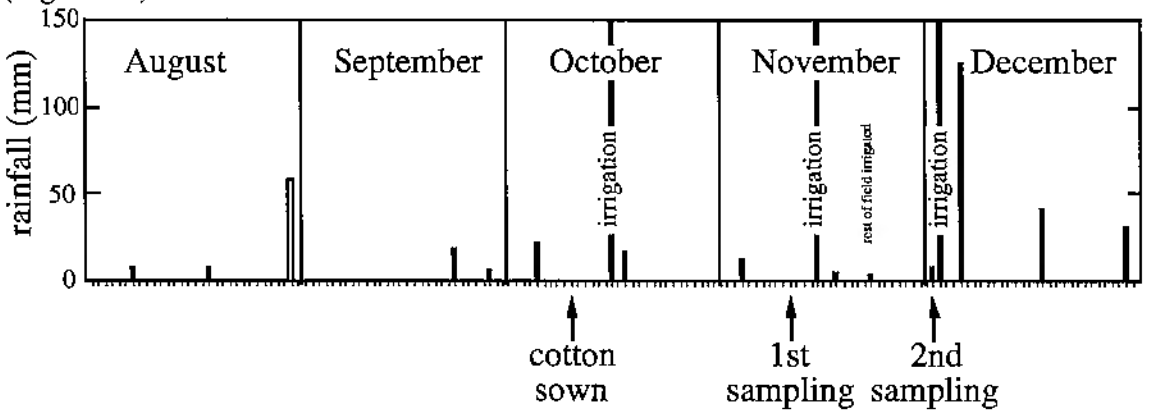

Figure 10. Precipitation, irrigation and sampling times in Experiment 5.

There was a significant, but marginal, increase in soil moisture at the first harvest (11 Nov) in plots that were irrigated at six days after sowing (Table 9). This early irrigation had no effect on cotton seedling growth (Table 9) and reflects the fact that soil moisture was high at the time of sowing, due to rainfall events (Figure 10).

Table 9. Effect of supplementary irrigations on cotton growth and yield in Experiment 5.

	Extra irrigations				
	16 Oct 15 Nov	- 15 Nov	16 Oct -	- -	Probability[z]
11 Nov 96					
Shoot dry mass (g plant^{-1})	0.27	0.28	0.28	0.28	NS
soil moisture %	30a	28b	30a	27b	p = 0.005
2 Dec 96					
Shoot fresh mass (g plant^{-1})	5.3a	5.9a	3.5b	3.5b	p ≤ 0.007
Shoot dry mass (mg plant^{-1})	2.6a	2.8a	1.9b	1.9b	p ≤ 0.007
Soil moisture %	26a	26a	20b	20b	p ≤ 0.006
28 Apr 97					
[y] Seed cotton (bales ha^{-1})	7.9	7.9	7.7	7.8	NS
[y] Micronaire	3.6a	3.6a	3.4b	3.4b	p < 0.001
[y] Staple length	1.138	1.152	1.141	1.144	NS

[z] In rows, values followed by the same letter were not significantly different at the stated probability level for pairwise comparison by the Scheffé test. NS = not significant.

[y] Data courtesy of Stefan Henggeler, Auscott Narrabri.

At the second harvest (2 December) cotton fresh mass and dry mass was increased by 60 and 40 % respectively following the irrigation in November (Table 9). At this stage in the season the soil was noticeably drier in the plots that weren't irrigated in November, reflecting the hot dry conditions which occurred in late November (Figure 10). Plants in the drier plots were showing signs of water stress at the time of harvest.

Yield tended to be greater in the plots which were irrigated in November but these increases were not significant. Micronaire was significantly lower in cotton from the plots which were not irrigated in November (Table 9) although both values for micronaire were below the penalty threshold value. There were some minor differences in staple length but these were not significant. The aim of Experiment 5 was to compare the growth and yield of cotton provided with extra irrigations, to that of cotton with irrigations scheduled by usual farm practice. Unfortunately, the extra irrigations did not meet this requirement satisfactorily. The irrigation after planting had no effect on growth because soil moisture was already high. The irrigation in mid-November resulted in substantially greater early season growth but this was in comparison to plants which were stressed at the time, following hot dry conditions in late November. This stress led to lower micronaire values later in the season. Although the differences in growth between plots were still visually discernible in Jan 1997, the long warm finish to the season allowed yields to equalise across treatments.

Experiment 6

Furrow irrigations, over and above the normal irrigation schedule, were applied to large plots at the north end of Auscott Field 8. Stunting is normally severe in this part of the field, while cotton growth is good at the southern end. The extra irrigations were applied in a 2 x 2 factorial design (four replicates) with plots either irrigated or not on 12 November, and half of these plots either irrigated or not on 11 January. In these four treatments, cotton was sown into uncultivated stubble remaining from a crop of oats that was grown as a covercrop, sprayed with glyphosate and slashed prior to planting. In the rest of the field the oats crop was incorporated by disk cultivation before planting. An extra 3 replicate plots were assessed at both the northern and southern ends of the field to account for the effects of incorporation of the oats. In all cases, plots were 32 rows wide by the length of the furrows.

The additional irrigation in November increased shoot fresh mass, dry mass and N uptake in December by 66 %, 44 % and 30 % respectively (Table 10), suggesting that the extra water gave the plants greater access to nutrient reserves in the soil. However, this early growth increase did not result in greater boll production. By January the N content of cotton given the November irrigation had fallen below that of cotton with the conventional irrigation schedule (Table 10).

Table 10. Effect of supplementary irrigation and oat stubble treatment on early season cotton growth and boll production in Experiment 6.

Oat stubble:	Standing/slashed		Incorporated		
First extra irrigation:	November	-	-	-	
Site location:	North	North	North	South	
12 December 1997					
Shoot fresh mass (g m^{-1})	307b	185c	173c	441a	$p \leq 0.006$
Shoot dry mass (g m^{-1})	49b	34c	33c	74a	$p \leq 0.043$
Shoot N content (%)	6.0	4.6	4.0	5.0	NS[z]
10 January 1998					
Shoot fresh mass (g m^{-1})	1123	1019	-	-	NS
Shoot dry mass (g m^{-1})	226	209	-	-	NS
Boll dry mass (g m^{-1})	10.0	13.2	-	-	NS
YFEL N content (%)	5.8	6.0	-	-	NS

[z] Significant (p = 0.017) when the sites where oats were incorporated were excluded from the statistical analysis.

Although cotton given the extra irrigation was larger in December and had 15 % more lint in open bolls in March, it 'cut out' early, perhaps because the N supply was prematurely depleted, and there was ultimately an 8 % yield penalty (Table 11). The additional irrigation in January did not affect maturity or yield (Table 11). This lack of effect probably reflects the frequent number of irrigations at Auscott at the time (eg Figure 9). Yield results are not yet available for all the plots where oats were incorporated. However, in one of the plots where oats were incorporated, at the northern end of the field, yield was 6.6 bales ha^{-1}, suggesting that a potential yield increase of 23 % was due solely to leaving the oat stubble standing. During the season, the beds in plots where the oats were left standing and slashed appeared to hold their shape, while beds elsewhere in the field, where the oat crop was incorporated were visibly slumped. Following rainfall events there was more water sitting in the furrows where the oat crop was incorporated, than in the standing oats plots. These qualitative observations suggest that the standing oats crop helped maintain good soil structure and increase infiltration.

Table 11. Effect of supplementary irrigation on lint maturity and total yield in the plots where oats were left standing and slashed, in Experiment 6.

First extra irrigation	November	November	-	-	
Second extra irrigation	-	January	-	January	
11 March 1998					
Seed cotton (kg ha^{-1})	3299a	3383a	2881b	2943b	$p = 0.039$
21 March 1998					
Total yield (bales ha^{-1})	7.6b	7.3b	8.1a	8.1a	$p = 0.001$

Experiment 7

In this experiment supplementary soil water was applied to plots (10 m long x 4 rows wide) in furrow irrigated fields that were affected by bacterial stunt to a greater (Field 19) and a lesser (Field 4) degree. The extra water was applied periodically by either drip irrigation or a wick running down the centre of the 2 m beds. Extra N was applied to these plots as occurred in the constant water table (CWT) in Experiment 3. This wick system emulated the CWT used in Experiments 3 and 4. Hay mulch was used in these treatments to prevent evaporative loss. At Auscott field 19, rainfall and irrigations were frequent enough during the season to maintain adequate soil water (Figure 11).

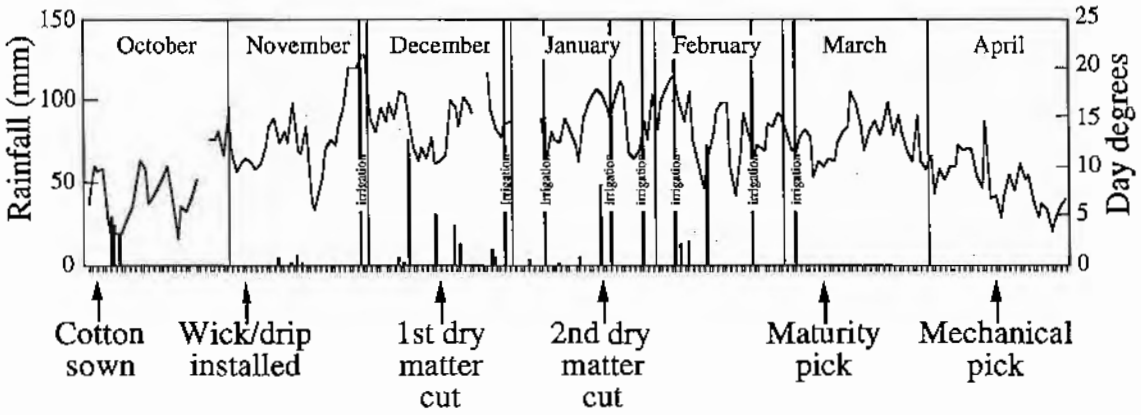

Figure 11. Precipitation, irrigation, day degrees and sampling times at Auscott field 19 in Experiment 7.

As occurred with the constant water table (Experiments 3 and 4), early cotton growth and subsequent boll production were increased by the drip irrigation and wick irrigation treatments (Table 12). Despite the early increases in growth and boll production in Field 19, the control plants (bare) finished as well as those in the other treatments (Table 12). Yield was higher in the drip irrigated plots but not significantly.

Table 12. Effect of supplementary irrigation on growth, maturity and seed cotton yield at Auscott field 19 in Experiment 6.

	Hay Drip	Hay Wick	Control		Increase over control (%) Drip Hay	Wick Hay
16 December 1997						
Shoot fresh mass (g plant^{-1})	67a	58ab	41b	$p = 0.012$	64	42
Shoot fresh mass (g m^{-1})	800a	625a	367b	$p \leq 0.021$	118	71
Shoot dry mass (g plant^{-1})	12.1a	10.8a	6.7b	$p \leq 0.019$	80	61
Shoot dry mass (g m^{-1})	142a	115a	60b	$p \leq 0.007$	135	91
YFEL N content (%)	5.3ab	4.3b	5.7a	$p \leq 0.019^{x}$	-24	
20 January 1998						
Shoot height (mm)	83	82	82	NS		
Shoot dry mass (g m^{-1})	449	426	483	NS		
Boll production (boll m^{-1})	36	39	27	NS		
Boll dry mass (g m^{-1})	119	121	81	NS		
Verticillium (% plants)	5.7	6.6	6.6	NS		
15 April 1998						
Seed cotton middle rows (kg ha^{-1})	7030	7362	6755	NS		
Seed cotton outside rows (kg ha^{-1})	6188	6099	6074	NS		

At ACRI, irrigations in Field 4 (Figure 11) were not as frequent during January and February as at Auscott Field 19 (Figure 11). Plants in the control plots were water stressed (visible wilting) just prior to the first irrigation in February. Nevertheless, plants were not noticeably stressed during the first half of the season.

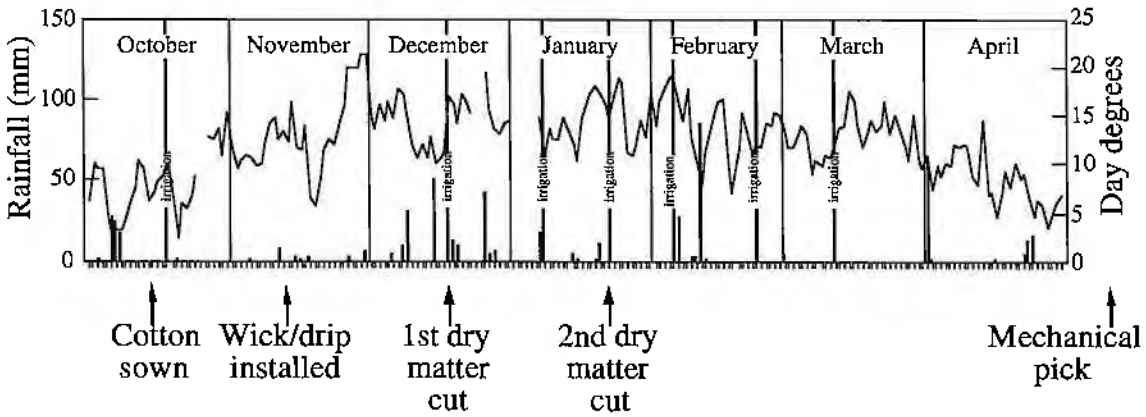

Figure 12. Precipitation, irrigation, day degrees and sampling times at ACRI field 4 in Experiment 7.

In Field 4 at ACRI, the drip the wick treatments both increased shoot dry mass in December by 45 %, and these gains were not due to better N nutrition (Table 13). Shoot dry mass maintained a lead over the control plots through to January while boll production did not. Hence, in contrast to the CWT experiments where early season increases in shoot growth were reflected in greater boll production in January (Tables 8

and 9), a greater proportion of extra photosynthate, produced in the drip and wick treatments, was allocated to vegetative rather than reproductive growth (Table 13).

The drip and wick treatments increased seed cotton yield, in the middle two rows of the plots, by 17 % and 19 % respectively, but not significantly (Table 13). However, there was a significant increase (32 %) in seed cotton yield in the outside rows of the wick plots (Table 13). Hence the competitive effect of the outside rows over the adjacent buffer rows, and also over the centre rows of the plot, may have prevented the centre rows from reaching their yield potential. Larger plot sizes would resolve this issue. Assuming that the yield increase of approximately 19 % in the centre rows may have been significant in a broadacre context, then the potential benefit from mulching and providing supplementary water would be approximately 1.5 bales ha^{-1}.

Table 13. Effect of supplementary irrigation on growth, maturity and seed cotton yield at ACRI field 4 in Experiment 7. Wick = periodic application of extra water with a wick buried in the centre of the 2 m bed. Drip = periodic application of extra water with drip lines on top of the bed. Hay = hay mulch. Bare = untreated.

	Hay Drip	Hay Wick	Hay	Control		Increase over control (%) Drip	Wick
18 December 1997							
Shoot fresh mass (g $plant^{-1}$)	105a	97a	73ab	60b	$p \leq 0.043$	76	62
Shoot dry mass (g $plant^{-1}$)	16a	16a	12ab	11b	$p \leq 0.048$	47	44
YFEL N content (%)	6.6	6.7	6.7	6.5	NS		
22 January 1998							
Shoot fresh mass (kg m^{-1})	3.87	4.85	3.54	3.03		28	60
Boll production (boll m^{-1})	41	67	47	43	NS		
Boll dry mass (g m^{-1})	111	177	134	125	NS		
Shoot dry mass (g m^{-1})	601ab	704a	525b	447b	$p \leq 0.030$	34	57
Verticillium (% plants)	18ab	24a	11ab	5b	$p = 0.019$	250	375
YFEL N content (%)	7.2	7.1	7.0	6.4		12	11
12 May 1998							
Seed cotton, middle rows (kg ha^{-1})	5993	6082	5804	5104	NS		
Seed cotton, outside rows (kg ha^{-1})	6161ab	6962a	5879b	5280b	$p \leq 0.024$[z]	17	32

[z] Mean comparison by Fisher's LSD test.

As observed in previous experiments, the mulches and supplementary irrigation did not increase the incidence of verticillium wilt (Tables 12 and 13).

CONCLUSIONS

Objective 1. Detection of the disease

The pathogenic species of *Pseudomonas* can be selectively isolated using King's B medium and phenotypic characteristics. PCR primers developed by researchers at the University of Queensland will enable definitive identification. Polyclonal antibodies proved to be ineffective for detecting the pathogenic pseudomonad. Monoclonal antibodies may be a feasible alternative but the cost of production cannot be justified in view of the other options now available.

The Biolog® system seems to have good potential for characterising temporal changes in soil microbial communities. However, its failure to distinguish between stunted-crop and normal-crop sites, and the lack of reproducibility of results, prevents its use as a predictive tool for the presence of bacterial stunt in cotton. Greater resolution may be possible with a large increase in the number of replications, but the cost of Biolog® plates ($10.00 each) would then become prohibitive.

A protocol for diagnosis of bacterial stunt at the farm level and at the laboratory level has been developed. Information will be extended to consultants and growers in a CRC publication currently in preparation.

Objective 2. Distribution of the disease

Bacterial stunt and the pathogenic species of *Pseudomonas* are widely distributed in cotton growing regions in NSW and Queensland. Significant stunting, that was not attributable to fungal pathogens, occurred in 40 % of the 43 fields examined using a pot bioassay. As reported in Project UNE7C, severe stunting is associated with heavy clay soils. Yield losses are most readily discernible when different soil types occur within the one field. Yield losses of up to 50 % occur in the field, although losses are commonly much less. A low level of bacterial stunt probably occurs in most cotton crops.

Objective 3. Additional information on the cause of the disease

This objective was not fully satisfied because a greater proportion of time was allocated to expanding on the success of the first experiments with mulches and the constant water table. Nevertheless, the experiments and observations conducted to satisfy the other three objectives have provided some useful information.

The failure to distinguish between microorganism communities from stunted-crop soil and normal-crop using the Biolog system suggests that bacterial stunt is dependent upon factors other than the diversity of the microbial commnnity. This is consistent with the fact that the pathogenic species of *Pseudomonas* can be found in cotton roots in nearly all cotton growing soils. Hence, the behaviour of the bacterial community under different soil conditions appears to be a more important factor in the disease. The survey of the distribution of bacterial stunt provided further confirmation that the severity of bacterial stunt is determined by soil types.

Wet conditions are an exacerbating factor in most soilborne diseases. The experiments with mulches indicated that high soil water content was not a cause of stunting. Hence the detrimental effects of waterlogging on cotton appear unrelated to bacterial stunt.

Objective 4. Control of the disease

There was no evidence of resistance to infection by the pathogenic species of *Pseudomonas* in any of the lines that were tested. There seems to be little prospect of selecting for resistance, at least from the existing breeding program. Furthermore, there

were no outstanding lines in terms of early season growth in the field. Nevertheless, yield performance did vary, with Sicala V2 and Siokra V16 performing best. Hence commercial cultivars with a proven track record of good agronomic characteristics should be used in fields affected by bacterial stunt.

The prospects for application of chemical and biological control agents are also poor. The lack of effect by the chemical agents may reflect the high clay content of the stunted-crop soils. Since stunting involves colonisation of the whole root system by bacteria, even incorporation of chemical control agents would be required. Even incorporation of reactive chemicals would be a difficult task. If even incorporation were achieved, the buffering capacity of these montmorillinite clays is likely to compromise the availability of chemical agents.

Maintaining a high soil water, especially near the soil surface, using mulching and supplementary irrigation techniques has proved to be the most promising method to improve cotton growth in stunted-crop soils. Cracking clay soils in the Namoi valley are more fertile at the surface (Table 14). As the soil surface dries roots lose access to the most fertile part of the soil profile. The growth increases caused by mulching and maintaining higher soil moisture were associated with proliferation of roots in the top 20 cm of soil and did not increase the incidence of Verticillium wilt or other diseases. Furthermore, the proportion of roots was greater near the surface, suggesting that greater access to the fertile layer of topsoil was responsible for the improvement.

Table 14. Characteristics of clay soils in the Edgeroi Data Set (McGarry et al., 1989). Values are the means of 134 sites that had > 40 % clay. Depths are significantly different (P<0.001). N = nitrate-N (mg kg^{-1}); P = bicarbonate-P (mg kg^{-1}); K = exchangeable-K (mmol (K^+) kg^{-1}); Na = exchangeable-Na (mmol kg^{-1}); ESP = exchangeable sodium percentage; OC = organic carbon (%).

Depth	N	P	K	Na	ESP	pH	OC
0-10 cm	17.61	39.31	16.08	14.65	3.649	7.86	1.31
10-20 cm	9.115	21.66	10.83	21.35	5.032	8.383	0.95

These treatments did not necessarily reduce the presence of pathogenic bacteria in the rhizosphere. Root browning, which is caused by microorganisms, was not consistently affected by the mulching and irrigation treatments. The increases in early season growtb were achieved by optimising conditions for the plant in these nutrient rich soils. These techniques are also applicable to clay soils that are not badly affected by bacterial stunt (ie Field 4 at ACRI).

While the early gains in cotton growth with mulching and supplementary water often advanced crop maturity, this was not reflected by yield increases. The experiments were conducted in fields that were managed for conventional crops, not the experimental treatments. The challenge now, is to convert the gains in early season growth into yield increases. The treatments need to be adapted for broadacre farming: covercrops and drip systems are the subject of further research in project DAN122C.

RECOMMENDATIONS AND APPLICATION TO INDUSTRY

Despite the widespread occurrence of bacterial stunt, growers are largely unaware of the presence of bacterial stunt and will probably not show concern while ever crops are profitable in those fields. The capacity for cotton growth to accelerate mid-season, in soils with bacterial stunt, masks the potential losses occurring when only part of a field is affected. Other parts of the field may bring yield up to an acceptable average for the whole field. Frequently, fields with bacterial stunt are the last to be harvested, having delayed maturity even if there is no loss in yield.

The experiments with mulches and irrigation in this project showed that there is potential for growth and yield improvement, not only in soils where bacterial stunt is severe, but also in soils where it is not severe. The application of mulches by spreading hay imported to the field, as occurred in these experiments, is not practical for broadacre crops. A cereal cover crop during the winter is a more feasible alternative. The potential advantages of using a cover-crop to provide a mulch include:

* Maintenance of higher water content in soil near the surface, enabling greater access by roots to soil nutrient reserves
* Improved soil structure and infiltration
* Reduced soil erosion
* Reduced establishment of weeds where the mulch gives a complete cover
* Improved predator populations

The potential disadvantages of using a cover-crop mulch include:

* Inability to cultivate for weeds
* Potential increase in wireworm population
* Utilisation of N by the cover crop and elevated levels of denitrification in wet soil

Clearly the successful use of mulches will require an integrated approach to managing the crop. Control of weeds may be achievable using shielded and spot spraying technology, in combination with herbicide resistant cotton cultivars. Application of N by gas rig is likely to destroy part of the mulch cover but alternative application methods (aerial, irrigation) are available.

There are two options for application of irrigation water: furrow irrigation and drip irrigation. Drip irrigation would enable the greatest control over soil water and N content near the soil surface but is more costly that furrow irrigation. With the reduced evaporation provided by a mulch cover, there may be savings in irrigation requirements overall.

Apart from selecting cultivars with good agronomic characteristics, manipulation of soil water content and maintenance of good soil structure using cover-crop mulches and modified irrigation are the best options for improving early season growth of cotton affected by bacterial stunt. It is anticipated that if cotton crops with a mulch cover are managed to prevent early cut out, then the increases in early season growth observed here can be converted to yield increases, and this is the objective of research in part of Project DAN122C.

COMMUNICATION OF RESULTS

Publications

Refereed papers

Nehl, D. B., Allen, S. J. & Brown, J. F. (1996). Mycorrhizal colonisation, root browning and soil properties associated with a growth disorder of cotton in Australia. *Plant and Soil* 179, 171-182.

Nehl, D. B., Allen, S. J. & Brown, J. F. (1997). Deleterious rhizosphere bacteria: an integrating perspective. *Applied Soil Ecology* 5, 1-20.

Hulugalle, N. R., Entwistle, P. C., Cooper, J. L., Allen, S. J. & Nehl, D. B. (1998). Effect of long-fallow on soil quality and cotton lint yield in an irrigated, self-mulching, grey Vertosol in the central-west of New South Wales. *Australian Journal of Soil Research* 36, 621-639.

Nehl, D. B., Allen, S. J. & Brown, J. F. (1998 in press). Slow arbuscular mycorrhizal colonisation of cotton caused by environmental conditions in the soil. *Mycorrhiza.*

Nehl, D. B., McGee, P. A., Torrisi, V., Pattinson, G. S. & Allen, S. J. (submitted). Patterns of arbuscular mycorrhizal colonisation of crop plants and associated colonisation potential of soil are poorly related. *New Phytologist.*

Refereed book chapters

Allen, S. J. & Nehl, D. B. (1997). Survival and dispersal of soilborne inoculum. pp. 219-230. In: J. F. Brown & H. J. Ogle (Eds), *Plant pathogens and plant diseases,* University of New England: Armidale, NSW, Australia. 556 p.

Refereed conference proceedings

Raghuwanshi, A., Fegan, M., Nehl, D. B., Hayward, C. & Sly, L. (1999 in press). Identification and molecular detection of a pseudomonad causing bacterial stunt of cotton. *Proceedings 1st Australian Soilborne Disease Symposium.*

Conference proceedings

Nehl, D. B., Allen, S. J. & Brown, J. F. (1996). Bacterial stunt of cotton: a balance between beneficial and harmful soil microbes. In *Proceedings of the 8th Australian Cotton Conference* vol. pp. 683-690. Australian Cotton Growers Research Association: Broadbeach, Australia.

Nehl, D. B., Mondal, A. H. & Allen, S. J. (1997). Using mulches to improve the growth of cotton affected by bacterial stunt. In *Proceedings of the Cropping Systems Forum, 1997,* pp. 50-51. Cotton Research and Development Corporation: Narrabri, NSW, Australia.

Nehl, D. B., Allen, S. J. & Brown, J. F. (1997). Plant-microbe-soil interactions in a soilborne disease of cotton. In *11th Biennial Conference, Australasian Plant Pathology Society* vol. pp. 42. Australasian Plant Pathology Society: Perth, Australia.

Hickman, M., Rochester, I.J., Tennakoon, S., Hare, C., Hulugalle, N. R., Charles, G., Allen, S. J, Nehl, D. B., Scott, F., Cooper, J. and Conteh, A. (1998). Rotation crops: what is the impact on an irrigated farming system. In *Proceedings of the 9th Australian Cotton Conference.* pp. 49-59. Australian Cotton Growers Research Association: Broadbeach, Australia.

Nehl, D. B., Mondal, A. H. & Henggeler, S. (1998). Roots and shoots in cahoots: Improving the growth of cotton affected by bacterial stunt. In *Proceedings of the*

9th Australian Cotton Conference, pp. 573-576. Australian Cotton Growers Research Association: Broadbeach, Australia.

Extension articles

Nehl, D. B. & Allen, S. J. (1996). Bacterial stunt of cotton: balancing beneficial and harmful soil microbes. *Australian Cottongrower* 17, 34-38.

Reports

Allen, S. J., Putcha, V. S. & Nehl, D. B. (1997). *Submission to the CRDC Research Review on Cotton Diseases and Microbiology* . Australian Cotton Research Institute: Narrabri, NSW, Australia.

Allen, S. J., Lonergan, P. A. & Nehl, D. B. (1997). Black root rot at the Warren Farming System site. In *Macquarie Valley Cotton Trial Reports, 1996-97* vol. pp. 93-96. Ed., D. Kelly. NSW Agriculture.

Field day notes

Nehl, D. B. & Allen, S. J. (1997). Control of bacterial stunt. In *Lower Namoi Annual Cotton Field Day Notes* . Australian Cotton Research Institute: Narrabri, NSW, Australia.

Nehl, D. B., Mondal, A. H. & Allen, S. J. (1998). Better early season growth of cotton. In *Lower Namoi Annual Cotton Field Day Notes*, Australian Cotton Research Institute: Narrabri, NSW, Australia.

Posters

Nehl, D. B. & Allen, S. J. (1996). *Managing Mycorrhizas in Cotton* . 8th Australian Cotton Conference: Broadbeach, Australia.

Nehl, D. B. & Allen, S. J. (1996). *Bacterial stunt. A soilborne disease of cotton* . 8th Australian Cotton Conference: Broadbeach, Australia.

Publications in preparation

The following papers are in preparation for submission to refereed journals.

Bacterial stunt of cotton: the effects of mutualistic and pathogenic soilborne microorganisms on plant growth. *Phytopathology*

Bacterial stunt of cotton: isolation and characterisation of a pathogenic species of *Pseudomonas*. *Phytopathology*

The distribution bacterial stunt in Australian cotton. *Australian Journal of Experimental Agriculture.*

Increased growth of cotton in irrigated cracking clays using mulches and supplementary water. *Australian Journal of Experimental Agriculture.*

Characterisation of microbial diversity in the rhizosphere of cotton using Biolog. *Plant and Soil.*

Other extension activity

Apart from participation in each of the Australian Cotton Conferences, the author has made presentations at field days and at meetings of growers and consultants on 13 occasions during the course of this project, and given lectures on VAM and bacterial stunt to the CRC cotton production course.

APPENDIX - BUDGET

Total funds contributed to DAN100C by the CRDC.

Year	DAN100C
1995-96	64 716
1996-97	58 692
1997-98	97 201
Total	$220 609